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TITLE: ERF is a Potential ERK-Modulated Tumor Suppressor in Prostate Cancer

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14. ABSTRACT Half of all prostate cancers contain an oncogenic gene fusion between the androgen-regulated upstream elements of the TMPRSS2-gene with the consequently upregulated ETS transcription factor ERG. Despite this high prevalence, detecting the presence of TMPRSS2-ERG in patients' tumors has little-to-no useful clinical utility, in part due to a lack of understanding of its mechanisms of oncogenesis. I have characterized a gene, ERF, which functions as a putative tumor suppressor. I had hypothesized that ERF is outcompeted by the TMPRSS2-ERG gene product. Currently, we have identified how ERF and ERG may compete with each other, and as a result, have opposing effects on cancer cell proliferation. I am currently now investigating the tumor suppressor function of ERF in prostate cancers lacking TMPRSS2-ERG.					
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ERF IS A POTENTIAL ERK-MODULATED TUMOR SUPPRESSOR IN PROSTATE CANCER

INTRODUCTION

Metastatic prostate cancer is the second most frequent cause of cancer death among American men and remains incurable. Half of such cancers contain a gene fusion, placing a slightly truncated ERG transcription factor downstream of the androgen-regulated promoter of TMPRSS2. This leads to significant upregulation of ERG, which drives tumorigenesis. ERG belongs to the ETS family of transcription factors, which contain the highly conserved ETS DNA-binding domain. There are differences in the biology of fusion-positive tumors versus those lacking the ERG fusion (fusion-negative). However, there is no targeted clinical strategy for treating either fusion-positive or fusion-negative cancers. This is partly due to our limited understanding of how exactly ERG promotes oncogenesis, as well as a lack of awareness of complimentary pathways in fusion-negative tumors. A recent multi-institutional effort sequencing metastatic prostate cancers yielded ERF as 1 of only 19 genes achieving the highest statistical criteria for containing cancer-relevant mutations, with several frameshift mutations as well as point mutations of key conserved residues noted in the critical ETS domain, presumably corresponding to loss-of-function mutations. ERF has known tumor suppressor ability and belongs to the same transcription factor family as ERG, contains a virtually identical DNA-binding ETS domain, but is a strong transcriptional repressor unlike the transcriptionally activating ERG. Most interestingly, all tumors with ERF mutations were fusion-negative. We hypothesize that in fusion-negative tumors, ERF functions as a tumor suppressor by occupying ETS binding sites and inhibiting gene expression, consistent with its repressor function. On the other hand, in fusion-positive tumors, loss of ERF function confers no discernible growth advantage, since ERF is already outcompeted at ETS binding sites by the upregulated ERG oncogene, which in turn activates gene transcription.

KEYWORDS

prostate cancer, tumor suppressor, TMPRSS2-ERG

ACCOMPLISHMENTS

Goals and Accomplishments

I. Specific Aim 1: To investigate how ERF and ERG may compete and have opposing effects on cancer cell proliferation

A. Major Task 1: Evaluate role of ERF in negatively regulating ERG-dependent genes

To investigate the possibility of competition between ERF and ERG, we used the ERG-positive VCaP cell line (that contains wild-type ERF alleles) as a model. The expression of ERG or ERF was separately inhibited via shRNA (Figs. 1A-C). The androgen transcriptome was analyzed by treated with and without dihydrotestosterone. Consistent with earlier work showing that ERG expands the AR transcriptome in mouse prostatic tissue, inhibition of ERG expression (“ERG-low”) resulted in a constricted androgen transcriptome compared to control VCaP cells (“ERG-high”) (Figs. 1D-left, 1E). Conversely, ERF inhibition increased the level of AR target gene expression and doubled the size of the androgen transcriptome (Figs. 1D-right, 1E). ERG expression was unaffected by ERF knockdown and vice versa (Fig. 1C); therefore, the opposing effects of ERF and ERG knockdown on AR output are unlikely due to one ETS factor directly affecting expression of the other.

