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Review Article

Oncogenic activation of *ERG*: A predominant mechanism in prostate cancer

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Abstract

Prevalent gene fusions involving regulatory sequences of the androgen receptor (AR) regulated genes (primarily *TMPRSS2*) and protein coding sequences of nuclear transcription factors of the *ETS* gene family (predominantly *ERG*) result in unscheduled androgen dependent *ERG* expression in prostate cancer (CaP).Cumulative data from a large number of studies in the past six years accentuate *ERG* alterations in more than half of all CaP patients in Western countries. Studies underscore that *ERG* functions are involved in the biology of CaP. *ERG* expression in normal context is selective to endothelial cells, specific hematopoetic cells and pre-cartilage cells. Normal functions of *ERG* are highlighted in hematopoetic stem cells. Emerging data continues to unravel molecular and cellular mechanisms by which *ERG* may contribute to CaP. Herein, we focus on biological and clinical aspects of *ERG* oncogenic alterations, potential of *ERG*-based stratification of CaP and the possibilities of targeting the *ERG* network in developing new therapeutic strategies for the disease.

Keywords: ERG, prostate cancer, TMPRSS2-ERG, oncoprotein, androgen receptor, patient stratification

BACKGROUND

Key molecular genetic alterations in prostate cancer

Prostate cancer (CaP) is the most common malignancy that affects men worldwide, with high frequency in the United States, Western Europe[1] and low reported frequency in Asia.[2,3] Risk factors associated with CaP include age, family history and ethnicity.[1,4] Although precise molecular events that contribute to such variation in the CaP incidence are not well established, the differences may be attributed to factors such as genetics, diet, lifestyle, and male hormone levels.[4-6] Despite the recent advances in early detection and continued refinements in treatment strategies, CaP is still the second leading cause of cancer mortality in American men.[1] Discovery of CaP-specific gene expression and/or mutational alterations have contributed to a significant impact on designing molecular markers to distinguish indolent from more aggressive forms of cancers as well as molecular pathways to develop effective novel therapeutic approaches to combat the disease.[7-12]

CaP susceptibility loci with germ-line mutations of *RNAseL, ELAC2, MSR1, BRCA* 1 and *2, HPCX, KLF6,* and *HPC20*have been reported in primary CaP.[13,14] However, low penetrance and disease heterogeneity have precluded the validation of CaP susceptibility genes. Recent genome wide association studies (GWAS) have identified multiple CaP risk alleles towards defining genetic determinants of CaP risk.[15,16] A "gene less 1.18 Mb region" between FAM84B at centromeric end and *C-MYC* at telomeric end on chromosome 8q24

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has been consistently found to be associated with CaP risk.[17-21] The8q risk allele specific for African ancestry showed an association with higher pathologic stage of CaP in African American men.[22] Functional evaluations of a risk allele on chromosome 10 suggested its impact on regulation of expression of *NCOA4* (*AR* co-activator) and *MSMB*.[23] Overall, a combinatorial assessment of the risk alleles has shown a significantly increased predictive power of CaP risk.[19,24]

Chromosome loci harboring putative proto-oncogenes or tumor suppressor genes (TSGs) have been extensively evaluated toward identifying specific gene mutations and expression signatures in CaP. Mutations, amplifications or over-expression of the androgen receptor (*AR*), and mutations in tumor suppressors such as *p53* and *PTEN,* are frequently identified subsets of advanced CaP.[8,9,25-28] Among the recurrent allelic losses of 8p21-22, 6q16, 7q31, 10q23-25 and 16q24 loci detected in primary CaP,[8,29] deleted 8p21-22 locus harbors a widely studied tumor suppressor gene *NKX3.1*.[30] While early studies showed *PTEN* mutations in subset of advanced cancers, more recent reports underscore higher frequency of *PTEN* hemizygous deletions in primary CaP.[31] In addition, frequent gains of chromosome 8q24, as well as over-expression of *C-MYC* and prostate stem cell antigen (*PSCA*) within this locus have been reported.[13]

Identification of common CaP specific gene signatures have enriched mechanistic as well as translational research investigations. Expression of genes such as *NKX3.1*[32] and *GSTP1*[33] have been studied extensively for their biological roles in onset of CaP. The virtual absence of *GSTP1* expression due to promoter methylation has led to blood- and urine-based assays for diagnosis.[34] Overexpression of AMACR and absence of p63 in most prostate tumors have already led to the use of these two proteins in diagnostic pathology.[35] Striking overexpression of a prostate tissue specific gene, *DD3/PCA3* in CaP have led to extensive evaluations for its diagnostic utility as a marker in urine based assays.[36] Although CaP specific gene alterations are increasingly studied, the most validated oncogenic alteration to date is *ERG*. This observation led to multi-faceted investigations towards defining the cancer specific characteristics of *ERG,* and is discussed in the following sections.

