Proto-Oncogene ETS-Related Gene in Prostate Cancer

Subjects: Biochemistry & Molecular Biology

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The ETS-related gene (ERG) is proto-oncogene that is classified as a member of the ETS transcription factor family, which has been found to be consistently overexpressed in about half of the patients with clinically significant prostate cancer (PCa). The overexpression of ERG can mostly be attributed to the fusion of the ERG and transmembrane serine protease 2 (TMPRSS2) genes, and this fusion is represented about 85% of all gene fusions observed in prostate cancer. Clinically, individuals with ERG gene fusion are mostly documented to have advanced tumor stages, increased mortality, and higher rates of metastasis in non-surgical cohorts.

Keywords: gene fusion ; prostate cancer ; tumorigenesis

1. Structural Characteristics and Allosteric Autoinhibition of *ERG*

The ERG transcription factor has a primary structure that consists of 486 amino acids and a corresponding molecular weight of 54kDa [1]. A distinguishing characteristic of ETS family proteins is the presence of a DNA-binding domain called the ETS DNA binding domain (EBD). This EBD domain is made of 85 amino acids and contains 3 alpha-helices that are further supported by a 4-stranded anti-parallel beta sheet [2]. The EBD domain plays a critical role in DNA recognition as well as in AP-1 and co-activator recruitment [3]. Within the EBD, there are three highly conserved tryptophan residues that serve as a hydrophobic core to facilitate the helix–turn–helix binding domain in proteins [4]. ERG analysis by means peptide sequencing has also predicted the presence of phosphorylation sites for protein kinase C and a pointed (PNT) domain in the N-terminus [1]. The PNT domains are a part of a larger sterile alpha motif (SAM) family that is involved in many diverse protein–protein interactions that can allow for self-association [5]. This PNT domain has also been shown to facilitate the heterodimerization of ERG with other proteins, including other members of the ETS family, DNA-dependent kinases, AP-1 complex, and the androgen receptor (*AR*) [6].

2. The Function of *ETS-Related Gene* (*ERG*) in Normal Cell Types

ERG has a multitude of physiological functions that differ based on the type of cells or the organism's developmental stage. During embryogenesis, ERG has been observed to be highly expressed in the mesoderm and endothelium, where it plays a crucial role in vasculogenesis and in the development of bone [7]. In adults, it regulates vascular homeostasis and angiogenesis by activating the transcription of endothelial specific genes, such as vascular endothelial (VE)-cadherin, an adhesion molecule that promotes vascular stability by maintaining and controlling endothelial cell contact [8]. In addition to this, VE-cadherin also plays a central role in cell proliferation and apoptosis and modulates endothelial growth factor receptor functions [9]. Other endothelial genes that are positively regulated by ERG include the vascular endothelial growth factor (*VEGF*), von Willebrand factor, and endoglin, which are all involved in endothelial cell differentiation and angiogenesis [4][10].

3. Prominent *TMPRSS2-ERG* Gene Fusion Found in PCa

In prostate cancer cells, a surprisingly common occurrence involves the fusion of *ERG* to *TMPRSS2*, which forms the fusion product of *TMPRSS2-ERG*. The most common mechanism by which these two genes fuse involves the deletion of intronic sequences on the long arm of chromosome 21 via an intron deletion between TMPRSS2 and ERG on chromosome 21q22.2-3 (**Figure 1**). This fusion mechanism has been identified as being prevalent in approximately 50% of prostate cancer patients [11]. The frequent occurrence of this fusion protein can be attributed to the presence of a homogenous deletion site that is present between *ERG* and *TMPRSS2* [12]. Moreover, this deletion site is separated into two different classifications according to various start sites. In both of the deletion products, the 5′ end of the *TMPRSS2* gene has been ligated to the 3′ end of *ERG*. *TMPRSS2-ERG* fusion results in *ERG* overexpression due to the androgen responsive promoter of the *TMPSS2* gene allowing for the constitutive transcription of *ERG,* which has been shown to be

correlated with increased cell proliferation, cell invasion, angiogenesis, and invasiveness in PCa cells [13][14]. In addition, this *TMPRSS2-ERG* fusion enhances the transcription and activates downstream oncogenes [15].

