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SHORT COMMUNICATION

TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates *C-MYC* and abrogates prostate epithelial differentiation

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The high prevalence of *TMPRSS2-ERG* rearrangements (~60%) in prostate cancer (CaP) leads to androgenic induction of the *ETS*-related gene (*ERG*) expression. However, the biological functions of *ERG* overexpression in CaP remain to be understood. *ERG* knockdown in *TMPRSS2-ERG* expressing CaP cells induced striking morphological changes and inhibited cell growth both in cell culture and SCID mice. Evaluation of the transcriptome and specific gene promoters in ERG siRNA-treated cells and investigation of gene expression signatures of human prostate tumors revealed ERG-mediated activation of *C-MYC* oncogene and the repression of prostate epithelial differentiation genes (*PSA* and *SLC45A3*/Prostein). Taken together, these data combining cell culture and animal models and human prostate tumors reveal that *ERG* overexpression in prostate tumor cells may contribute to the neoplastic process by activating *C-MYC* and by abrogating prostate epithelial differentiation as indicated by prostate epithelial specific markers.
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Molecular genetic evaluations of prostate cancer (CaP) are defining mutations and expression alterations of critical genes involved in disease onset and/or progression (De Marzo *et al*., 2007; Witte, 2007). Prevalent gene fusions leading to the activation of *ETS* transcription factors (predominantly *ERG*) through the androgen receptor (AR)-regulated *TMPRSS2* gene promoter underscore the critical roles of *ERG* overexpression in CaP (Lin *et al*., 1999; Petrovics *et al*., 2005; Tomlins *et al*., 2005; Demichelis and Rubin, 2007; Turner and Watson, 2008).

ETS factors including ERG have been implicated in diverse cancers (Seth and Watson, 2005; Turner and Watson, 2008). As *TMPRSS2-ERG* fusions represent the majority (>95%) of *TMPRSS2-ETS* factor alterations described in CaP thus far, this study focuses on tumor biologic functions of *ERG* overexpression in CaP. *EWS–ERG* fusions have been described in a small subset of Ewing's sarcoma, whereas *ERG* overexpression without fusion was highlighted in acute myeloid leukemia and acute T-lymphoblastic leukemia (Marcucci *et al*., 2005; Baldus *et al*., 2006). *TMPRSS2–ERG* fusion in CaP cells may lead to androgen-dependent overexpression of near full-length ERG-encoded products. Among the nine reported splice variants of *ERG*, *ERG1–3* encode prototypical proteins with ERG3 being the longest (Owczarek *et al*., 2004).

Comparison of the wild-type ERG3 and *TMPRSS2-ERG3*-encoded proteins revealed truncation at the N-terminus of ERG (Figure 1a) due to the *TMPRSS2–ERG* fusion A (Tomlins *et al*., 2005). Thus, the *TMPRSS2-ERG* fusion transcripts may encode truncated ERG proteins at the amino terminus. Thus, due to N-terminal alterations of the *TMPRSS2–ERG*-encoded protein(s) and the complex nature of *ERG* splice variants, we reasoned that targeting knockdown of the known *ERG* splice variants, would provide an effective strategy to evaluate the functions of the *TMPRSS2* promoter-driven *ERG* overexpression in CaP. We initially evaluated the effects of *ERG* knockdown on cell biologic and tumorigenic properties of VCaP cells (Korenchuk *et al*., 2001) that harbor overexpression of *TMPRSS2-ERG* type A fusion, frequently detected in human prostate tumors (Demichelis and Rubin, 2007; Furusato *et al*., 2008). Five ERG siRNAs were screened by assessing the inhibition of ectopically expressed ERG