Prevalence of *TMPRSS2-ERG* fusion in prostate cancer

Identification of *ERG* proto-oncogene overexpression in CaP transcriptome led to focused evaluations of *ERG* alterations in CaP.[37-39] Quantitative expression assessment of *ERG* mRNA in matched benign and malignant prostate cells from a large patient cohort confirmed the tumor cell specific *ERG* overexpression in 60-70% patients.[39] Over expression of *ERG* due to fusions between androgen regulated *TMPRSS2* gene promoter and the coding regions of *ERG* has been identified as the most common genomic alteration.[40] These observations also led to the development of a combined CaP gene panel (*PCA3, ERG* and *AMACR*) with diagnostic potential in which overexpression of at least one of three genes associated with virtually all of prostate tumor specimens.[39] Discovery of prevalent gene fusions involving promoters of the androgen receptor (*AR*) regulated genes (*TMPRSS2, SLC45A3, NDRG1, Herv-K22q11.23, CANT1 and KLK2*) and coding sequences of *ETS* gene family (*ERG, ETV1, ETV4, ETV5*)marked a major milestone towards defining molecular mechanisms of prostate carcinogenesis.[11,41] Of the fusions involving *TMPRSS2* and *ETS* factors in CaP, majority (>90%) involve *ERG,* and *ETV1, ETV4* and *E*TV5 represent very low frequency (1-5%).[11] *TMPRSS2* gene is mapped to 21q22.3 between markers *ERG* and D21S56, and transcribed as 3.8 kb mRNA.*TMPRSS2* promoter analysis revealed the presence of a non-canonical ARE as a *CIS*-regulatory target of AR action.[42] *TMPRSS2* is predominantly expressed in prostate tissues with low levels of expression in pancreas, kidney, lung, colon and liver.[43,44] Gene fusions between *TMPRSS2* and *ERG* or *ETV1* appears to be CaP specific and are potentially mediated by AR-induced proximity of fusion gene partners in the presence of genotoxic factors[45,46] followed by topoisomerase-2b-mediated recombination event.[47] Comprehensive evaluations of gene fusions involving *ETS* factors have been covered in excellent reviews.[11,48]

ERG gene structure and transcription

ERG is a member of the *ETS* gene family[49,50] which is one of the largest families of transcriptional regulators consisting of at least 27 members, subdivided into 5 subfamilies.[51] Conserved PNT/SAM domain and an ETS domain are the common features of members of ETS related proteins. These domains play key roles in regulating downstream target genes that are crucial for several biological processes such as cellular proliferation, differentiation, development, transformation, and apoptosis.[52] *ERG* consists of 17 exons and is transcribed to generate several alternately spliced forms[53] [Figure 1]. At least five splice variants are translated into proteins: ERG-1 (p41), ERG-2 (p52), ERG-3 (p55), ERG-4 (p49) and ERG-5 (p38)[54] by a combination of alternative mRNA splicing and/or use of alternative polyadenlyation sites.[50,55] Most characteristic of the family is the evolutionarily conserved 85–amino acid ETS domain, which facilitates binding to purine-rich DNA with a GGAA/T core consensus sequence.[51,56]

ERG is among a small number of transcription factors

that exhibit an endothelial cell and hematopoietic cell restricted expression pattern in various species. In developing mouse, *Erg* mRNA is expressed in mesodermal tissues such as endothelial cells, mesenchymal condensations during precartilaginous depositions, and in urogenital regions.[57] Similarly, *ERG* protein is predominantly detected in endothelial cells, hematopoietic tissues and transiently in pre-cartilage.[58] *Erg* is expressed transiently during early T-cell development, early pre-B and continue to express in mature B cells.[59,60] Later in development, *Erg* functions in cell survival maintaining the differentiation of endothelial cells of vascular and lymphatic origins.[61,62] Thus, highly restricted expression of *Erg* mRNA or *ERG* protein during early phases of lymphocytic, hematopoietic, chondrocytic and endothelial lineage differentiations appears to be crucial in lineage specification function.[58,63-65] Intriguingly, *ERG* protein is not detected in any epithelial tissues including prostate epithelium, or in infiltrating lymphocytes that are occasionally seen in the prostate environment.

Normal biological functions of *ERG*

Biological functions of *ERG* have been studied in xenopus, zebra fish, mouse and humans.[57,66-71] Angiogenesis is an essential process by which new vessels are developed from preexisting ones, during normal development, as well as in pathologic conditions, including tumor development. Widespread expression of *ERG* in endothelial cells suggests for its biological roles in these specialized cells. In addition to VE-cadherin, other endothelial specific factors such as, von Willebrand factor, endoglin, and intercellular adhesion molecule-2 are also regulated by *ERG* supporting its role in endothelial cell differentiation and angiogenesis.[62,72] Endoglin is an accessory receptor for TGF-β and both endoglin and TGF beta receptor type II are positively regulated by Erg.[73,74] Recently, using a functional mutation in mouse models, *Erg* has been shown to regulate the normal platelet development, stem-cell function, definitive hematopoiesis and the normal megakaryopoiesis.[70] Although, *ERG* is considered as critical regulator of hematopoiesis, *Erg* is dispensable during early embryonic hematopoietic development, hematopoietic specification from the mesoderm and is required to sustain definitive hematopoiesis. During this process, *ERG* acts as a direct regulator of critical transcription factors such as Runx1 and Gata2.[75] During hematopoiesis, adult hematopoetic stem cells require *ERG* for self-renewal and differentiation.[76] *ERG* is also documented as a transcription regulator of embryonic stem cell (ES) towards differentiation of early endothelial lineage[77] and exhibits anti-inflammatory responsein endothelial cells by suppressing IL 8.[72]

Prostate cancer associated *TMPRSS2- ERG* transcripts

Several types of *TMPRSS2-ERG* fusion transcripts involving various exons of the *TMPRSS2* and *ERG* have been identified in CaP specimens.[66,78-83] These transcripts were identified on the basis of *TMPRSS2* fusions with the 5' end of the *ERG* and are broadly classified into 8 different groups. In the context of full length transcripts, 2 major forms were identified on the basis of mRNA splicing, cDNA and deduced amino acid sequences.[81] Although, several fusion transcripts are generated from *TMPRSS2-ERG* fusions, it is not clear whether these transcripts are expressed from a single or multiple foci of CaP. Evaluation of *TMPRSS2-ERG* transcripts in multi-focal CaP have shown inter-focal heterogeneity with respect to the presence of fusion positive or negative foci in malignant prostate glands.[82,84-86]