4. Functional *ERG* Overexpression in Prostate Cancer Cells

In prostate cancer cells, *ERG* overexpression increases the rate of epithelial to mesenchymal transitions via the EMT pathway, enhancing the ability of PCa cells to invade and metastasize. *ERG* achieves this by upregulating matrix metalloproteinases (*MMPs*), *CXCR4*, and *Osteopontin* (*OPN*), which have been correlated with higher rates of cell invasion and metastasis among patients [16]. Additionally, the *TMPRSS2-ERG* pathway reveals the epithelial to mesenchymal transition via the *ZEB1/ZEB2* axis in PCa [17]. The constitutive expression of ERG also hyperactivates the inflammatory pathway in PCa cells by binding to *Toll-like receptor 4*; this activates the NF-kb pathway, increasing the transcription of target genes such as *TNFA*, *IL6*, *BCLXL*, *BCL2*, *BCLXS*, *XIAP*, and *VEGF* [18][19]. These proteins trigger tumor growth and progression by enhancing cell proliferation, survival, and angiogenesis. Tumor growth is further accentuated by the activation of the *EZH2* promoter by *ERG.* This relieves the epigenetic inhibition of tumor suppressor genes such as *NKX3.1*, resulting in the constitutive expression of the *TMPRSS2-ERG* fusion gene. In addition to its role in regulating tumor cell invasion and proliferation, *ERG* also plays an important role in negatively regulating tumor cell differentiation by inhibiting the transcription of genes such as *KLK3*/*PSA* and *SLC45A3*/*Prostein* [20]. Altogether, the overexpression of *ERG* in prostate cancer is a key modulator of tumor progression and aggressiveness, as it can regulate the transcription of the proteins that mediate inflammation, cell invasion, differentiation, and oncogenesis [21].

5. TMPRSS2-ERG Fusion Upregulates CXCR4 Enhances Tumor Adhesion and Aggregation

Previous ones have been concluded that there are eight ERG/Ets factor binding sites near the promoter of chemokine receptor type 4 (*CXCR4)* (**Figure 1**) and that *TMPRSS2-ERG* expression enhances the function and expression of *CXCR4* [22][23]. CXCL12 is a known CXCR4 receptor ligand, and the interactions between the two functions enhance tumor aggressiveness and increase the ability of cancer cells to adhere to the extracellular matrix [24][25]. The *CXCR4/CXCL12* axis has been shown to increase MMP expression, which promotes cell migration and growth in PCa [22]. An analysis of PCa demographics revealed relatively higher CXCR4 expression during PCa progression and in metastatic bone tissue compared to benign PCa cells that were reported. In vitro, a direct correlation between *TMPRSS-ERG* and CXCR4 was observed when ERG was knocked down [25].

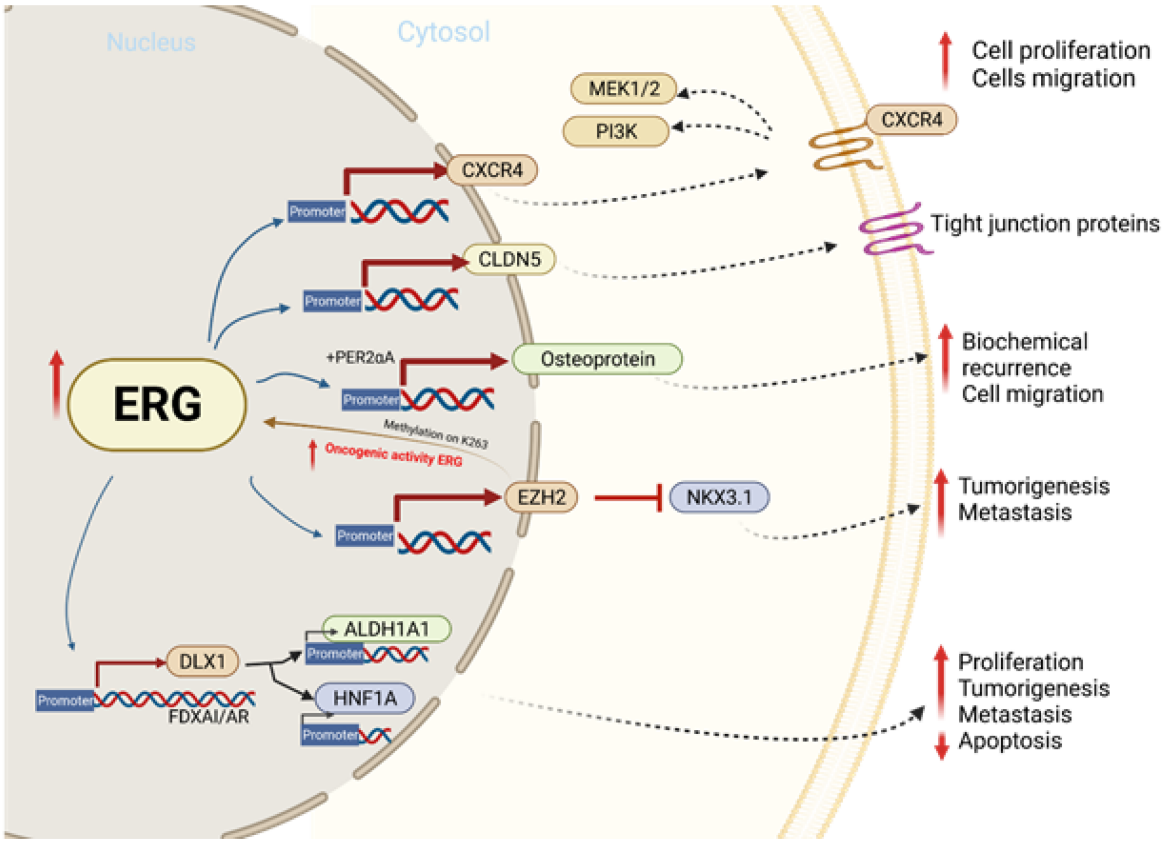

Figure 1. The molecular mechanism involved in ERG overexpression and related outcomes in tumor cell proliferation, migration, invasion, and metastasis.

