Expanding ERG's empire: ChIP-Seq in clinical samples reveal altered chromatin landscapes and dysregulated transcription in prostate cancer.

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Mini abstract:
ERG overexpression in prostate cancers leads to the development of wide-spread changes in gene expression and chromatin landscapes leading to redistribution of key transcription factors in TMPRSS2-ERG fusion gene positive prostate cancers. The overexpression of ERG is further assisted by the development of super-enhancer in the *ERG* locus.

The story so far…
Prostate cancer is the most common cancer in men above 50 years. Investigations into the molecular basis of prostate cancer identified several candidates and genetic alterations [1-3]. Out of these, *TMPRSS2-ERG* (T2E) fusion gene arising due to genetic rearrangement leading to fusion of the transmembrane protease serine 2 gene (*TMPRSS2*) and the transcription factor gene *ERG* (ETS-related gene) has been on the center stage as it was found to be associated in nearly half of prostate cancer cases [4-7]. How does the T2E fusion gene drive the development of prostate cancer? In a recent report, Kron et al. used genomic approaches on patient samples to demonstrate altered chromatin leading to enhanced *ERG* transcription in T2E prostate cancer. The modified chromatin landscape in conjunction with expanded transcriptional activity leads to upregulation of other target genes involved in prostate development and cancer.

First, the authors compared T2E prostate cancer (in which ERG is overexpressed) and non-T2E prostate cancer (no overexpression of ERG) from patient samples and identified significant changes in gene expression and chromatin landscape marked by the active histone modification, H3K27ac, between the two cancer types. T2E prostate cancers were typified by global acquisition and activation of several cis-regulatory elements (CRE's) particularly

at the enhancers of several target genes of ERG. Further, that differences in gene expression patterns observed between the two cancer types could be attributed to activation of CRE's in T2E prostate cancer.

How does ERG modify chromatin and transcriptional profile of T2E prostate cancers? The authors discovered that ERG recruits and physically binds to other transcription factors important in prostate biology like FOXA1 and HOXB13 and initiates transcription from new CRE's. This association in turn recruits androgen receptor and possibly initiates tumorigenesis in T2E prostate cancers. Additionally, ERG overexpression was also found to give rise to novel super-enhancers with high levels of H3K27ac. Super-enhancers (which are large regulatory elements comprising of multiple enhancers) are strongly associated with increased gene expression and have important roles in cancer [8-10]. As a result of this, there is an upregulation of genes concomitant with changes in acetylation levels of the super-enhancers. Further, in the case of T2E prostate cancers, the authors discovered the presence of a super-enhancer within the *TMPRSS2* promoter that expanded into the *ERG* locus and could aid in amplification of *ERG* expression. And finally, the altered CREs in T2E prostate cancers are vulnerable to NOTCH signaling as a NOTCH inhibition led to a significant reduction in cell migration in ERG-dependent prostate cancer cell line.

The road ahead…

Previous work demonstrated that ERG overexpression in T2E prostate cancer was driven by the Androgen response element (ARE) present in the TMPRSS2 promoter [11]. The present report adds an additional level of complexity by uncovering an expansion of the super-enhancer into rearranged ERG allele, leading to increase in ERG expression revealing a more complex association than what was thought previously. Along with that, these findings also raise questions that remain to be answered. For example, most of the downstream molecular players are present in T2E and non-T2E prostate cancers. Then, how are distinct cellular behaviors generated? And importantly, how does this knowledge translate towards development of newer diagnostic and prognostic biomarkers? Likewise, the role played by ERG overexpression

needs further clarity with respect to initiating the carcinogenesis in T2E prostate cancers. How much of ERG overexpression is needed before driving a cell towards carcinogenesis in T2E prostate cancer? Similarly, the nature of the feedback loops that operate to maintain levels of ERG expression in T2E cancers need better characterization.

The functional relevance of chromatin alterations in T2E prostate cancers need detailed investigations. While the authors observed increased acetylation of histones in T2E prostate cancers and related gene expression changes, what is the consequence of such an altered gene expression in driving T2E prostate cancer? How much of this altered gene expression leads to alterations in the proteome in case of T2E positive prostate cancers? An additional offshoot of this investigation could be in identifying newer drug target and developing potential therapeutics. Further, what is the role of other epigenetic factors in T2E prostate cancers? What is the interplay between different histone modifications around key target genes of ERG in T2E prostate cancer? Similarly, what are the roles for other epigenetic factors in T2E prostate cancer? Future experiments would be needed to identify exact steps connecting ERG overexpression to chromatin modifications in T2E prostate cancers and to tease apart the direct and indirect effects of ERG overexpression.