Despite the heterogeneity of *TMPRSS2-ERG* fusions, most common fusion is in between *TMPRSS2* exon 1 and *ERG* exon 4, which results in the deletion of first 32 amino acids from the N-terminus of *ERG* protein.[87] The expression of *TMPRSS2* exon 2 with *ERG* exon fusion 4 mRNA associated with PSA recurrence and seminal vesicle invasion.[78] The most common full length *TMPRSS2-ERG* transcripts (Type I) translate into full length proteins (ERG1, ERG2, ERG3) containing protein-protein interacting (pointed/SAM) and DNA-binding (ETS) domains.[81,87] The most predominant of the proteins generated from the fusions is the N-terminal truncated ERG3 protein. Whereas the type II *TMPRSS2-ERG* transcripts code for ERG8 and a new variant, TEPC, with deletion of 32 amino acids at N-terminus and contain only pointed/SAM domain[81] [Figure 1]. Importantly, higher ratio of type I over type II *TMPRSS2-ERG* splice forms are shown to correlate well with unfavorable prognostic features of CaP, such as poorly differentiated tumors, higher Gleason sum, positive margin, and biochemical recurrence.[81] Additional studies are needed to assess prognostic association specific *TMPRSS2-ERG* fusion transcripts with CaP progression. Since *ERG* is the most common cancer gene activation in CaP, *ERG* expression and function in normal and other cancer contexts may be illustrative in further understanding the biological roles of *ERG* in CaP.

Prostate cancer associated functions of *ERG*

Since the discovery of *ERG*, several reports have shown that *ERG* transforms epithelial cells[49,88-91] and functions through mitogenic signals including the *MAP* kinases.[88] Acute myeloproliferation and megakaryocytic differentiation are the main features of hematologic diseases associated with Down syndrome (trisomy of chromosome 21), in which *ERG* expression is found to be elevated.[92] Myeloproliferation and acute megakaryocytic leukemia were experimentally demonstrated in a genetically engineered Down syndrome mouse model Ts(17(16))65Dn.[92] Similarly, in cell culture system, over expression of *ERG* in erythroleukemia cell line,

K562 induced erythroid to megakaryoblastic phenotype[91] suggesting a critical role for *ERG* in malignant hematologic disorders in Down syndrome. In addition, *ERG* promotes expansion of megakaryocytes from hematopoietic progenitor cells[93] and function as a megakaryocyte oncogene.[94]

In diverse neoplasms, *ERG* is either over expressed abnormally or fused to other genes due to chromosomal translocations and expressed as a chimeric protein. *ERG* gene fusions were initially described in Ewing's sarcoma (EWS) and acute myeloid leukemia (AML).[90,95] In a small subset (about 5-10%) of Ewing's sarcoma, *EWS-ERG* fusions resulted into a chimeric protein containing amino-terminal end of EWS and the carboxy-terminal *ERG* including the DNA binding ETS domain.[96] Majority (95%) of *EWS* fusion involve *EWS* and *FLI*, the closest homolog of the *ERG*.[97] Similarly, *ERG* fuses with *TLS/FUS* in certain acute myeloid leukemias.[98] These fusions generate chimeric proteins abnormally regulate downstream genes due to altered transactivation and DNA binding activities.

As noted above *TMPRSS2-ERG* fusions in CaP leading to androgen dependent expression of *ERG* are exclusive to prostate tumor cells. *ERG* regulates the expression of *C-MYC*, a widely studied oncogene, by physically interacting with the ETS binding element within the P2 promoter region.[71] Consistent with the above observations a positive correlation between *ERG* and C-*MYC* expression suggests that *ERG* mediates oncogenic process through *C-MYC* and may be one of the potential mechanisms in CaP. In addition to the positive regulation of *C-MYC*, *ERG* negatively regulates the expression of a number of prostate differentiation genes such as *KLK3/ PSA, SLC45A3/ Prostein* and abrogates the prostate epithelial differentiation program.[71,99] Of note, knock-down of either *ERG* or *C-MYC* in *TMPRSS2-ERG* positive CaP cells showed similar effects on cellular morphology and expression of prostate differentiation related genes.[71]

In the majority of cancers, cell invasion and migration are the key features of aggressive nature of tumors towards metastasis. *ERG* regulates invasion and migration related genes in CaP

