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Molecular Mechanism of Activation of Transforming Growth Factor Beta/Smads Signaling Pathway in Ets Related Gene-Positive Prostate Cancers

Jinbo Fang, **Huali Xu**, **Chunshu Yang**, **Shubha Kayarthodi**, **Roland Matthews**, **Veena N. Rao**, and **E. Shyam P. Reddy***
Georgia Cancer Center for Excellence, Cancer Biology Program, Department of OB/GYN, Morehouse School of Medicine, Grady Health System, 80 Jesse Hill Jr. Drive, Atlanta, GA 30303, USA

Abstract

Transforming growth factor beta (TGF-β) signaling pathway is involved in diverse cellular processes, including cell proliferation, differentiation, adhesion, apoptosis, and some human diseases including cancer. Smad proteins function as mediators of intracellular signal transduction of TGF-β. Following their phosphorylation by TGF-β receptor I, Smad2 and Smad3 form a heteromeric complex with Smad4 and then are translocated into the nucleus where they bind to other co-factors and regulate the expression of target genes. ERG (Ets Related Gene) belongs to the ETS family of transcriptional factors. Chromosomal rearrangement of TMPRSS2 gene and ERG gene has been found in the majority of prostate cancers. Over-expression of full length or truncated ERG proteins is associated with a higher rate of recurrence and unfavorable prognosis. In this review, we focus on recent understanding of regulation of TGF-β/Smads signaling pathway by ERG proteins in prostate cancer.

Keywords

TGF-β; Smad3; Phosphorylation; ERG; TMPRSS2-ERG

Since transforming growth factor-β1 (TGF-β1) was discovered in 1983, it is well accepted that TGF-β signaling pathway affects cell proliferation, differentiation, migration, adhesion, apoptosis, embryonic development, and is even involved in human diseases, including cardiovascular, fibrosis, reproductive, wounded healing disorders and cancer (Drabsch, 2012; Larsson, 2005; Sakaki-Yumoto, 2013). TGF-β1 isoform is expressed abundantly and ubiquitously in all cells and is secreted as a complex with many proteins into extra-cellular matrix. It is not very well known how TGF-β activates downstream signaling pathways. Once activated, the TGF-β ligands regulate cellular processes through binding to cell surface receptors (Elliott, 2005; Kang, 2009). TGF-β1 signaling involves its binding to the TGF-β receptor type II (TGF-βRII) (Fig. 1), which allows it to recruit TGF-β receptor I (TGF-βRI)

*Author to whom correspondence should be addressed. ereddy@msm.edu.

and assemble it as a heterodimeric receptor complex. TGF-βRII phosphorylates TGF-βRI, which, inturn, phosphorylates R-Smads (Receptor-regulated Smads, including Smad2 and Smad3 proteins). Phosphorylated Smad2 and Smad3 interact with common Smad (Smad4) to form a complex and then translocate into the nucleus where they bind to transcriptional coactivators or corepressors and regulate transcription of target genes (Fig. 1) (Feng, 2005; Massague, 2012). Smads contain a highly conserved *N*-terminal domain (MH1) and a conserved *C*-terminal domain (MH2). The conserved domain MH1 functions as a DNA-binding domain whereas MH2 domain transiently interacts with TGF-βRI. Activated TGF-β receptor complex phosphorylates R-Smads at the sequence SSXS in the *C*-terminal tail of the MH2 domain. This phosphorylation of R-Smads is required for their activation. Smad4 is not phosphorylated because it lacks SSXS motif (Yue, 2001). In order for the Smad4-R-Smad complexes to bind to specific target genes, they need to recruit transcriptional coactivators, corepressors, and chromatin remodeling factors. Through this complex interaction, they regulate hundreds of target genes at once (Massague, 2008).

Ubiquitin-dependent protein degradation plays a key role in various biological processes. Smad proteins are degraded by the ubiquitin-proteasome pathway. After TGF-β binding to membrane receptor, phosphorylated R-Smad and Smad4 complexes are formed and translocated into nucleus, where they activate or repress target genes. Heterologous Smad complex can remain for several hours in the nucleus, and then R-Smads are dephosphorylated at a low rate. This dephosphorylation results in the dissociation from Smad4. In the nucleus, Smad3 interacts with a RING finger protein, ROC1, through its *C*-terminal MH2 domain. Smad3 bound to ROC1-SCF^{Fbw1a} (an E3 ubiquitin ligase complex) is then exported from the nucleus to the cytoplasm where it is subjected to proteasomal degradation (Fukuchi, 2001; Ten Dijke, 2004). This ubiquitin-proteasome mediated degradation of Smads regulates the levels of Smads in cell (Derynck, 2003). Despite the fact that Smads are not enzymes they still function as key regulators of TGF-β signaling and, thereby show profound effect on cellular responses with small changes in Smad protein levels (de Caestecker, 2000).

