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ETS Gene Fusions as Predictive Biomarkers of Resistance to Radiation Therapy for Prostate Cancer

PRINCIPAL INVESTIGATOR:

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CONTRACTING ORGANIZATION:

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14. ABSTRACT

The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in preclinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a translational scientist. This grant proposal was approved as a five-year award; the current annual report summarizes accomplishments over the fourth year of the grant, from July 15, 2013 to July 15, 2014.

Overall, the first four years of this grant have been very successful. The work accomplished as a result of this grant resulted in five publications (including a sixth in submission), five presentations, and five grants (three from the Prostate Cancer Foundation, one from Celgene, and one from the Fund for Cancer Research). Additionally, Dr. Feng has met the training achievements specified in his original grant.

The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mechanistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents a primary treatment modality for localized prostate cancer. In the fourth year of this grant period, we have accomplished another two subtasks, for a total of 17 out of 20 originally proposed subtasks accomplished over the first four years of this grant. These two subaims included one aimed at studying the functional significance of the interaction between ETS fusions and the DNAPK protein, as well as a third towards our biomarker goals. This work builds on our findings from our first three years that ERG (the predominant ETS gene fusion product) confers radioresistance in preclinical models of prostate cancer and that this radioresistance can be reversed with DNA-PK inhibition. In total, our findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers.

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Prostate cancer, ETS gene fusions, ERG, radiation resistance, DNA-PK					
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Introduction

This annual report will summarize the accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to Felix Feng, M.D. This award included both research goals and training goals. The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in preclinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a translational scientist, with the ultimate goals of submitting a NIH-level grant as an independent investigator and developing a translational clinical trial. This grant proposal was approved as a five-year award; the current annual report summarizes accomplishments over the fourth year of the grant, from July 15, 2013 to July 15, 2014.

Body

Research achievements: Tasks and Subtasks

As outlined in the original Statement of Work, this grant proposal was comprised of three specific aims, subdivided into 7 tasks, which were further divided into 20 subtasks. In year 1, I accomplished seven subtasks (1A, 1B, 3A, 3B, 4A, 4B, and 4C), resulting in completion of Tasks #1 and #3. In year 2, I performed subtasks 2A, 2B, 6A, 7A, and 7B, resulting in progress in Tasks #2, #6, and #7. In year 3, I was able to complete an additional three subtasks (5A, 5B, and 6B), resulting in progress in Tasks #5 and #6. In year 4, I made progress in subtasks 4D and 6C, resulting in completion of Tasks #4 and progress in Task #6. In total, I have now completed 17 out of 20 proposed subtasks over the first three years of my grant, which puts me ahead of the schedule outlined in my initial statement of work (16 subtasks to be completed over the first 4 years). The findings associated with these subtasks and tasks from year 4 are detailed below.

The goal of Task #4 was to determine which ERG domains are responsible for interacting with DNAPK, and which phenotypes are mediated by this interaction. Specifically, Task #4 consisted of 4 subtasks. Subtask 4A was to create FLAG-tagged ERG mutants, subtask 4B was to use immunoprecipitation to assess for interactions between ERG mutants and DNAPK, subtask 4C was to overexpress these ERG mutants in prostate cells, and subtask 4D was to determine which ERG-mediated phenotypes depend on the ERG-DNAPK interaction. In year 4, our major findings are a) that both ERG-mediated invasion and ERG-mediated radioresistance depend on the ETS subdomain of ERG and b) the ERG-DNAPK interaction is likely necessary for these phenotypes.

As shown in Figure 1 below, we generated created a series of FLAG-tagged ERG mutants, with mutated sequences including the conserved PNT and ETS domains within the ETS gene family, as well as the intervening regions between these domains and the carboxy/amino termini. We had, in previous years, shown that the ETS subdomain, which spans from amino acids 310-393, was necessary for a physical interaction with DNAPK. We now stably expressed all of these ERG mutants in RWPE prostate cells, which do not endogeneously overexpress ERG. We then utilized Boyden chamber assays to quantitate the invasive capabilities of RWPE cells harboring these ERG mutants.