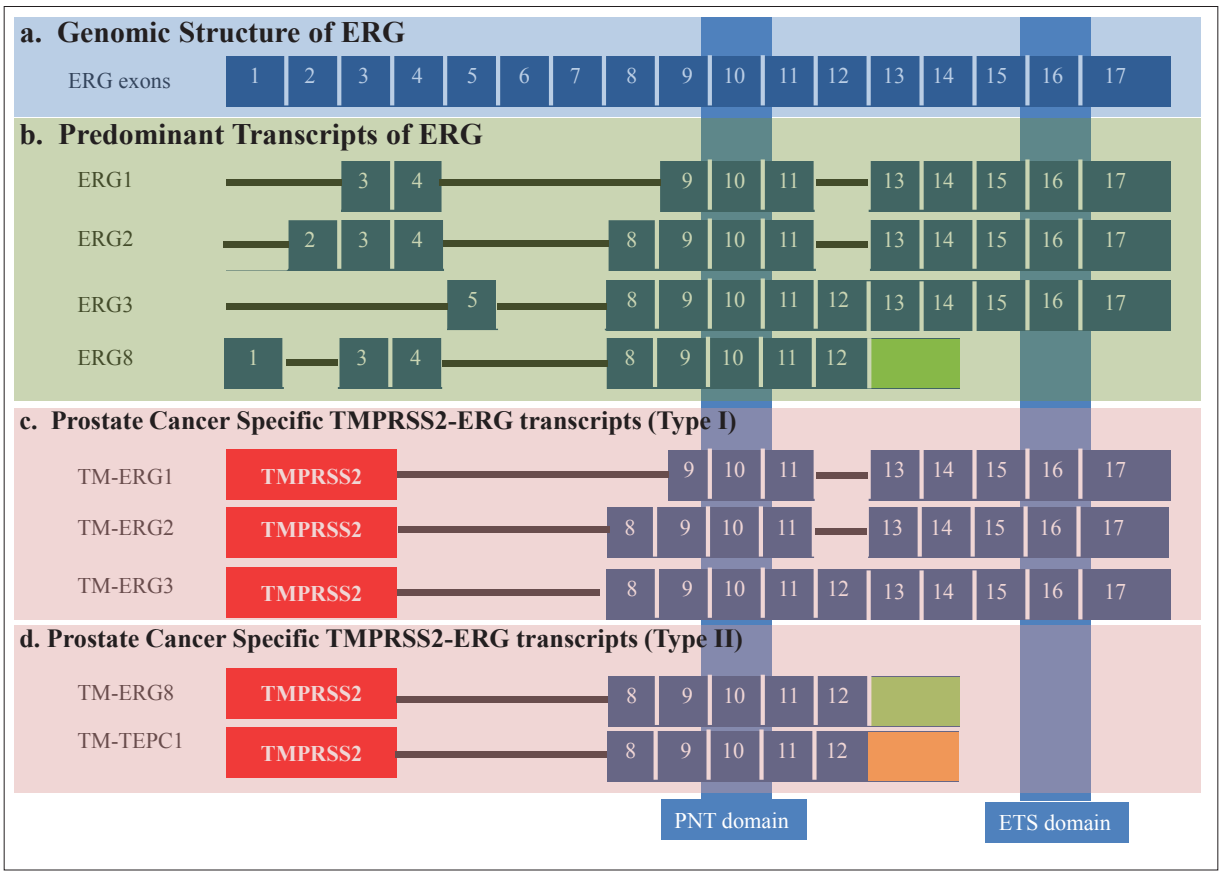

Figure 1:Genomic structure and transcripts of human ERG gene. (a) Genomic structure depicting ERG Exons (blue boxes) numbered from 1-17.[53] (b) Structure of expressed ERG transcripts.[53] (c) Prostate cancer specific TMPRSS2-ERG fusion transcripts containing protein-protein interaction domain (pointed/SAM) and DNA binding (ETS) domain (Type I).[81] (d). TMPRSS2-ERG fusion Type II transcripts containing only pointed/SAM without ETS domain.[81] Note: In prostate cancer, the original ERG exon 8[53] is numbered as 4.[40,78,87]

such as *MMP1, MMP3, MMP9,* and *ADAM19*, the urokinase plasminogen activator (*PLAU*), and the plasminogen activator inhibitor type1 in CaP.[99-101] *ERG* enhances cell invasion and metastasis through regulating *CXCR4*, a chemokine receptor.[27,102] *ERG* also induces the expression of osteopontin (*OPN*) through ETS binding sequences within the promoter.[103] *OPN*, a member of a Small Integrin-Binding Ligand, N-linked Glycoprotein (SIBLING),and a key regulator of metastasis of a wide variety of cancers is up-regulated in several cancers including prostate. Phenotype of human prostate cancer such as metastasis has been correlated with increasing levels of *OPN* expression.[104]

Accumulating data suggests that *ERG* mediates epigenetic regulatory function[105] through *EZH2*, a polycomb group (PcG) protein in CaP.[106] *EZH2* promotes cancer formation and progression through activation of oncogenic signaling cascades and inhibition of pro-differentiation pathways.[10] In CaP, *NKX3.1* expression is negatively regulated by *ERG* induced *EZH2* interactions.[106] Interestingly, *NKX3.1* negatively regulates *TMPRSS2* promoter that is frequently fused to *ERG*.[107] Therefore inhibition of *NKX3.1* either by *ERG/EZH2* or loss of *NKX3.1* due to recurrent 8p21 deletions may fuel *TMPRSS2* dependent *ERG* expression in CaP. Other epigenetic factors include histone acetyl transferases (*HATs*) and histone deacetylases *(HDACs*) which are frequently altered in majority of cancers including CaP.[108] *ERG* binds to and inhibits HAT activity to deregulate protein acetylation and also activates *HDAC* to deacetylate histone proteins.[109,110] Interestingly, *ERG* has been shown to play critical role in epithelial-to-mesenchymal transition (EMT) by repressing epithelial specific genes and inducing mesenchymal specific genes through *WNT* signaling components.[109,111] EMT has received considerable attention as a conceptual paradigm to explain invasive and metastatic behavior during cancer progression. During this transition, the epithelial cells lose their polarity and cohesiveness, acquiring migration and invasive properties.[112] Recent genome wide screening of *ERG* candidate genes and subsequent validation revealed ERG-enriched targets that include both canonical and non-canonical *WNT* signaling genes: *WNT11, WNT2, WNT9A, CCND1* and *FZD7*.[113] Both *ERG* and *WNT11* expression were elevated in high-grade prostate tumors.[114,115] *FZD4*, one of the members of *WNT* signaling pathway, is often co-expressed with *ERG* in clinical specimens. Down regulation of *ERG* or *FZD4* releases the transcriptional block on both *β1-Integrin* and *E-cadherin* to maintain epithelial phenotype.[109] Interestingly, *ERG* also up regulates EMT facilitators such as *ZEB1* and *ZEB2* that negatively control the *E-cadherin*[111] potentially through *SNAIL1* and *2* pathway in CaP.[116] Although EMT is not a prerequisite for invasive cancer development, this process can play an important role in cancer cell dissemination from the tumor due to altered expression of *E-cadherins.*