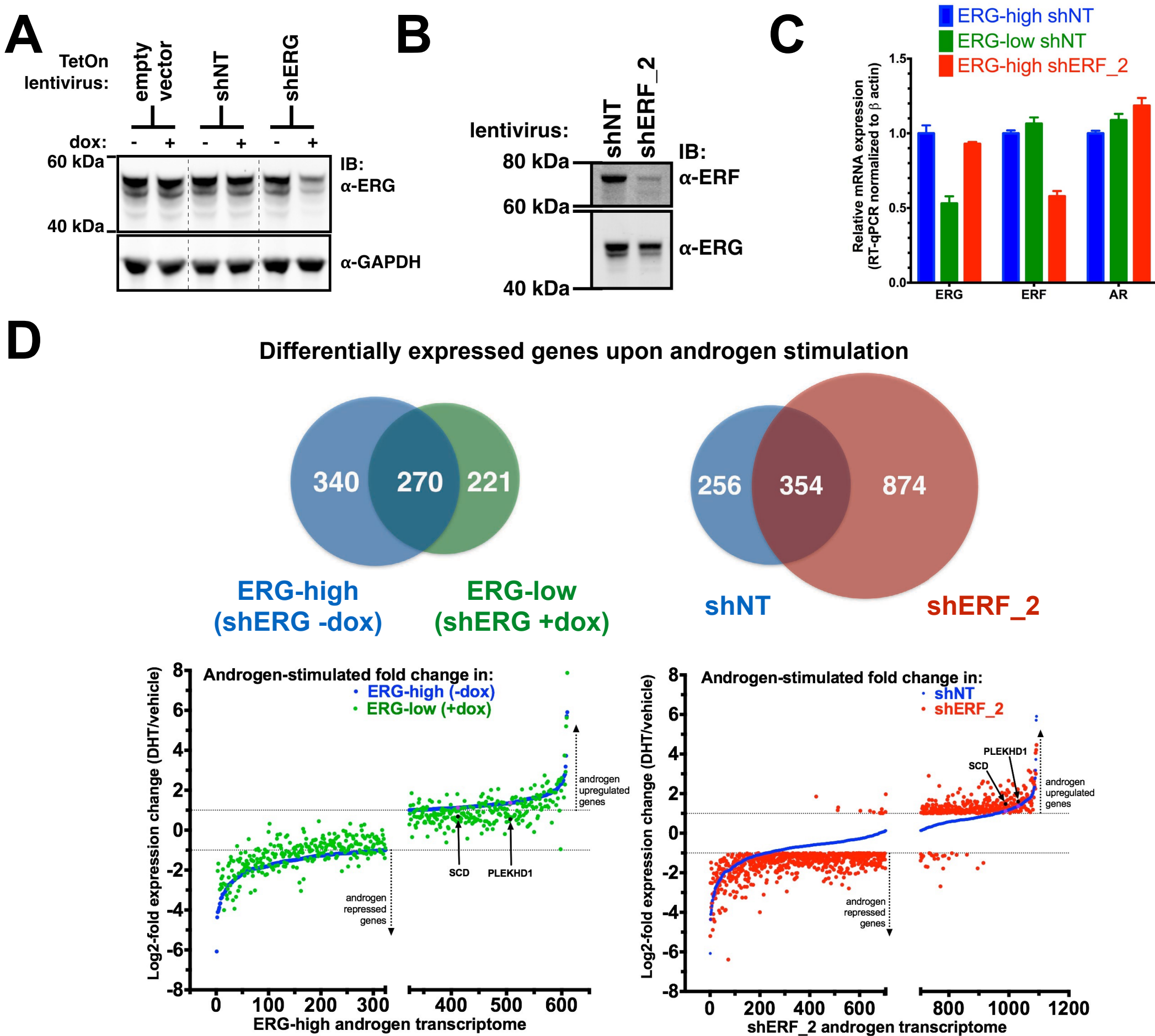


Figure 1 - A) VCaP cells were infected with a Tet-inducible non-targeting shRNA (shNT) or shRNA targeting ERG (shERG) and treated with or without 100ng/mL doxycycline (dox). From here on, cells with shERG without dox will be referred to as ERG-high and with dox as ERG-low. **B)** VCaP cells were infected with non-targeting shRNA (shNT) or shRNA targeting ERF (shERF_2). **C)** RT-qPCR (mean \pm s.e.m.) from VCaP cells infected with non-targeting shRNA (shNT) or shRNAs targeting ERF, as well as a doxycycline (dox)-inducible shRNA targeting ERG, +vehicle (ERG-high) or +dox (ERG-low). **D)** Top – Venn diagrams of androgen regulated genes (>2 fold, $FDR < 0.05$ with 16 hours of 1nM DHT treatment). Bottom – Comparisons of androgen-stimulated fold change of gene expression between the indicated conditions.