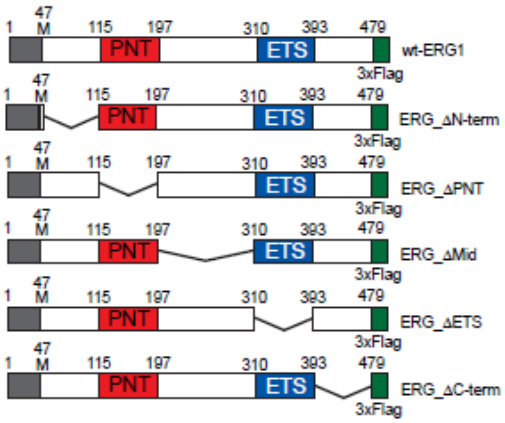

Figure 1: Schematic of wild-type ERG and ERG mutants: ΔN (deletion of 47-115), ΔP (deletion of 115-197), ΔM (deletion of 197-310), ΔE (deletion of 310-393), and ΔC (deletion of 393-479). All are FLAG-tagged and constructed using PCR amplification approaches

As shown in Figure 2 below, overexpression of full-length ERG (wild-type) resulted in a 6-fold relative increase in invasion compared to the LACZ control. Deletion of amino acids 47-197 did not impact the ability of these cells to invade, suggesting that these subdomains of ERG are not necessary for ERG-mediated invasion. However, deletion of amino acids 310-393 (which constitute the ETS subdomain within the ERG protein) significantly decreased invasion. Given that our previous studies have demonstrated that this subdomain (310-393) is necessary for binding of ERG to DNAPK, these findings suggest that the ERG-DNAPK interaction is necessary for ERG-mediated invasion. Of note, deletion of the subdomains adjacent to the ETS subdomain (amino acids 197-310 and amino acids 393-479) also decreased invasion, though not to as significant of an extent as deletion of 310-393. This suggests that perhaps the subdomains adjacent to the ETS subdomain may also contribute to binding of ETS to DNAPK to promote invasion, perhaps by maintaining a secondary structure necessary for ETS-DNAPK binding.

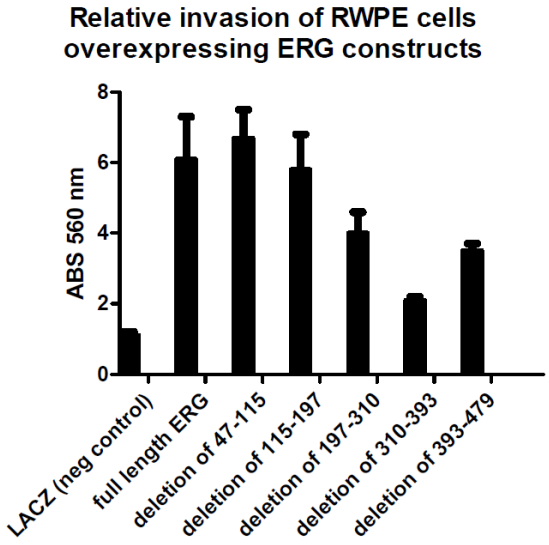

Figure 2: ERG constructs harboring full length ERG (positive control) or serial deletions in ERG were overexpressed in RWPE prostate cells. Boyden chamber assays were used to quantitate invasion of cells. Deletion of the ETS subdomain (amino acids 310-393) led to the most significant decrease in ERG-mediated invasion.

In addition to assessing the impact of these ERG mutants on invasion, we also performed clonogenic survival assays following radiation therapy, to determine which subdomains of ERG were necessary for radioresistance (see Figure 3). Our findings for radioresistance were very similar to those for invasion. Overexpression of full length ERG conferred resistance to radiation, as evidenced by an increase in clonogenic survival (following 3 Gy of radiation) from

0.11 to 0.34. Deletion of amino acids 197-310, 310-393, or 393-479 significantly decreased survival following radiation, consistent with decreases in ERG-mediated radioresistance. The greatest effect was seen with deletion of the ETS subdomain (310-393), again supporting the hypothesis that the ERG-DNAPK interaction mediates ERG-driven radioresistance. By identifying which subdomain of ERG is responsible for ERG-induced oncogenic phenotypes, our studies may inform the design of drug therapy directed against ERG.