ERG has also been shown to interface with genes linked to inflammation and DNA damage repair pathways. *ERG* activates *NF-kB* pathway through *toll-like receptor 4* suggesting for its role in inflammation related pathways.[117] *15-hydroxy-prostaglandin dehydrogenase (HPGD),*a tumor suppressor and prostaglandin catabolizing enzyme, is down regulated in variety of cancers such as lung, colon, breast and bladder cancers. Recent studies have shown a potential link between *ERG* and prostaglandin signaling and inflammation pathways in which *ERG* down-regulates the *HPGD* expression to induce carcinogenesis.[118] Proteomics evaluations of *ERG* binding proteins show that *ERG* interacts with Poly (ADP-ribose) polymerase (PARP) and catalytic subunit of DNA protein kinase (DNAPKcs) in a DNA independent manner.[119] This complex formation is required for *ETS* gene mediated transcription and cell invasion. *ERG* induced DNA damage in CaP cells can further be potentiated by PARP1 inhibition, an observation similar to effects of these inhibitory compounds in breast cancer with *BRCA1/2* mutations. As noted, most of studies addressing biochemical and cell biological functions of *ERG* in CaP have used VCaP cell line as this is the only well characterised TMPRSS2-*ERG* positive CaP cell line.[120] Since *ERG* downstream targets may be cellular context dependent, these data need to be interpreted with caution especially in cases when, findings have not been validated in human CaP specimens or complementary experimental models. Development of additional *ERG* positive CaP cell lines will also facilitate cell biologic evaluations of ERG.

Although, the presence of elevated expression of *ERG* in large number of CaP patients have been well characterized by several groups, it is not clear whether *ERG* is an initiating factor or expressed as a consequence of other aberrant genetic events. Towards this, several groups have developed *ERG* transgenic mice by prostate targeted expression of *ERG* driven by rat probasin promoter.[27,87,99,101] Prostatic intraepithelial neoplasia (PIN), a pre-invasive lesions of CaP was reported in the prostates of transgenic mice, which surprisingly did not progress to adenocarcinoma.[99,101] On the contrary, other studies did not observe PIN phenotype, however, developed of adenocarcinoma in combination with either phospho *AKT* overexpression or with loss of *PTEN*.[27,87] Similarly, in prostate tissue dissociation/ regeneration system, high levels of *ERG* expression could induce the initiation of neoplastic transformation of adult prostate epithelial cells and further developed adenocarcinoma in combination with *pAKT* or *AR*.[121] Recent evaluations of the association

*TMPRSS2-ERG*fusion with other genomic alterations in human CaP revealed significant associations with deletions of chromosomal regions, 10q23.31 and 17p13.1 harboring PTEN and p53 respectively.[122] Further, *ERG* fusions showed an intriguing association with CaP specific focal deletion of 3p14.1-p13 harboring several candidate TSGs.[122] While cooperation of *ERG* with PTEN/p-AKT has been shown in enhancing prostate tumorigenesis, interaction of *ERG* with other cancer genes needs to be further defined in engineered mouse models. Taken together, the studies focusing on *ERG* functions provide an emerging picture of the *ERG* network involved in the regulation of differentiation, cell invasion, epigenetic control, EMT inflammation and DNA damage, all of these support the biological role of *ERG* in CaP [Figure 2]. Further, interactions/cooperation of *ERG* with genes (*AR, C-MYC, NKX3.1* and *PI3K/PTEN* axis) functionally significant in CaP, defines potential role of *ERG* in common CaP pathways. These findings have potential to provide new therapeutic approaches for CaP.

ERG as diagnostic/prognostic marker for prostate cancer

Detection of gene fusions has led to a paradigm shift in the diagnosis, classification, and treatment options for hematologic cancers.[123-125] These gene fusions provide CaP specific markers which have promise in improving diagnosis, as well as molecular classification of prostate tumors.[126,127] The feasibility of detecting *TMPRSS2-ERG* fusion by FISH in prostate biopsies and prostatectomy specimens enhances the detection of CaP in diagnostic and prognostic settings.[128-131] The clinical value of *ERG* fusion in prostate biopsies needs to be further explored and validated in larger prospective studies.

Interrogation of the presence of *TMPRSS2-ERG* fusion or *ERG mRNA* in CaP was initially believed to provide prognostic information. However, in retrospective prostatectomy cohorts conflicting results have been reported regarding associations between *ETS* fusions and cancer aggressiveness.[11,48] For example, presence of *TMPRSS2-ERG* fusion predicted cancer recurrence after surgery or lethal outcome in a watchful waiting cohort.[79,132] However, association of the fusion or *ERG* expression with favorable outcome was also reported.[39,133,134] Since *ERG* expression in CaP is androgen dependent due to *TMPRSS2-ERG* fusion, alterations of AR transcription factor activity may result in altered *ERG mRNA* expression as noted in poorly differentiated tumors.[135] These data also suggest that *ERG* in combination