Drs. Reddy and Rao have identified the ERG gene (ETS Related Gene) that belongs to the ETS family of transcriptional factors (Rao, 1987; Reddy, 1987) and have shown that ERG functions as a sequence specific transcriptional activator (Siddique, 1993). ERG gene was also involved in chromosome translocations in Ewing family of tumors as well as in leukemias (Liu, 2013; Ohno and Prasad, 1994; Rao, 1988; Sreenath and Tsuzuki, 2011). It has also been shown to be a common genetic alteration of a recurrent gene fusion between *ERG* and the androgen responsive gene *TMPRSS2* (transmembrane protease, serine 2) on chromosome 21 in prostate cancer (Tomlins, 2005). Approximately 50% of prostate cancer patients have a fusion of *TMPRSS2* and *ERG* genes (Furusato, 2008; Shah, 2009). In these prostate cancers, *ERG* gene expression is significantly up regulated by the androgen-responsive promoter of *TMPRSS2*. To date, the role of TMPRSS2-ERG fusion protein in prostate cancer is not well understood (Brase, 2011; Hossain, 2013; Rosen, 2012). Recent results suggest that over-expression of ERG may be useful as a biomarker for prostate cancer diagnosis (Hossain, 2013).

ERG-positive patients have a low rate of high Gleason grade, poor differentiation, and African American ethnicity compared to ERG-negative patients (Hu, 2008). Consistent with this view, it was also shown that the frequency of ERG-positive tumors was significantly greater among Caucasian Americans than among African Americans (Rosen, 2012). Some studies also suggest a causal role of ERG protein in prostate cancers (Klezovitch, 2008). *TMPRSS2-ERG* gene fusions may be cancer-initiating, and expressed at both RNA and protein levels in prostate cancer stem cells (Klezovitch, 2008; Polson, 2013). Recently, Dr. Reddy's group has shown that an anti-epileptic drug targets ERG-positive prostate cancer cells through the activation of tumor suppressors and nuclear receptors (Fortson, 2011). Similar results were also observed in the Ewing family of tumors (Kayarthodi and Reddy et al., unpublished observations).

TGF-β/Smad signaling plays an important role in the regulation of growth of normal and cancer cells (de Caestecker, 2000; Tian, 2011; Yue, 2001). This signaling pathway has been acknowledged to have a dual role in tumor progression, which is a tumor suppressor for normal epithelial and early stages of cancer cells. It is also a tumor promoter in the last steps of the metastatic disease (Kocic and Miles, 2012). However, it is not clear how this signaling pathway plays a role in ERG-positive prostate cancers, and if there is crosstalk between ERG onco-protein and TGF-β/Smads signaling pathway. Recent studies have shown that ERG protein regulates TGF-β/Smads pathway (Fang and Reddy unpublished observations). We find that ERG can enhance the activity of Smad3 in absence or presence of TGF-β (Fang and Reddy unpublished observations). Furthermore, these results revealed that ERG onco-protein physically interacts with P-Smad3, and stabilized phospho-Smad3 protein levels (Fig. 1). Possible implications of the above mechanism are: first, ERG binds to P-Smad3 and make latter not to bind to other proteins especially involved in ubiquitination pathway and thereby reduce the amount of ubiquited Smad3 and, secondly, ERG bind to P-Smad3 and, thereby inhibits the dephosphorylation of P-Smad3, which leads to inhibition of export of Smad3 from nucleus to cytoplasm. The above-mentioned two novel possibilities may result in an increased amount of phosphorylated-Smad3 in the nucleus and enhance the activity of TGF-β/Smads (Fig. 1). These results provide the first direct evidence that ERG onco-protein contributes to prostate cancer progression by enhancing TGF-β/Smads-signaling pathway in ERG-positive prostate cancers (Fang and Reddy unpublished observations). Therefore, it is possible that therapeutic agents that interfere with the interaction of Smad3 and ERG onco-protein can be used to treat ERG-positive prostate cancers.