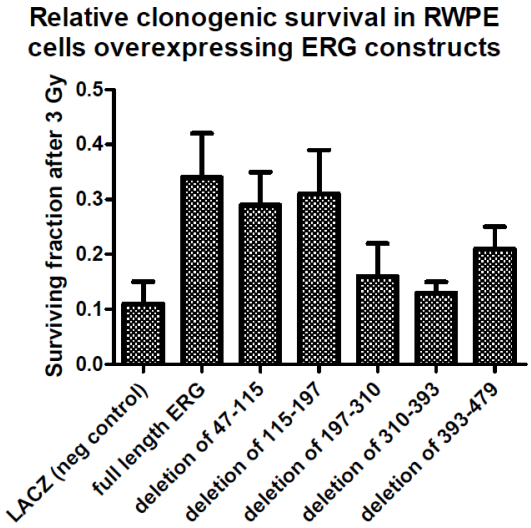

Figure 3: ERG constructs harboring full length ERG (positive control) or serial deletions in ERG were overexpressed in RWPE prostate cells. Clonogenic survival assays were utilized to quantitate cell survival following 3 Gy of radiation. Deletion of the ETS subdomain (amino acids 310-393) and the surrounding subdomains (amino acids 197-310 and 393-479) led to significant decreases in ERG-mediated radioresistance.

Subtask 6C required my research team to optimize fluorescence in-situ hybridization (FISH) assays for detection of ERG fusions in prostate cancer cells. While multiple groups have previously published on FISH-based approaches to detect ERG, my research team needed to establish this methodology within my laboratory. As shown in Figure 4, we have now successfully optimized this approach and can robustly perform FISH for ERG translocations (shown) and ERG deletions in prostate cells, and this positions us well for the biomarker studies proposed in Task 7 (subtask 7C).

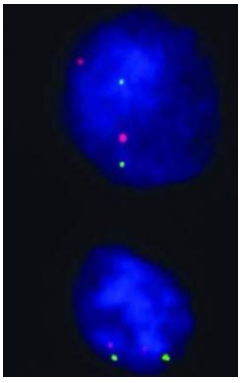

Figure 4: Fluorescence in-situ hybridization (FISH) assays were performed using break-apart probes for ERG in prostate cancer cells. A representative case of ERG translocation is shown.

Thus, to summarize, Year 4 of my grant period is notable for completion of two subtasks. In particular, subtask 4D represented quite an extensive effort, as we functionally and phenotypically characterized the impact of each ERG subdomain. I have now completed 17 of the proposed 20 subtasks over the first four years of this grant, which puts me ahead of the schedule outlined in my initial statement of work (16 subtasks to be completed over the first 4 years). Overall, the first four years of this grant have been quite successful.

Research achievements: Milestones

In the original Statement of Work, 11 milestones were identified, and targeted over the 5 year course of this grant. To date, I have completed 8 out of 11 milestones (Milestones #1, #2, #4, #5, #6, #7, #8, and #9) during the first 4 years of this proposal, which is exactly on schedule (the original target on the Statement of Work was 8 milestones achieved by the end of year #4).

Training achievements

In my original grant application, I highlighted a series of training program activities which I hoped would contribute substantially to my scientific development. Over the past year, as proposed, I have continued to attend a number of basic science seminars, hosted by the Departments Medicine, and Molecular and Cellular Biology, which have broadened by scientific knowledge within my field. I have also regularly attended Gene Fusion and Cancer Biology Research Meetings, run by my mentor Arul Chinnaiyan, as well as the Pathology and Radiation Oncology Research Seminars, run by the two departments with which I am affiliated. Additionally, I have renewed my "Training in the Responsible Conduct of Research" certification, and presented at the national meetings noted above in the milestones section. I have presented my research at national conferences, including the *AACR Prostate Cancer* meeting, the *ASCO* meeting, the *Prostate Cancer Foundation* annual meeting, and the *ASTRO* annual meeting. Finally, I have met regularly with my mentors, Drs. Arul Chinnaiyan, Ted Lawrence, and Tom Carey, as planned in my original proposal.