Finally, there has been considerable interest in looking at 3D genome organization in cancer [12]. In the field of prostate cancer, previous studies have highlighted the role of DNA looping to achieve coordinated gene expression, particularly from the androgen receptor [13, 14]. Hi-C analysis in benign prostate epithelial cells, RWPE1, revealed wide spread changes in the topology of chromatin. Furthermore, ERG binding was associated with the formation of a hotspot region where greater number of chromatin interactions were observed leading to overexpression of target genes leading to cancer [14]. It would be interesting to see results from a similar approach using primary T2E prostate cancer tissue as against from a cell culture model to reflect more accurate the *in vivo* situation. A tantalizing opportunity would be to test the effect of abrogation of T2E prostate cancer-specific DNA loops and assay the downstream

consequence. This is valuable considering that ERG overexpression leads to the development of super-enhancers that further drive the cell towards an oncogenic outcome. Hence, would an abrogation of this association lead to amelioration of the disease pathology?

In conclusion, chromatin landscape alteration and ectopic expansion of ERG binding in T2E prostate cancer raises several interesting questions. Hopefully, these answers would lead us closer to understanding the biology of prostate cancer as well as to develop molecular medicines to tackle prostate cancer.

References:

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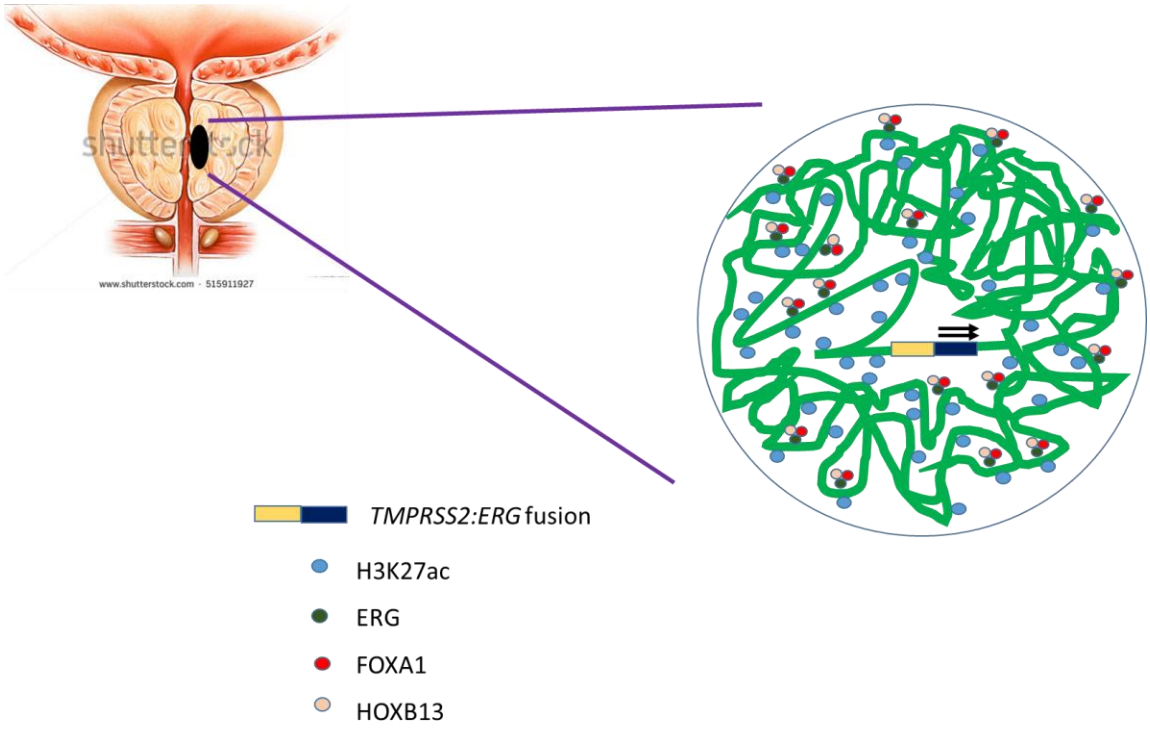
Figure 1



H3K27ac ChIP-Seq reveals an upregulation of genome-wide H3K27ac signals as shown by the blue circles. ERG expression expands in T2E positive prostate cancer and leads to ectopic binding to transcription factors like FOXA1 and HOXB13 shown as a complex of green, red and pink circles. Large blue circle shows the nucleus of a T2E prostate cancer cell.