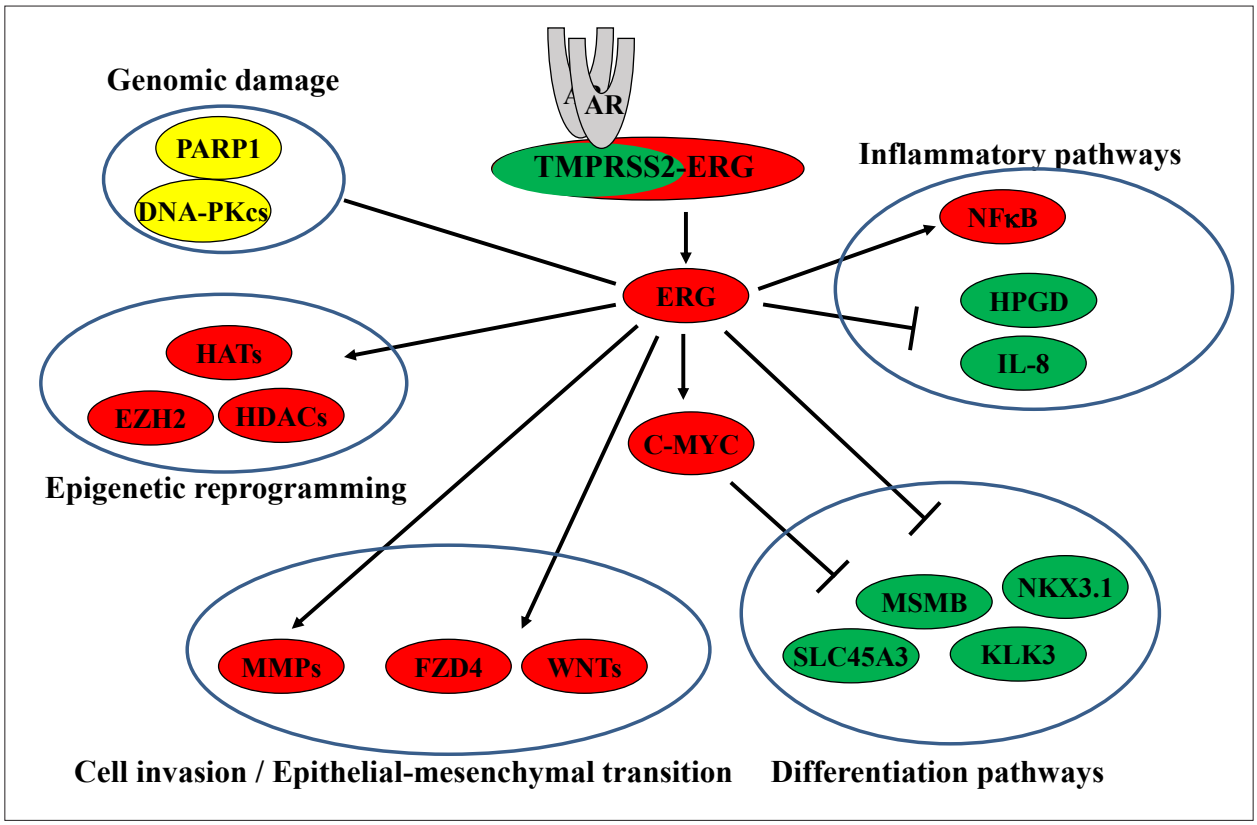

Figure 2: ERG regulated prostate cancer pathways.ERG regulates the expression of target genes associated with cancer initiation and progression pathways such as DNA damage, inflammation,epigenetic control,regulation of differentiation, EMT, cell proliferation and cell invasion. (Red: upregulated;Green:down regulated;Yellow: protein-protein interactions)

with a panel of androgen receptor regulated genes (*PSA, PMEPA1, NKX3.1, ODC, AMD*) may serve as a biomarker panel for Androgen Receptor Function Index (ARFI) in CaP. Thus, ARFI may provide new opportunities in AR function based stratification of CaP, where *ERG* expression evaluation could play important role in over half of CaP.[135] These findings may provide potential biologic basis for initial observations on association of decreased or no *ERG mRNA* expression with poor prognosis of CaP.[39] *TMPRSS2-ERG* fusion isoforms have variable tumor promoting biological activities and certain isoforms are correlated well with more aggressive disease[55] and others with favorable prognosis.[136] Similarly, the ratios of full length splice forms type I and type II also shown to have prognostic association.[81] However, some studies have reported no significant association of *TMPRSS2-ERG* fusion or *ERG* expression with disease progression after prostatectomy.[83,137,138] Therefore, larger and better designed studies are needed for further clarification. The observations of combination of *TMPRSS2-ERG* fusion and *PTEN* deletions associating with poorer prognosis have been supported with functional studies showing cooperation of these genes in mouse models of CaP.[27,87,121,139] Further assessment of the utility of combinatorial prognostic markers is warranted.

Utility of detection of *TMPRSS2-ERG* fusion or *ERG* transcripts in post-digital rectal examination (post-DRE) urine are also being evaluated for improving CaP diagnosis using minimally invasive assays.[140-142] Promising results from evaluations of highly CaP specific non-coding RNA, *PCA3*, in post-DRE urine specimens, have led the way for evaluation of additional CaP specific expression markers.[143-145] A CaP gene panel (*PCA3, ERG* and *AMACR*) with diagnostic potential in which overexpression of at least one of three genes associated with virtually all of the LCM derived prostate tumor specimens suggested for careful evaluation of such panels in post-DRE urine.[39] Evaluation of ERG[141] or *TMPRSS2-ERG*[140] transcripts in post-DRE urine have provided promising data on diagnostic potential of *ERG* in this minimally invasive bio-specimen. A recent multi-center study of 1312 men showed promising data with respect to association of *TMPRSS2-ERG* in post-DRE urine with clinically significant CaP.[142] This study further showed utility of the combination of *TMPRSS2-ERG* and *PCA3* in post-DRE urine in comparison to serum PSA for detecting clinically significant CaP in specimens.[142]