B. Major Task 2: Evaluate role of ERF in competing for DNA binding with ERG

Given the similarity of the ETS domains between ERF and ERG, as well as the ability of ETS factors to displace one another from canonical GGA(A/T) motifs, we postulated that the opposing effects of ERF and ERG on androgen signaling could be explained by competition for AR-associated ETS binding sites. To explore this possibility, we performed ERF ChIP-seq in the ERG-high and ERG-low states (Figs. 2A-C). De novo motif analysis of the ChIP-seq peaks identified the canonical ETS motif as the primary ERF binding site, with 5,566 binding sites in the ERG-high condition (Fig. 2Ai-iii). Remarkably, an additional 19,592 ERF binding sites were observed in the ERG-low state, nearly all of which were bound by ERG prior to ERG knockdown (Fig. 2Ai-iv). Furthermore, the ChIP-seq signal intensity of the smaller number of ERF peaks observed in the ERG-high state was increased in the ERG-low state in almost all cases (Fig. 2Ai). Finally, ERF peaks in the ERG-low state largely overlap with AR-binding peaks, as previously reported for ERG. PLEKHD1 and SCD are two AR-regulated genes that illustrate how ERG and ERF levels can modulate their expression. In the ERG-high state, both genes have prominent AR- and ERG-associated binding sites but only limited ERF binding (Fig. 2D). However, ERF binding is substantially increased in the ERG-low state (Fig. 2D) in parallel with a decrease in androgen-induced expression (Fig. 1D-left). On the other hand, ERF inhibition yields only a small increase in androgen-induced expression for these two genes (Fig. 1D-right), consistent with minimal ERF binding at baseline. These data suggest a model whereby the relative levels of ERF and ERG influence the size and output of the AR transcriptome and compete for ETS binding sites.