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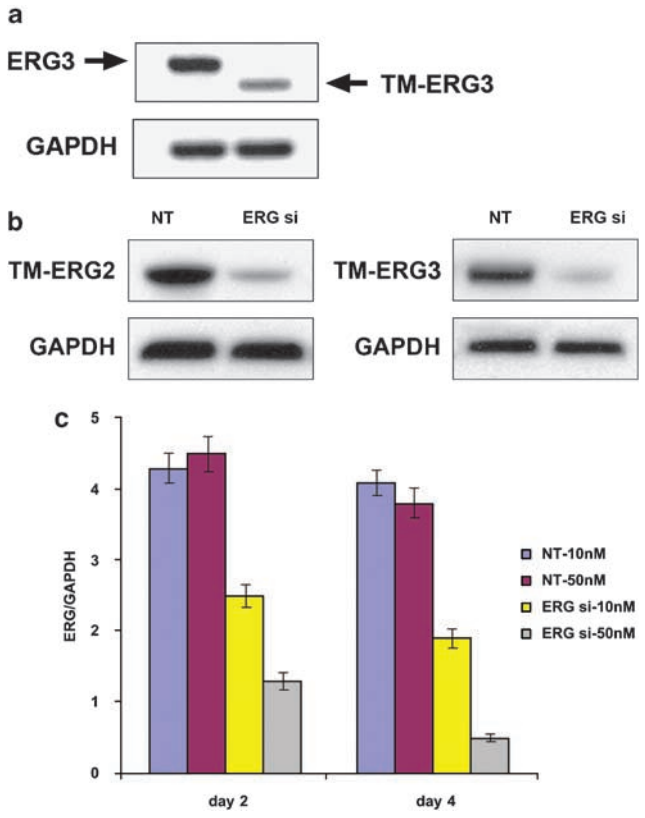
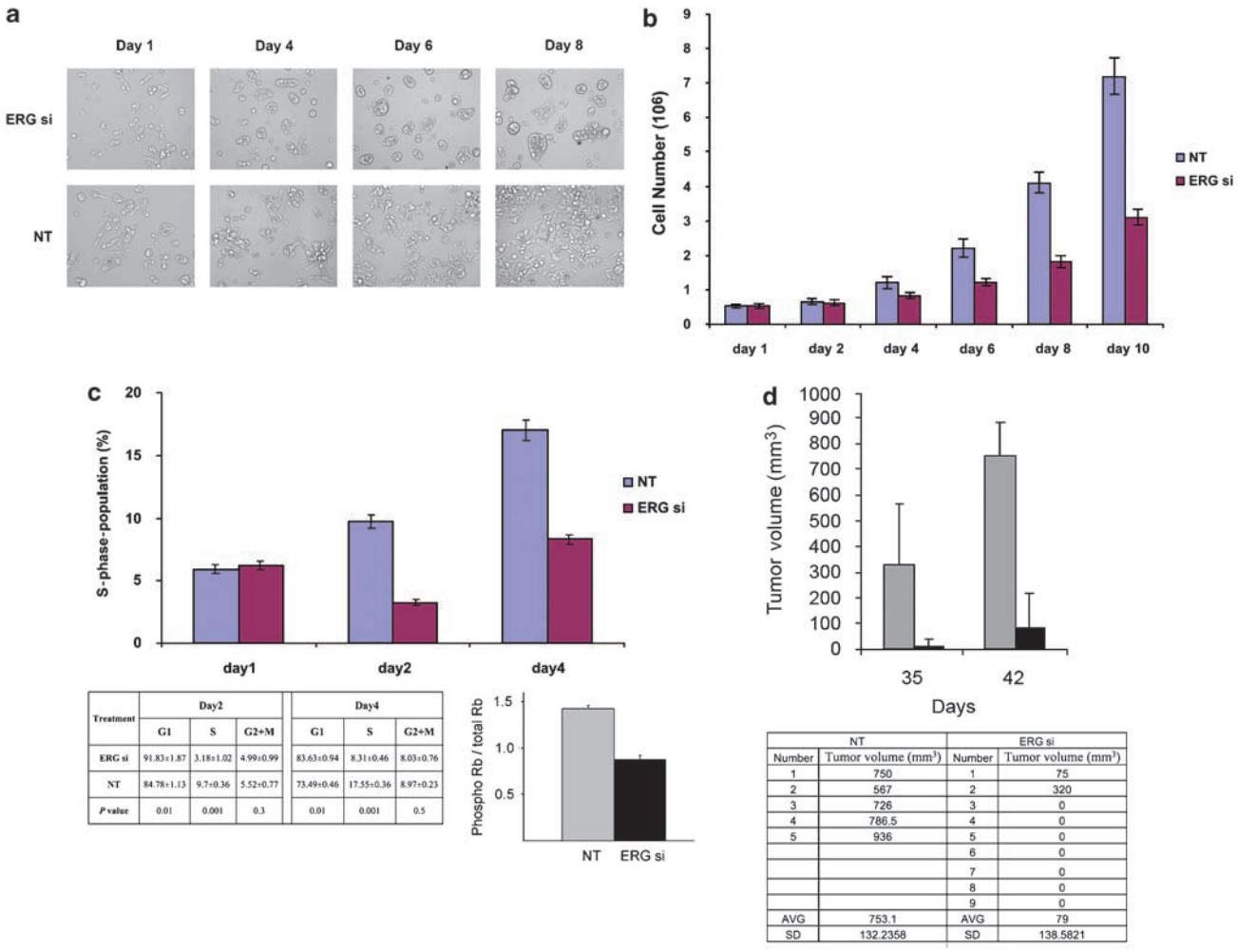