New insights into detection of *ERG* oncoprotein in prostate cancer

Accurate molecular analysis of ERG oncoprotein in CaP has been a challenge as ETS family of proteins share high homology among the family members. Recent development and evaluation anti-ERG monoclonal antibodies have paved the way for evaluation of ERG protein in routine pathologic specimens. Through exhaustive analysis of 132 whole-mount prostates sections (261 tumor foci and over 200,000 benign glands) for the ERG oncoprotein nuclear expression by an anti-ERG mouse monoclonal antibody (clone 9FY), this study demonstrated 99.9 % specificity for detecting tumor cells in prostate.[138] The ERG oncoprotein expression correlated well with fusion transcript or gene fusion in selected specimens. Strong concordance of ERG positive prostatic intraepithelial neoplasia (PIN) lesions with ERG positive carcinoma (82 out of 85 sections with PIN, 96.5%) affirmed the biological role of ERG in clonal selection of prostate tumors in 65% (86 out of 132) of patients[138] [Figure 3]. These observations lend a support to the functional role of ERG in initiation of preneoplastic lesions.[99,101] Evaluations of anti-ERG rabbit monoclonal antibody (EPR 3864) in CaP tissue microarrays from 207 established correlation between detection of ERG protein expression by IHC and ERG rearrangement by using fluorescence *in situ* hybridization (FISH). Detection of the ERG protein expression in CaP exhibited 95.7% sensitivity and 96.5% for the presence ERG rearrangement. Further, presence of ERG protein in CaP also correlated with less common ERG rearrangements. Since ERG expression is almost exclusive to prostate tumor cells and IHC is easier to perform in comparison to FISH. It is expected that ERG protein detection in pathologic specimens will greatly facilitate the evaluations of biological and clinical utility of ERG antibodies in CaP. Among the currently known CaP biomarkers, detection ERG oncoprotein offers unprecedented opportunities in the diagnostic setting [Figure 4]. With the availability of highly specific ERG monoclonal antibodies, better and more effective monitoring, treatment, and therapies

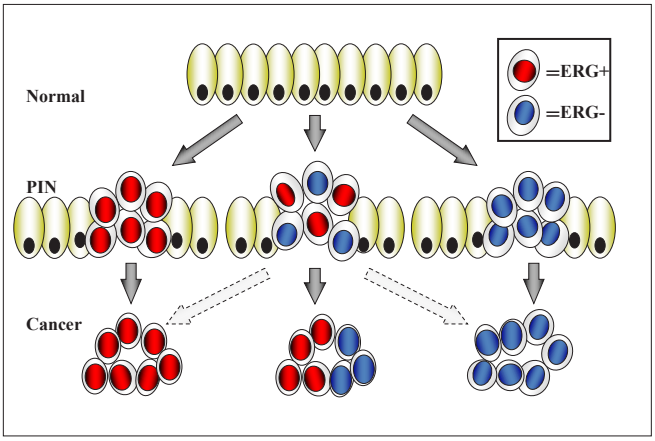

Figure 3: ERG-dependent Clonal Selection of Prostate Tumors. Model describing the ERG-dependent clonal selection of prostate tumors from prostatic intraepithelial neoplasia (PIN) to prostate cancer. Other precursor lesions which may not progress through the PIN morphological stage are not represented by this model. Normal prostate epithelial cells are marked by green color

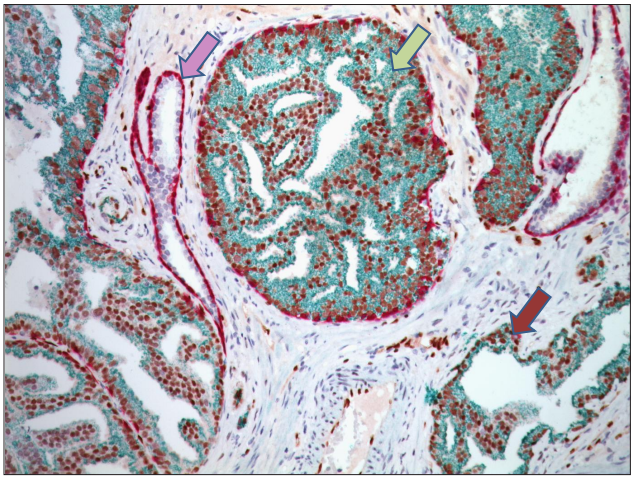

Figure 4: Detection of PIN and prostatic adenocarcinoma by the combination of ERG, AMACR, p63 and CK5 markers in immunohistochemistry.Tumor cells are positive for nuclear ERG (brown) and cytoplasmic AMACR (green), whereas, absence of p63 (purple) and CK5 (purple) indicate the lack of basal cell layer. By contrast, in normal prostatic glands prominent staining with p63 and CK5 distinctively demarcate intact basal cell layer.In PIN disrupted basal cell layer and prominent ERG and AMACR staining is apparent (×400). (Image: Courtesy of Dr. David Tacha, Biocare Medical Inc, Concord, CA, USA)

may also be available in future to patients with CaP.[146,147]

Since ERG MAb 9FY is highly ERG specific as illustrated by lack of recognition of its closest homolog, FLI,[58,138] the presence of ERG protein in hemangiomas, lymphangiomas, angiosarcomas, epithelioid hemangio-endotheliomas and Kaposi sarcomas[148] serve as an excellent new marker for vascular tumors. Similar studies are also warranted in Acute Myeloid leukemia where *ERG* has been suggested as prognostic marker based on mRNA based studies.[58,148]