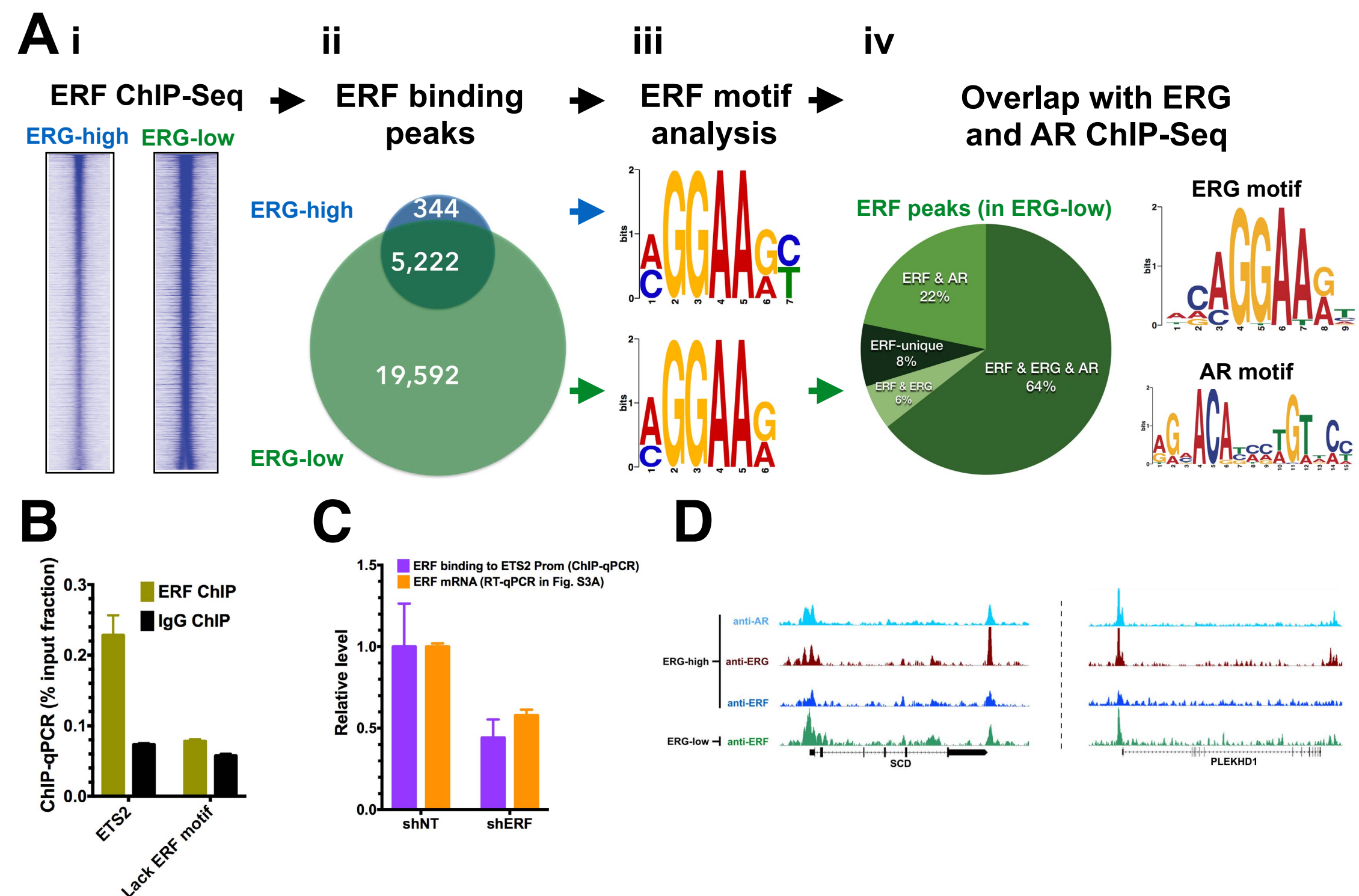


Figure 2 - A) i – Heatmaps depicting ERF ChIP-seq read density in +/- 2kb regions of the ERF peak summits. The same number of ChIP-seq reads were loaded into the software seqMINER for creating the two maps, sorted by the read densities in the ERG-low condition. ii – Venn diagram showing the overlap of ERF ChIP peaks in the ERG-high and ERG-low states. iii – De novo motif analysis from the ERF peaks in the ERG-high (top) and ERG-low (bottom) states. iv – Pie chart illustrating the overlap between ERF ChIP-seq peaks with ERG and AR peaks, as well as the top motifs enriched in the ERG and AR ChIP-seq peaks. **B)** ChIP-qPCR (mean +/- s.e.m.) in VCaP cells for the ETS2 promoter region that contains a known ERF binding site as a positive control, and with an upstream element of PSA lacking the ERF binding motif noted in panel Aiii, as a negative control. **C)** Comparison of effect of ERF shRNA inhibition on ERF binding to ETS2 promoter by ChIP-qPCR versus ERF mRNA by RT-qPCR (mean +/- s.e.m.). **D)** ChIP-seq signals for the SCD and PLEKHD1 loci.

C. Major Task 3: Evaluate for in vitro role of shERF

To the phenotype of ERF loss *in vitro*, we infected normal murine prostate organoids with shRNA targeting murine Erf (shErf) (Fig. 3A). The shErf organoids acquired morphologic characteristics of ERG overexpression: they formed single-cell luminal structures lacking basal cells (Fig. 3B) and RNA-seq profiling revealed significant enrichment for genes whose expression is upregulated by androgen in human prostate cancer cells (Fig. 3C).