Figure 1 Inhibition of ERG expression by ERG siRNA. (**a**) HEK 293 cells were transfected with ERG3 or TMPRSS2-ERG3 (TM-ERG3) expression vectors. Protein levels were measured by western blotting using antibody against ERG or GAPDH. (**b**) HEK 293 cells were cotransfected with ERG siRNA (ERG si) or non-targeting siRNA (NT) and TMPRSS2-ERG2 (TM-ERG2, left panel) or TMPRSS2-ERG3 (TM-ERG3, right panel) expression vectors. (**c**) VCaP cells were transfected with 10 or 50 nM of ERG si or NT and incubated for 2 or 4 days. Knockdown of endogenous ERG by ERG si was assessed by RT–PCR relative to GAPDH. Bars represent three independent experiments, purple, NT 10 nM, maroon, NT 50 nM, yellow, ERG si 10 nM and gray, ERG si 50 nM. Additional information on Materials and methods are provided in Supplementary Information. CaP, prostate cancer; ERG, *ETS*-related gene; RT–PCR, reverse transcriptase PCR.

proteins in HEK-293 cells transfected with TMPRSS2-ERG2 or TMPRSS2-ERG3 expression vectors or by assessing the inhibition of ERG in VCaP cells (data not shown). Inhibitory effects of the selected ERG siRNA for further studies and experimental controls are shown in Figures 1b and c and in Supplementary Figure 1. This ERG siRNA is targeted to a 19 bp region in exon 10 and is predicted to inhibit all known ERG splice variants (Owczarek *et al*., 2004). For detecting ERG protein, an anti-ERG peptide antibody was raised against the DFHGIAQALQPHPPESSLYKYPSDLPYMGSYHA HPQKMNFVAPHPPAL polypeptide in our laboratory (Supplementary Methods). ERG expression knockdown in VCaP cells resulted in striking cell morphologic alterations as evidenced by slow growing and clumped cells within 6–8 days (Figure 2a and Supplementary Figure 2). Also, there was significant inhibition of the androgen-dependent cell proliferation, S phase of the cell cycle and p-Rb in response to *ERG* knockdown in VCaP cells (Figures 2b and c). However, no significant apoptosis was observed. ERG siRNA did not have any effect on LNCaP cells that do not express detectable ERG due to the lack of *TMPRSS2-ERG* genomic rearrangement in this cell line (data not shown). When ERG siRNA-transfected VCaP cells were injected in SCID mice, striking inhibition of the tumorigenicity was noted (Figure 2d). Only two of nine mice developed detectable tumors (22%) at day 42. By contrast, five of five (100%) mice in the control group developed large tumors (Figure 2d table). Taken together, these data provide the first insight into the critical function of ERG in the growth of prostate tumor cells in cell culture and in mice. These data support recent transgenic mice studies showing that the over-expression of ETV1 (Tomlins *et al*., 2007) or ERG (Klezovitch *et al*., 2008; Tomlins *et al*., 2008) in mouse prostate may induce the features of early CaP and also provide important new insights into the therapeutic potential of the ERG knockdown in CaP cells.