Career achievements

The overall goal of my DOD Mentored Physician Research Training Award was to help me develop a career as a physician scientist committed to prostate cancer research. This grant has really helped me accomplish this goal, both directly and indirectly. Because of my need to obtain tissue specimens to fulfill Aim 3 of this grant, I approached the Radiation Therapy Oncology Group (RTOG), and began regularly attending their Genitourinary Cancer Translational Research Committee meetings. Because of my increasing involvement with this group, I was appointed as chair of this committee. As chair of this committee, my role is to help direct RTOG-based prostate cancer research on a national level. This role has resulted in national recognition, as I was asked to present my research from this DOD grant in the 2011 AACR Prostate Cancer conference and the 2012 ASTRO meeting. Similarly, I moderated one of the 3 sessions at the 2013 ASCO GU conference (my session was focused on translational research in prostate cancer), and I have been asked to chair and organize a session on localized prostate cancer at the 2015 AACR Annual Meeting. Over the past few years year, I have also served as a grant reviewer for the NIH Cancer Biomarker Study Section (four times), DOD study sections (twice), Prostate Cancer Foundation Young Investigator and Challenge grants (six times), and Prostate Cancer Canada/Movember grants (once). Additionally, my reputation as a translational prostate cancer researcher led to my nomination and subsequent election to the National Cancer Institute Genitourinary Cancer Steering Committee, which reviews national cooperative group clinical trial proposals in prostate cancer. Also, I was named as the Chair of the Biology Scientific Track for ASTRO (American Society of Radiation Oncology), the national organization for radiation oncologists – in this role, I lead and organize the biology scientific sessions for this organization. Because of these successes, my chairman recently appointed me as Division Chief of a newly formed division (the Division of Translational Genomics) in the Department of Radiation Oncology at the University of Michigan. I will also be submitting my application for promotion (to Associate Professor) and for tenure in the upcoming year.

My DOD-sponsored project has led to the preliminary data necessary for several grants that I have received over the past four years, including a Celgene Translational Award ($500,000 over 2 years) and five separate Prostate Cancer Foundation (PCF) Challenge Award ($1,000,000 split among co-Principal Investigators over 2 years). This includes two PCF Challenge Awards focused on DNA repair (entitled "*Interrogation of Aberrant DNA Repair in Sporadic Prostate Cancer"* and "*Targeting DNA Repair Pathways to Improve Treatment for Advanced Prostate Cancer"*), which stem directly from the findings in this DOD grant and seek to translate some of these findings into the clinic. The other three PCF Challenge grants, which include a genomic sequencing study to identify biomarkers of radioresistance and two additional studies seeking to develop targeted therapies for prostate cancer, build upon skill sets that I have developed in the course of completing the subtasks and training program specified in this DOD grant. In addition, I was funded for five additional prostate cancer-based grants. One of these grants, from the Fund for Cancer Research ($75,000 for 1 year), was based directly on extending the work initiated in Aim 1 of this DOD PCRP grant. Two of the other four grants (I am co-PI of project within a NIH SPORE grant, entitled "Development of Novel BET Bromodomain Inhibitors for the Treatment of Advanced Prostate Cancer" and am a key co-investigator of a U10 grant entitled "*Integrated Translational Genoproteomics Center at Washington University"*) are NIH grants. The other two grants (Mazzone grant and Medivation/Astellas Investigator Grant) are not directly related to the work included in this DOD PCRP grant, but do focus on different aspects of prostate cancer. In total, during the four years of this DOD PCRP grant, I have received In addition, over the past year, I have received 10 additional grants, from both NIH and Foundation sources. Much of this success has been based upon the data generated and experienced gained from this DOD Grant. In addition, I have had four manuscripts accepted for publication, and a fifth one in submission, based on work from this proposal (detailed in the reportable outcomes section below). In addition to these 5 manuscripts (see references 1-5 below), I have published 61 additional manuscripts over the four years of this grant, including over 20 from the last year. I would like to thank the DOD for making all of this possible for me.