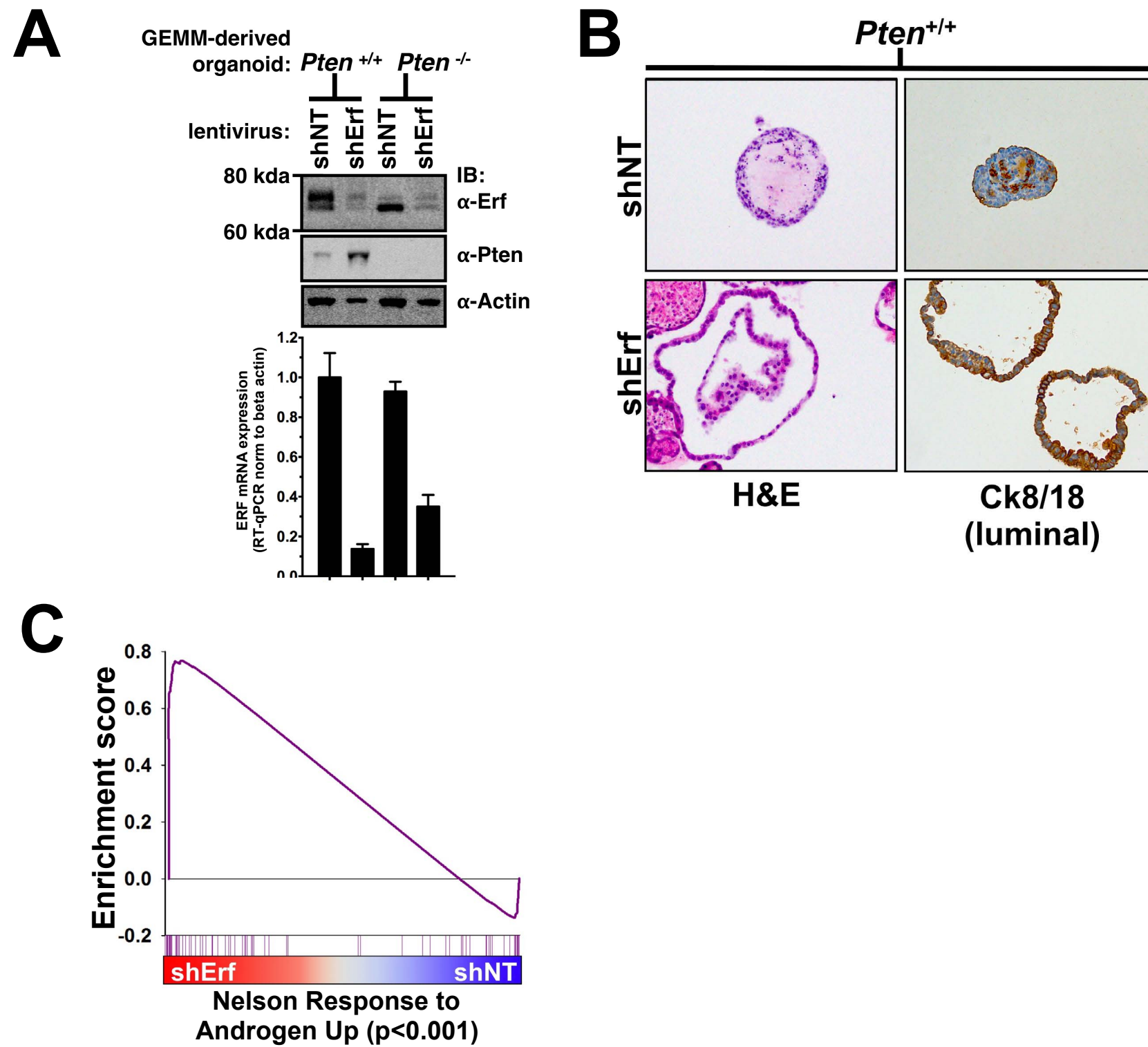


Figure 3 - A) Organoids derived from *Pten*^{+/+} and *Pten*^{-/-} GEMM prostates infected with non-targeting shRNA (shNT) or mouse Erf (shErf). Western blot (top) and RT-qPCR (bottom). Error bars represent s.e.m. from 3 biological replicates **B)** Histology and IHC analysis of the murine prostate organoids. **C)** Expression profiles of shErf and shNT in normal prostate compared by GSEA for an androgen expression signature