To delineate potential biochemical mechanisms of ERG siRNA-mediated cell growth and tumor growth inhibitory effects, ERG siRNA-regulated transcriptome was analyzed in VCaP cells. Cells were treated with 50 nM of either ERG siRNA or nontargeting siRNA (NT) control. The efficiency of ERG inhibition was assessed by monitoring ERG protein levels (Figure 3a, insert). Gene expression changes were evaluated by using Affymetrix HG U133 Plus 2.0 high-density oligonucleotide human genome arrays. Comparison of gene expression profiles between NT and ERG siRNA by using a functional cocitation-based program (BiblioSphere, Genomatix GmbH, Munich, Germany (Scherf *et al*., 2005)) highlighted key genes commonly involved in cancer-specific regulatory nodes such as *C-MYC, PCNA* and *p53* (Supplementary Figure 3). Evaluations of common gene expression alterations between the ERG siRNA-regulated transcriptome in VCaP cells and gene expression alterations associated with *ERG* overexpressing human prostate tumors from our and others data (Iljin *et al*., 2006) prompted us to further analyze *C-MYC* (Figure 3a, Supplementary Figure 4), one of the major players in cancer pathways. Downregulation of *C-MYC* by ERG siRNA was confirmed at RNA and protein levels in VCaP cells (Figure 3b). Furthermore, recruitment of ERG to the *C-MYC* P2 promoter (Meulia *et al*., 1992) downstream ETS element (V$ETSF_PDEF = Prostate-derived ETS factor element (Oettgen *et al*., 2000)) in VCaP cells was detected by chromatin immunoprecipitation (ChIP) assay. ERG siRNA consistently decreased ERG recruitment to this *C-MYC* promoter downstream ETS element. These data suggest that *ERG* expression under androgenic control may lead to the elevated expression of *C-MYC* in CaP cells.

ERG siRNA-regulated transcriptome in VCaP cells revealed striking upregulation of a number of genes (*KLK3*/PSA, *SLC45A3*/prostein, *C15ORF, MSMB*/PSP94, *SCGB1D2*) whose expression are restricted to prostate epithelium, suggesting that ERG interferes with prostate epithelial differentiation (Supplementary Figure 3). While these data are in agreement with similar



Treatment	Day2			Day4		
	G1	S	G2+M	G1	S	G2+M
ERG si	91.83±1.87	3.18±1.02	4.99±0.99	83.63±0.94	8.31±0.46	8.03±0.76
NT	84.78±1.13	9.7±0.36	5.52±0.77	73.49±0.46	17.55±0.36	8.97±0.23
P value	0.01	0.001	0.3	0.01	0.001	0.5

NT		ERG si	
Number	Tumor volume (mm^3)	Number	Tumor volume (mm^3)
1	750	1	75
2	567	2	320
3	726	3	0
4	786.5	4	0
5	936	5	0
		6	0
		7	0
		8	0
		9	0
AVG	753.1	AVG	79
SD	132.2358	SD	138.5821

Figure 2 ERG knockdown inhibits prostate tumor cell growth *in vitro* and *in vivo*. VCaP cells were transfected with 50 nM of ERG si or NT. (**a**) Cell morphology from triplicate experiments was monitored on day 1, 4, 6 and 8. (**b**) Cells transfected with ERG si (maroon) or NT (purple) were counted at day 1, 2, 4, 6, 8 and 10 ($P = 0.001$) in triplicates. (**c**) S-phase population of cells (upper panel) transfected with ERG si (maroon) or NT (purple) and distribution of cells in G1, S and G2 + M phases (table) were assessed by FACS analysis on the indicated time points from three independent experiments. Phospho-Rb to total Rb ratios were measured at day 4 by western blot assay. (**d**) VCaP cells transfected with ERG si (solid bars) or NT (gray bars) were injected into SCID mice. Tumor growth was monitored at day 35 ($P = 0.0072$) and 42 ($P = 0.0072$). s.d. and average tumor volumes (AVG) at day 42 are shown in the table. CaP, prostate cancer; ERG, *ETS*-related gene; FACS, fluorescence-activated cell sorting; NT, nontargeting siRNA.

observation (Tomlins *et al*., 2008), we show direct role of ERG in the modulation of prostate epithelium differentiation markers. Consistent with our GeneChIP data, we observed inverse correlation between C-MYC and PSA expression in response to ERG knockdown in VCaP cells (Figure 3c left panel). Furthermore, consistent with the ERG siRNA data, adenoviral expression of ERG in *TMPRSS2-ERG* negative LNCaP cells or in VCaP cells revealed significantly diminished PSA levels (Supplementary Figure 5). Immunostaining of the ERG siRNA-treated VCaP cells by PSA and cytokeratin 8 and 18 (CK8/18) antibodies confirmed striking alteration of cellular morphology and elevated PSA (Figure 3c, right panel), suggesting for modulation of the prostate epithelial differentiation markers by ERG.