6. TMPRSS2-ERG Binds to EMT Key Regulators *ZEB1/ZEB2*

Prior one was showed that *TMPRSS2-ERG* can directly bind to the Zinc finger e-box binding homeobox 1 (*ZEB1*) promoter and binds to *ZEB2* modulators such as ILIR2 and SPINT1 and increases their overall expression [26]. *ZEB1* and *ZEB2* are members of the *ZEB* family of transcriptional factors and are key regulators in the EMT and disease progression pathways [27]. Furthermore, *ZEB1* knockdown revealed a significant decline in the migration and invasion capacity of *TMPRSS2-ERG*-expressing cells.

7. EZH2 Enhances ERG Oncogenic Activity

It appears that the enhancer of zeste jomolog 2 (*EZH2*), which is a histone H3K27 methyltransferase, catalyzes the methylation of *ERG* at the lysine 263 residue. This interaction promotes the translocation and DNA binding of *ERG* in the nucleus, which results in the enhancement of *the* oncogenic activity of ERG [28][29]. It has been shown that the methylation of *ERG* at the lysine362 residue is associated with metastatic properties and increased tumorigenic characteristics in cell lines [28]. Further, these interactions seem to enhance the progression of PCa from non-invasive lesions to invasive adenocarcinomas in Erg/Pten mice [28].

8. *ERG* and Tight Junction Protein CLDN5

Recently it has been shown that ERG is positively correlated with the expression of an important tight junction protein known as Claudin 5 (CLDN5); it has been observed that there are two major ERG binding sites near the promoter of the *CLDN5* gene, making ERG a direct transcriptional regulator of *CLDN5*. Moreover, CLDN5 is known as a member of the 24 tetraspan transmembrane protein family and one of the major components of the tight junction strands that are responsible for regulating barrier functions [30]. This protein is involved in many of the processes related to vascular homeostasis. Research has shown that the knockout of ERG expression in mice resulted in increased endothelial cell permeability due to decreased CLDN5 expression [31]. Interestingly, *CLDN5* upregulation results in decreased the cell migration and invasion ability of lung cancer cells due to the decreased permeability of the cell membrane due to the enhancement of the CLDN5 tight junctions [32][33]. In addition to ERG's function in maintaining vascular homeostasis, it also plays a critical role in maintaining the population of hematopoietic stem cells (HSC) by regulating their differentiation. A recent study demonstrated that *ERG* is necessary for arresting HSCs in a dormant G_0 phase by analyzing the number of cells within each phase of the cell cycle. *CLDN5* has been implicated as a negative regulator of many biological processes such as angiogenesis, cell migration, and vascular permeability; alternatively, it has also been shown to be a positive regulator of tight junction assembly, cell population differentiation, protein binding, and endothelial barrier development [32][34][35]. Furthermore, *TNF*-alpha has been classified as a direct inhibitor of *ERG* and *CLDN5* by extension [30].

9. *ERG* Upregulates Distal-Less Homeobox-1(DLX1)

It is thought that *ERG* upregulates distal-less homeobox 1 (*DLX1*) by interacting with enhanced bound *AR* and *FOXA1* [36]. To support this hypothesis, it has been observed that when *ERG* and therefore *DLX1* transcription is inhibited via BET inhibitors, there is a significant reduction in the oncogenic effects of *DLX1* [36]. *DLX* is a transcriptional factor and is part of the homeobox-containing family [37]. Several malignancies, such as those including the prostate, have been linked to the deregulation of the homeobox gene, and therefore, *DLX1* has been validated as a potential PCa biomarker [36][38].

10. GSK3B and WEE1 Induces TMPRSS2-ERG Degradation

Hong et al. [39] revealed that the glycogen synthase kinase 3 beta (*GSK3B*) and WEE1 induce *TMPRSS2-ERG* degradation via the dual phosphorylation of ERG threonine-187 and tyrosine-190. Such phosphorylation allows for the recognition and degradation of the *ERG* oncoprotein by the E3 ubiquitin ligase FBW7. *GSK3B* and *WEE1* have been found to be associated with the DNA damage that is induced proteasomal degradation in PCa [40]. The relationship between *TMPRSS2-ERG* and *PTEN* has been described previously, but it is interesting to note that this degradation pathway is eradicated for *PTEN* loss or *GSK3B* inactivation. This has further been implicated with the growth of chemoresistant PCa cell lines in culture and in mice.

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