Two paths for stabilization of ERG in prostate carcinogenesis: TMPRSS2-ERG fusions and speckle-type pox virus and zinc finger protein mutations

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Speckle-type POZ (pox virus and zinc finger protein) protein (SPOP) is an E3 ubiquitin ligase adaptor protein that specifically promotes the ubiquitination and proteasome degradation of proteins. SPOP mutations are frequent in prostate cancer, and in a previous study, An et al. demonstrated that SPOP induced the degradation of the androgen receptor (AR) suggesting that SPOP is important in maintaining prostate homeostasis. In this current highlighted report, An and colleagues showed that ERG, which has been implicated as an oncoprotein in prostate cancer, contains putative SPOP-binding consensus (SBC) motifs 42ASSSS and 42VTSSS in the N- and C-terminal of ERG, respectively. The authors went on to demonstrate that SPOP promotes the ubiquitination and degradation of ERG through binding to the degron/SBC motif at the ERG N-terminus. SPOP mutations in the MATH domain prevented recognition and targeting of ERG for ubiquitination and degradation. In addition, N-terminal truncated ERG proteins encoded by the most frequently identified TMPRSS2-ERG rearrangements in prostate cancer (T1-E4 and T1-E5) were resistant to SPOP-mediated degradation, resulting in the stabilization of truncated ERG proteins. Stabilization of ERG protein through either SPOP mutation or TMPRSS2-ERG fusions induced proliferation and invasion in prostate cancer cells. This study along with a recently published similar report provides two previously unrecognized mechanisms for the upregulation of ERG proteins frequently observed in prostate cancers. These findings generate great enthusiasm for the development of targeted therapeutic strategies designed to eliminate ERG protein in prostate cancer cells.

SPOP is a substrate-binding adaptor protein for the Cullin-RING E3 ubiquitin ligase, which catalyzes the specific ubiquitination and proteasome degradation of multiple target proteins. Mutations in SPOP frequently appear in prostate cancer¹ but do not co-occur with other prostate cancer-associated gene mutations or TMPRSS2-ERG fusions² and appear to be quite rare in other cancers.³ The SPOP-Cullin 3-RING box 1 ubiquitin ligase complex binds to its substrates through the N-terminal MATH domain of the SPOP protein. Somatic SPOP mutations in prostate cancer reported thus far have all clustered in the MATH domain, potentially impacting substrate-binding. Furthermore, SPOP substrate proteins, which include Macro H2A, Puc, Daxx, and Gli, are characterized by an SPOP-binding consensus motif⁴ and any alteration of the substrate-binding complex (SBC) might also impair SPOP-binding to its substrates. In a previous study, An et al. performed a protein motif search for SPOP-binding motifs in androgen receptor and ERG proteins. They initially reported that SPOP could bind to the hinge domain of the androgen receptor (AR), resulting in the degradation of full-length AR and inhibition of AR target genes and prostate cancer cell proliferation.⁵ SPOP was unable to recognize and bind to AR variants, resulting in the degradation of AR but not AR variant proteins. In their recently published study, An and colleagues characterized the SPOP-binding motifs in ERG and explored the interaction between SPOP and ERG in prostate cancer cells.

Overexpression of ERG due to fusions between androgen-regulated TMPRSS2 gene promoter and the coding regions of ERG has been reported as the most common genomic alteration in prostate cancer.⁶ Normally, ERG protein is expressed predominantly in endothelial cells and is not detected in epithelial tissues including the prostate epithelium.⁷ However, the androgen responsive TMPRSS2 protein is preferentially expressed in normal prostate tissues and is overexpressed in the neoplastic prostatic epithelium. Androgen stimulation of TMPRSS2-ERG-positive cell lines has been shown to induce increased ERG expression.⁸ In a recent study in Molecular Cell,⁹ An and colleagues demonstrated that ERG was targeted by the SPOP-CUL3-RBX1 E3 ligase for ubiquitination in prostate cancer cells. The authors showed that ERG co-immunoprecipitates with SPOP and through knockdown and overexpression assays that SPOP regulates ERG protein levels. SPOP knockdown induced an increase in ERG protein as well as increased cell invasion, and this effect was abrogated by combined knockdown of SPOP and ERG suggesting that the increased invasion induced by SPOP knockdown was mediated by increased ERG.
Furthermore, the effects of SPOP knockdown on proliferation were also inhibited by ERG knockdown in AR-positive C4-2 cells; genes co-regulated by SPOP and AR were inhibited by concurrent knockdown of SPOP and ERG. These results support previous findings that ERG acts as a “pioneer factor” for activation of AR signaling. In a series of deletion mutant experiments, the authors showed that SPOP recognizes the ASSSS motif in ERG and binds to the MATH central groove. Mutations in the MATH domain of SPOP and TMPRSS2-ERG fusions that have been identified in prostate tumor specimens severely inhibit ERG binding to SPOP, resulting in the stabilization of ERG protein and the subsequent increase in cell proliferation and invasion in patient specimens exhibiting TMPRSS2-ERG fusions lacking the SPOP substrate-binding complex (SBC), elevated ERG protein was observed by immunostaining analysis.

These findings coincide with those of Gan and colleagues, also published in Molecular Cell. Gan and colleagues also showed that SPOP targets ERG for ubiquitination and degradation and that SPOP negatively regulates ERG-mediated cell migration and invasion. They showed that prostate cancer-associated mutations in SPOP and TMPRSS2-ERG fusions lacking the ERG N-terminal region result in the stabilization of ERG protein. In addition, Gan and colleagues demonstrated that SPOP-binding and degradation of ERG required casein kinase 1δ (CKIδ)-mediated ERG phosphorylation at the N-terminal serine residues 44–46. Etoposide treatment stimulated CKI-dependent phosphorylation of specific TMPRSS2-ERG fusions with a masked SPOP-binding site, and wild-type ERG restored SPOP-binding and degradation of ERG in prostate cancer cells.

Cumulatively, these studies demonstrate two previously undefined potential paths that could contribute to the accumulation of ERG in prostate epithelial cells resulting in a subsequent increase in a cancer phenotype (Figure 1). Since it is extremely rare for SPPOP mutations and TMPRSS2-ERG to appear concurrently, the restoration of SPPOP-mediated degradation of ERG fusion protein is a potentially effective treatment strategy for patients with TMPRSS2-ERG fusions. Gan and colleagues demonstrate that etoposide could promote the accumulation of CKIδ and conformational change exposing degron 1, thus triggering the SPPOP-mediated degradation of ERG. These studies illustrate that SPOP plays a critical role in prostate tumor suppression in part due to its targeted ubiquitination and degradation of ERG and provide promising evidence that therapies restoring this function could be effective in the treatment of prostate cancer.

COMPETING INTERESTS
All authors declared no competing interests.

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