ChIP assays revealed recruitment of ERG to the PSA enhancer (androgen responsive element (ARE) III) and to the *SLC45A3* (prostein) promoter upstream ETS element (V$ETSF_PDEF) (Figure 3c and d). As both of these genes are regulated by AR and frequent overlap between AREs and ETS-binding sites have been reported (Massie *et al*., 2007), we evaluated the ERG-dependent recruitment of the AR to the *PSA* enhancer (AREIII), and *SLC45A3* (prostein) ARE elements. In contrast to diminishing ERG binding, increased recruitment of AR was evident in response to ERG knockdown. These data suggest that the modulation of prostate epithelium-associated genes by ERG may involve AR. As the expression response of prostein showed the strongest effect of *ERG* knockdown in VCaP cells, we further evaluated the levels of prostein in a subset of human prostate tumors that were previously analyzed for the status of TMPRSS2-ERG fusion A transcript (Furusato *et al*., 2008). Although, prostein was detected in both benign and malignant epithelial cells showing variable expression in 26 evaluated

whole-mount radical prostatectomy specimens, there was an inverse correlation between prostein expression and *TMPRSS2-ERG* fusion transcripts status (Figure 3d). Of 17 patients with *TMPRSS2-ERG* fusion, only one patient (5%) showed high expression of prostein. On the other hand, six of nine (54%) patients lacking *TMPRSS2-ERG* fusion showed high expression of prostein. Interestingly, *SLC45A3* promoter was previously identified as a fusion partner of *ETV1* gene in a CaP case (Tomlins *et al*., 2007).