New therapeutic opportunities targeting *ERG* in prostate cancer

Studies have shown growth inhibitory effects of the *ERG si RNA* in *TMPRSS2-ERG* positive VCaP cells and VCaP derived tumors in SCID mice suggesting for therapeutic potential of *ERG* inhibition in CaP.[66,71] Further, these mechanistic data delineated the effects of *ERG siRNA* through inhibition of C-MYC and induction of prostate epithelial cell differentiation markers.[71] Recent reports in transgenic mice have shown cooperative effects of *ERG* overexpression with *PTEN/PI3K* axis alteartions, leading to progressive features of CaP.[27,87] Thus targeting the inhibition of *ERG* pathway may provide a promising therapeutic strategy. In addition to siRNA as a potential molecule to interfere with the *ERG* expression, YK-4-279, a derivative of the lead compound from the small molecule screen, has proven to effectively bind to ERG and subsequently down regulate its transcriptional activity as well as tumor cell invasion in cell culture model.[149,150] Inhibitors of HDACs are currently being considered as one of the potent anti-cancer agents. HDAC inhibitors, such as SAHA, MS-275, TSA and VPA have been evaluated both *in vitro* and *in vivo* prostate cancer models[108] and in a number of clinical trials.[151] HDAC inhibitors (VPA, TSA) induce apoptosis of prostate cancer cells (VCaP) through up-regulation of *p21/Waf1/CIP1* pathway. These inhibitors alsodown-regulate*TMPRSS2-ERG* and alter the acetylation status of p53.[110] Targeting nuclear transcription factors is often difficult in designing therapeutic strategies; hence, targeting components of the "*ERG* Network" may serve as an effective alternative strategy to combat the CaP. Recent findings showed physical interaction of ERG protein with PARP in inducing DNA damage and inhibition of PARP impaired *ERG* mediated cell invasion and tumorigenesis.[119] These findings suggest a promising therapeutic potential for PARP inhibitors for a large subset of CaP harboring oncogenic activation of the *ERG* or *ETV1*. In recent years, PARP inhibitors have been increasingly considered as a viable option in exploiting the DNA-repair defects of *BRCA1/2*-deficient tumors to induce cell death.[152-154] As CaP is heterogeneous and potentially involves multiple molecular pathways leading to complex phenotypes, development of small molecule inhibitors targeting multiple targets (*AR, ERG, PARP, PTEN, PI3K, AKT* and *mTOR*) may incorporate new therapeutic strategies for CaP.[155,156] Importantly, *ERG* network targeted therapy may be an effective strategy for more than half of CaP in early stages when cancer cells may be more responsive to treatment.

Concluding remarks

Androgen dependent expression of *ERG* transcription factor as a result of *TMPRSS2-ERG* fusion is detected in 50-70% of CaP patients in Western countries. Evaluations of *ERG* fusions represent one of the most studied and validated genomic alterations in CaP. Other gene fusions are low frequency events in CaP and need to be better understood. Since *ERG* fusions described in CaP are highly specific to this cancer type, numerous studies have evaluated clinical utility of *ERG* as a diagnostic or prognostic biomarker in CaP. Detection of *ERG* rearrangement by FISH or immunostaining of *ERG* protein has been streamlined in pathologic specimens and results from these studies suggest the role of *ERG* in clonal expansion of *ERG* positive PIN (pre-invasive lesion) to carcinoma. While *ERG* alteration is homogenous with in a tumor focus, heterogeneity of *ERG* alteration is apparent in mutli-focal tumor context by simultaneous presence of *ERG* positive and negative tumor foci in the malignant prostate of a patient. Detection of *ERG* alterations in tissue or urine based assays have promise in improving prostate cancer diagnosis and continued investigations are anticipated along these lines. Prognostic value of *TMPRSS2-ERG* fusion or

ERG protein expression is uncertain, however, combination of *ERG* alteration with other CaP gene alterations such as PTEN may define prognostic marker panels for progressive disease. Additional studies are also warranted to further assess the prognostic properties of specific *ERG* fusion type or relative abundance of type I and II splice *ERG* splice variants in CaP. *ERG* mRNA or *ERG* protein expression may serve as a surrogate of AR functional status in prostate tumors and therefore evaluation of *ERG* mRNA or protein expression in prostate tumors has potential in companion diagnostic setting for therapeutics targeting androgen/AR axis.

Functional evaluations of *ERG* in experimental models suggest causal role of *ERG* oncogenic activation in prostate tumorigenesis. *ERG* induces pre-invasive lesions and *ERG* in combination with *PTEN* loss, *AKT* or *AR* cooperate in neoplastic transformation. *ERG* knock-down inhibits prostate cancer cell growth. Studies focusing on *ERG* transcriptional targets in prostate cancer cells suggest role of *ERG* in regulating genes involved in oncogegnesis, differentiation, cell invasion, DNA damage, epigenetic control, inflammation and epithelial-mesenchyme transition. The emerging "*ERG* network" defines new facets of *ERG* functions in CaP and underscores the functional interface of *ERG* with genes (*AR, C-MYC, NKX3.1*, and *PI3K/PTEN* axis) known to have critical functions in CaP. Studies focusing on therapeutic targeting of *ERG* or its network are promising as shown by therapeutic potential of *PARP* inhibitors for *ERG* and *ETV1* positive tumors in preclinical models. Taken together, strategies developing *ERG* based biological classification of prostate tumors and therapeutic targeting of the *ERG* network in prostate cancer represent new paradigm in prostate cancer stratification and treatment.

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