Key Research Accomplishments:

The key research accomplishments from the past year of this grant proposal include the following:

- Generation of RWPE prostate cancer cells overexpressing ERG deletion constructs
- Discovery that ERG-mediated invasion depends on the ETS subdomain of the ERG protein
- Determination that ERG promotes radioresistance via its ETS subdomain
- Optimization of ERG FISH by my research team for subsequent biomarker studies

These accomplishments add to the findings from the first three years of the grant proposal, which showed that:

- ERG overexpression in prostate cancer cell lines confers radiation resistance
- This ERG-associated radiation resistance is mediated by increased efficiency of DNA repair in response to radiation
- ERG interacts with the repair protein DNAPK in a DNA-independent manner, at its tyrosine 373 site
- DNAPK knockdown or inhibition preferentially radiosensitizes ERG-positive vs ERG-negative cells, and can reverse ERG-mediated radiation resistance
- ERG is diffusely localized through the prostate cancer cell and does not redistribute upon genotoxic stress

Reportable Outcomes:

The past year of work from this grant proposal has resulted in the following reportable outcomes:

1) A manuscript, reviewing data from Aim 1 this grant, published in *Clinical Cancer Research*[1] (on which I am first author)
2) A manuscript, based on data from Aim 2 of this grant, submitted to *Cancer Cell* (on which I am co-senior author)[2]
3) A funded Challenge grant from the Prostate Cancer Foundation, entitled "*Targeting DNA Repair Pathways to Improve Treatment for Advanced Prostate Cancer"*

These outcomes add to the following reportable outcomes from the three years of the grant:

4) Publication of work from Task #4 in a *Cancer Cell* manuscript[3], co-published with my mentor and primary collaborator, Dr. Arul Chinnaiyan
5) Two publications on ETS gene fusions in prostate cancer, published in the journal *Curr Drug Targets*[4] and *Neoplasia*[5]
6) A publication on DNAPK in prostate cancer, published in the journal *Cancer Discovery*[6]
7) Oral presentation on work from Task #4, at the 2010 American Society of Therapeutic Radiology and Oncology Annual Meeting[6]
8) Poster discussion presenting work from Tasks #1 and #3, at the 2011 American Society of Clinical Oncology Annual Meeting[7]
9) Invited oral presentation on work from Tasks #1 and #3, at the 2011 Prostate Cancer Foundation Annual Meeting
10) A funded Young Investigator Award from the Prostate Cancer Foundation ($225,000 over 3 years), entitled "*Cooperativity between TMPRSS2:ERG Gene Fusions and PTEN Genomic Deletions in the Radiation Resistance of Prostate Cancer",* from January 2011 to January 2014
11) Oral presentation of work from this grant proposal, at the 2012 AACR Prostate Cancer Conference
12) An invited presentation, in which I reviewed data from this grant, at the Winship Cancer Center (at Emory)

13) A funded Challenge Grant from the Prostate Cancer Foundation ($1,000,000 over 3 years, split among 4 co-principal investigators, of which I am one), entitled "*Interrogating DNA repair aberrations in advanced prostate cancer*", from 8/2012-7/2015
14) A funded Translational Award from the pharmaceutical company Celgene ($500,000 over 2 years, on which I am PI), entitled "CC115 as a therapeutic approach for metastatic Ewing's sarcoma or prostate cancer", from 4/2012-4/2014
15) A funded grant from the Fund For Cancer Research, entitled "Investigating ETS Gene Fusions as Predictive Biomarkers of Radiation Resistance and Targets for Radiosensitization" ($75,000 over 1 year)

Conclusion:

This Annual Report summarizes the four-year accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to Felix Feng, M.D. Overall, the fourth year of this grant period has been successful, and has resulted in two manuscripts published or submitted (*Clinical Cancer Research* and *Cancer Cell)* and one funded grant (PCF Challenge Grant). These accomplishments add to those achieved during the first three year of this grant, including four publications (*Cancer Cell, Neoplasia, Curr Drug Targets, Cancer Discovery)*, four funded grants, and five presentations. In total, the first four years of this grant have resulted in five subsequent funded grants, six publications, five presentations, and several national leadership position. In addition, I have completed 17 out of the 20 subtasks proposed for this 5 year grant, and am ahead of the schedule proposed in the initial Statement of Work. Additionally, I have met the training achievements specified in my original grant.

The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mechanistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents one of the primary treatment modalities for localized prostate cancer. Our findings are that ERG confers radiation resistance in preclinical models of prostate cancer and that this radiation resistance can be reversed with DNAPK inhibition. These findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers. In addition, our accomplishments have now paved the way for us to continue with the necessary xenograft and human biomarker studies necessary to translate this work to the clinic.

I would like to thank the DOD review committee for providing me this grant to accomplish the proposed research.

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Appendices:

None