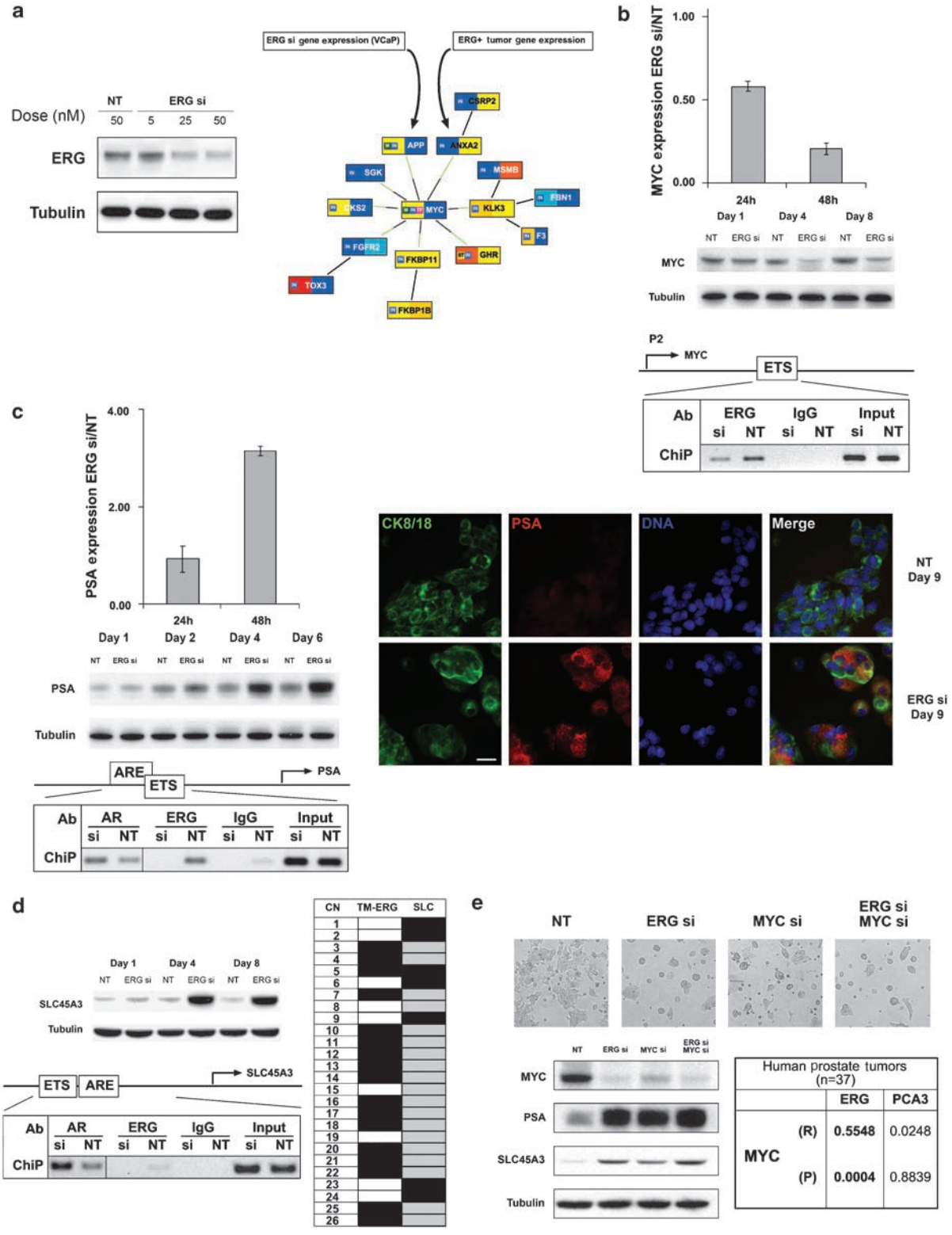


Figure 3

C-MYC may link ERG to a major oncogenic pathway in prostate cancer; therefore, we addressed morphological effects of C-MYC siRNA and ERG siRNA. (Figure 3e). Intriguing similarity was observed when C-MYC siRNA and ERG siRNA-treated VCaP cells were compared. Indeed, the upregulation of prostate differentiation genes characteristic to ERG inhibition, PSA and SLC45A3/prostein, was noted. We further evaluated quantitative gene expression features of *ERG* and *C-MYC* in a cohort of 37 human prostate tumors. The analysis revealed significant correlation between *ERG* and *C-MYC*. In contrast, *PCA3* that is frequently overexpressed in prostate tumors showed no correlation with *C-MYC*. These data establish that *C-MYC* expression is tightly linked to *ERG* expression in prostate cancer.

On the basis of the data presented, we propose a working model for ERG functions in prostate tumorigenesis (Figure 4). The tumor biologic functions of ERG are critical in neoplastic process, as evidenced by the dramatic effects of ERG knockdown on cell morphology/differentiation in culture and inhibition of tumor growth in mice. Although, recent studies using immortalized prostate epithelial cell culture models, such as RWPE and PrEC, have shown the effects of ERG on cell invasion (Klezovitch *et al*., 2008; Tomlins *et al*., 2008), VCaP cell used in our study did not exhibit *in vitro* features of cell invasion. Despite of this observation, VCaP cell model closely resembles prostate tumors that harbor *TMPRSS2-ERG* fusions and express prostate epithelial markers.

Taken together, elevated ERG, as a result of the *TMPRSS2-ERG* fusion, modulates the growth of CaP cells by upregulating *C-MYC* oncogene and by abrogating the differentiation of prostate epithelium. On the basis of these findings we have defined *C-MYC* as one of the critical targets of ERG in CaP cells. In summary, this report establishes the oncogenic functions of ERG in CaP cells and defines important downstream targets of ERG-mediated prostate tumorigenesis. Our study also suggests that in addition to ERG, C-MYC may also represent a rational therapeutic target in a large subset (>60%) of prostate cancers harboring *ERG* overexpression.

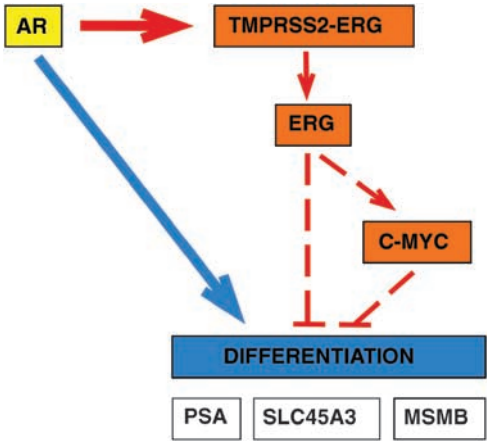


Figure 4 Model for ERG functions in prostate cancer. ERG upregulates C-MYC and interferes with differentiation in prostate cancer cells. ERG, *ETS*-related gene.