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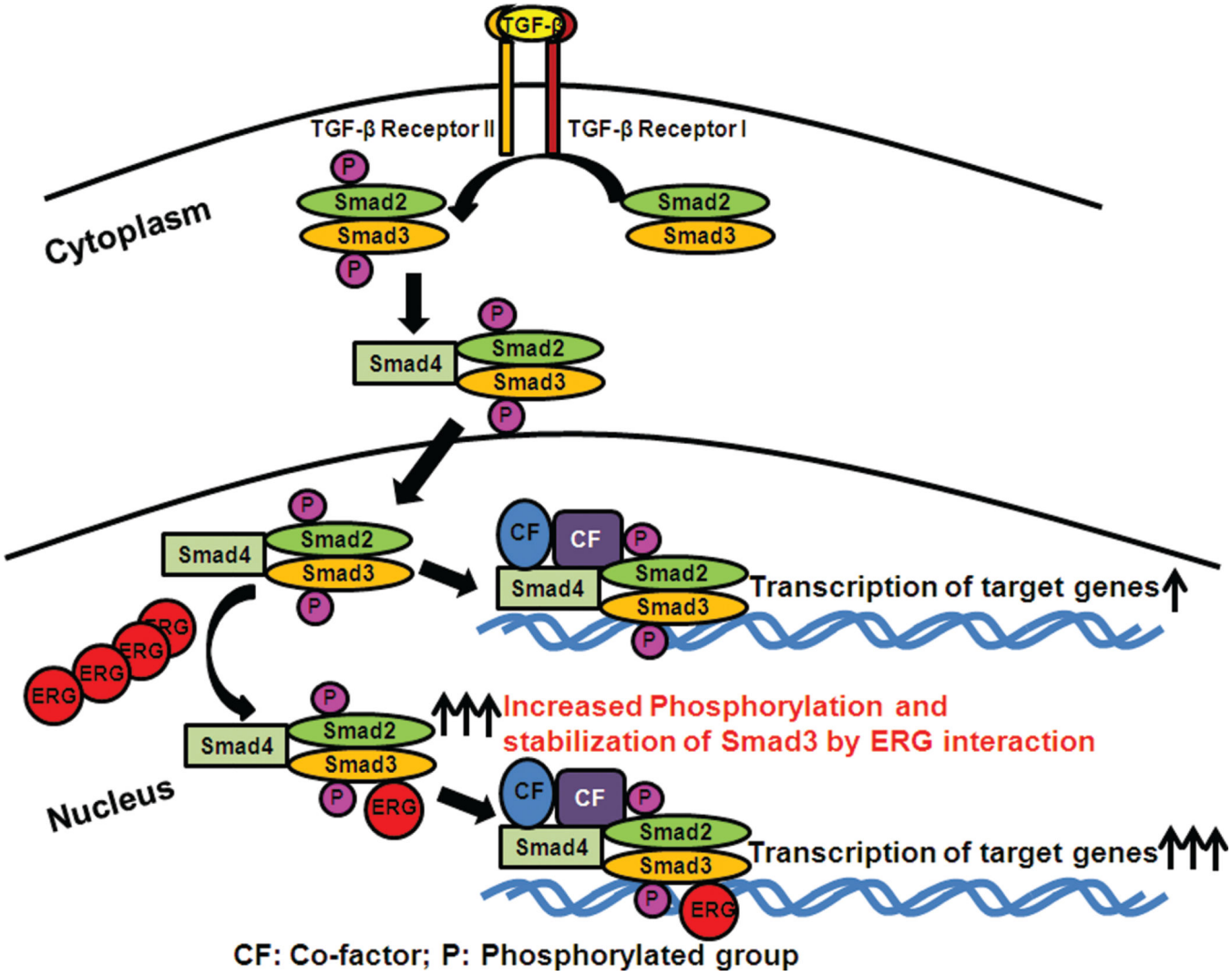

Figure 1.

Phosphortlated Smad3 is stabilized by ERG in ERG-positive prostate cancer cells. Smad3 is phosphorylated by TGF-β binding to receptor. Phosphorylated Smad3 forms a complex with phosphorylated Smad2 and the common Smad (Smad4) and translocates to the nucleus. Phosphorylated Smad3 is stabilized by binding to ERG in the nucleus.