Specific Aim 2: To evaluate for in vivo tumor suppressor function of ERF, and role of MEK/ERK inhibition

Nothing to report

Specific Aim 3: To interrogate and map how ERF may regulate androgen-mediate transcription via its novel interaction with AR

Nothing to report

Training Opportunities and Professional Development

I have been undergoing an intensive training program for a prospective prostate cancer physician-scientist. I have completed flow cytometry training. I continue to attend bioinformatic seminars necessary for the RNA-seq and ChIP-seq analysis in Aims 1 and 2. I attend oncology grand rounds weekly. I also attend the interdisciplinary bimonthly prostate cancer meeting organized by our Genitourinary Service, and attend the monthly inter-institutional prostate cancer meeting held amongst the prostate cancer-focused labs in New York City. Regarding conferences, I attended the Ft Lauderdale prostate cancer meeting earlier this year. In the past year, I completed my clinical fellowship training under the clinical guidance of Dr. Howard Scher, and have begun as instructor, with Dr. Scher continuing to provide mentorship.

Dissemination of Results

Nothing to report

Next Reporting Period

Aiming to make progress on the *in vivo* tumor suppressor function of ERF, a major component of Aim 2.

IMPACT

On principal discipline

The findings over the reporting period have furthered our understanding of tumor genetics responsible for prostate cancer progression. Specifically, this project has investigated the poorly

understood nature of the ERG fusion-negative tumors and further clarified the nature of the ERG fusion-positive tumors as well. The identification of an ETS factor, ERF, that serves as a tumor suppressor in fusion-negative tumors, but is outcompeted in ERG fusion-positive prostate cancer, is a significant contribution to our understanding of the tumor genetics of prostate cancer.

The results from this proposed research project could also have a major impact on prostate cancer care. Existing treatments for advanced prostate cancer increase survival only on the order of months, and can be associated with significant morbidities. Half of all such cancers lack ERG gene fusions, and at present, we lack a unifying understanding of the key regulators in such tumors. If the project hypothesis is correct, then we may be able to identify a novel treatment strategy for patients possessing fusion-negative tumors. This approach could lead to increased survival and decreased morbidity for men suffering from this disease. In addition to the clinical applications above, this project could shed light on how tumors with and without ERG mutations may actually be similar. This, in turn, would contribute to our understanding of the 50% who do have the ERG mutation, and assist in developing a targeted strategy for them as well.

On other disciplines

Nothing to report

On technology transfer

Nothing to report

Beyond science and technology

Nothing to report

CHANGES/PROBLEMS

Nothing to report

PRODUCTS

Publications, conference papers, and presentations

Presentations:

Bose R, Abida W, Karthaus W, Iaquinta P, Armenia J, Wongvipat J, Doran M, Hieronymus H, Watson P, Liang Y, Schultz N, Sawyers CL. Genomic Mutations of an ETS Tumor Suppressor Expand the ETS-related Subset of Prostate Cancer. Multi-institutional Prostate Cancer Program Retreat – Fort Lauderdale (2016)

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

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals

Name: Rohit Bose
Project Role: PI
ORCID ID: 0000-0002-6785-0697
Nearest person month worked: 10.8 months
Contribution to Project: PI, Designed, performed and analyzed experiments.

Change in Other Active Support

GC227928 (PI: Bose)	6/27/2016 – 6/27/2019	1.20
calendar		
Prostate Cancer Foundation (formerly CaP CURE)	\$ 75,000	
Epigenetic Sensitization of Enzalutamide Response		
Aims: I. To determine whether the methylation landscape of prostate cancer is associated with enzalutamide sensitivity		
II. To determine whether EHMT2 and KMT2B modulate enzalutamide sensitivity by affecting AR function		
III. To determine whether small-molecule inhibition of EHMT2 leads to enhanced enzalutamide sensitivity		
Agency Contact: Jonathan Simons, MD jsimons@pcf.org (t) 310.570.4712		
Overlap: None		

Other Organizations

Nothing to report

SPECIAL REPORTING REQUIREMENTS

None