Figure 3 *C-MYC* and prostate differentiation are targeted by ERG in prostate cancer cells. VCaP cells transfected with ERG si or NT were analyzed by Affymetrix HG U133 Plus 2.0 high-density oligonucleotide human genome arrays, QRT-PCR, chromatin immunoprecipitation and by western blots. (**a**) Functional cocitation-based network was obtained from overlapping genes within the comparative gene expression analyses of ERG expressing prostate tumors (ERG+ tumor gene expression, left side color codes) and ERG si-treated VCaP cells (ERG si gene expression, VCaP, right side color codes) by the BiblioSphere Software (Genomatix GmbH, Munich, Germany). Red and yellow colors mark the upregulation, shades of blue indicate the downregulation. For the human tumor gene expression analysis, microarray data set was selected from well-differentiated tumors of seven patients with 19- to 38-folds *ERG* overexpression (Shaheduzzaman *et al*., 2007). For assessing gene expression changes in VCaP cells by microarray, cells were transfected with ERG si and were analyzed 48 h posttransfection. The central node of combined ERG si and ERG+ tumor pathways highlights common changes in C-MYC (MYC) and PSA (KLK3) nodes. On the insert (upper left), inhibition of ERG protein expression in VCaP cells in response to various doses of ERG si 48 h post-transfection is shown. (**b**) *MYC* expression in response to ERG si inhibition was measured by QRT-PCR and western blots. Reduced recruitment of ERG to the *MYC* P2 promoter downstream ETS element was assessed at 48 h post-transfection by ChIP assay by using anti-ERG (ERG) antibody (Ab). IgG and input were used as controls. (**c**) PSA mRNA and protein expression were measured by QRT-PCR and western blot assays, respectively. Increased AR binding to the PSA enhancer (ARE) and decreased ERG recruitment to the overlapping ETS cognate element was measured by ChIP assay 48 h after transfection. On the right panel, VCaP cells at 9 days after treatment with control NT (upper photographs) and ERG si (lower photographs) were immunostained for CK8/18 (extreme left), PSA (second column from left) and DNA (third column from left) and the separate images merged (extreme right column). The scale bar is 25 μm. (**d**) SLC45A3 (prostein) expression in ERG si-transfected VCaP cells was measured by western blots (upper left). Recruitment of AR and ERG to the SLC45A3 promoter upstream ARE and ETS elements was assessed by ChIP assay 48 h post-transfection (lower left). On the table matrix representation of *TMPRSS2-ERG* expression and SLC45A3 (SLC) immunostaining in prostate tumors of 26 patients (CN, case number) is shown. Sections of whole mounted radical prostatectomy specimens were assessed by immunohistochemistry with anti- SLC45A3 (SLC) antibody. Strong (solid) or weak (gray) staining was correlated with the presence (solid) or absence (hollow) of TMPRSS2-ERG gene fusion transcripts in the same tumors. (**e**) VCaP cells were transfected with 50 nM of NT or ERG si or MYC si or the combination of 25 nM of ERG si and 25 nM of MYC si. Cell morphology at day 8 is shown. Cell lysates were prepared and assayed by immunoblots with anti- C-MYC or PSA or SLC45A3 (prostein) antibodies. Tubulin was used as the control. ERG-MYC correlation analysis was performed by assessing quantitative gene expression data of *C-MYC* and *ERG* or *C-MYC* and *PCA3* from 37 laser capture microdissected human tumors. *R* and *P*-values are shown in the table. AR, androgen receptor; CaP, prostate cancer; ChIP, chromatin immunoprecipitation; ERG, *ETS*-related gene; IgG, immunoglobulin G; NT, nontargeting siRNA; QRT-PCR, quantitative real time PCR.

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References

Baldus CD, Burmeister T, Martus P, Schwartz S, Gokbuget N, Bloomfield CD *et al*. (2006). High expression of the ETS transcription factor ERG predicts adverse outcome in acute T-lymphoblastic leukemia in adults. *J Clin Oncol* **24**: 4714–4720.

De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG *et al*. (2007). Inflammation in prostate carcinogenesis. *Nat Rev Cancer* **7**: 256–269.

Demichelis F, Rubin MA. (2007). TMPRSS2-ETS fusion prostate cancer: biological and clinical implications. *J Clin Pathol* **60**: 1185–1186.

Furusato B, Gao CL, Ravindranath L, Chen Y, Cullen J, McLeod DG *et al*. (2008). Mapping of TMPRSS2-ERG fusions in the context of multi-focal prostate cancer. *Mod Pathol* **21**: 67–75.

Iljin K, Wolf M, Edgren H, Gupta S, Kilpinen S, Skotheim RI *et al*. (2006). TMPRSS2 fusions with oncogenic ETS factors in prostate cancer involve unbalanced genomic rearrangements and are associated with HDAC1 and epigenetic reprogramming. *Cancer Res* **66**: 10242–10246.

Klezovitch O, Risk M, Coleman I, Lucas JM, Null M, True LD *et al*. (2008). A causal role for ERG in neoplastic transformation of prostate epithelium. *Proc Natl Acad Sci USA* **105**: 2105–2110.

Korenchuk S, Lehr JE, Mclean L, Lee YG, Whitney S, Vessella R *et al*. (2001). VCaP, a cell-based model system of human prostate cancer. *In Vivo* **15**: 163–168.

Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD *et al*. (1999). Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res* **59**: 4180–4184.

Marcucci G, Mrozek K, Bloomfield CD. (2005). Molecular heterogeneity and prognostic biomarkers in adults with acute myeloid leukemia and normal cytogenetics. *Curr Opin Hematol* **12**: 68–75.

Massie CE, Adryan B, Barbosa-Morais NL, Lynch AG, Tran MG, Neal DE *et al*. (2007). New androgen receptor genomic targets show an interaction with the ETS1 transcription factor. *EMBO Rep* **8**: 871–878.

Meulia T, Krumm A, Spencer C, Groudine M. (1992). Sequences in the human c-myc P2 promoter affect the elongation and premature termination of transcripts initiated from the upstream P1 promoter. *Mol Cell Biol* **12**: 4590–4600.

Oettgen P, Finger E, Sun Z, Akbarali Y, Thamrongsak U, Boltax J *et al*. (2000). PDEF, a novel prostate epithelium-specific ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. *J Biol Chem* **275**: 1216–1225.

Owczarek CM, Portbury KJ, Hardy MP, O'Leary DA, Kudoh J, Shibuya K *et al*. (2004). Detailed mapping of the ERG-ETS2 interval of human chromosome 21 and comparison with the region of conserved synteny on mouse chromosome 16. *Gene* **324**: 65–77.

Petrovics G, Liu A, Shaheduzzaman S, Furasato B, Sun C, Chen Y *et al*. (2005). Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. *Oncogene* **24**: 3847–3852.

Scherf M, Epple A, Werner T. (2005). The next generation of literature analysis: integration of genomic analysis into text mining. *Brief Bioinform* **6**: 287–297.

Seth A, Watson DK. (2005). ETS transcription factors and their emerging roles in human cancer. *Eur J Cancer* **41**: 2462–2478.

Shaheduzzaman S, Vishwanath A, Furusato B, Cullen J, Chen Y, Banez L *et al*. (2007). Silencing of lactotransferrin expression by methylation in prostate cancer progression. *Cancer Biol Ther* **6**: 1–8.

Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS *et al*. (2007). Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* **448**: 595–599.

Tomlins SA, Laxman B, Varambally S, Cao X, Yu J, Helgeson BE *et al*. (2008). Role of the TMPRSS2-ERG gene fusion in prostate cancer. *Neoplasia* **10**: 177–188.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW *et al*. (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310**: 644–648.

Turner DP, Watson DK. (2008). ETS transcription factors: oncogenes and tumor suppressor genes as therapeutic targets for prostate cancer. *Expert Rev Anticancer Ther* **8**: 33–42.

Witte JS. (2007). Multiple prostate cancer risk variants on 8q24. *Nat Genet* **39**: 579–580.

Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)