

Supplementary Material

Figure S1. AR induces chromosome conformational change juxtaposing *TMPRSS2* and *ERG*

A. Noncrosslinked LNCaP nuclear extract was digested with EcoRI followed by ligation using T4 DNA ligase. B. Conventional PCR was performed at T_m 55°C with primers adjacent to the EcoRI sites along chromosome 21q22.3 including *TMPRSS2* as targets and *ERG* primer as bait. The gel image shows equimolar amounts of ligated fragments. C. Compared to vehicle (EtOH), 1 hour exposure to 100 nM DHT induced chromosome conformation change as shown by the 3C assay, which detected colocalization of *TMPRSS2* and *ERG* DNA fragments in LNCaP cells. The schematic below the graph shows the interaction on chromosome 21q22.3 demonstrated by the assay.

Figure S2. *TMPRSS2-ERG* rearrangement in different NKX3.1-expressing cell lines. A. LNCaP cells expressing three different siRNA constructs were assayed for *TMPRSS2-ERG* rearrangements after 24 hour exposure to etoposide and 1-hr treatment with 100 nM DHT. LNCaP(si3098) is engineered to knock down NKX3.1 expression and has been previously described (Bowen and Gelmann, 2010). B. NKX3.1 rescue of *TMPRSS2-ERG* gene rearrangement in LNCaP(si471) cells exposed to retroviral vectors with the indicated gene inserts. C. NKX3.1 knockdown affects *TMPRSS2-ERG* rearrangement in LAPC4 cells. The left panel shows quantitation by FISH assay of LAPC4 cells transfected with either control or NKX3.1 siRNA (smart pool siRNA from Dharmacon). After 3 days cells were exposed to 100 nM DHT +/- 10µM etoposide for 24 hours. The right panel shows an immunoblot for the indicated proteins at the time of cell collection

Figure S3. NKX3.1 has no effects on *TMPRSS2-ERG* gene rearrangement in the absence of AR

A. PC-3 cells are known to harbor a subset of AR positive cells (1) confirmed by immunoblots

staining of native PC-3 cells, but PC-3 cells engineered to express NKX3.1 are an AR-negative clonal derivative. B. FISH proximity assay with 3' probe (red dot) for *ERG* and 5' probe (green dot) for *TMPRSS2* showed no juxtaposition in both PC-3 and PC-3(NKX3.1) cells after exposure to 100 nM DHT for 1 hr. C. Quantitation of FISH results from analysis shown in panel B. There was no difference in colocalization frequency between vehicle (EtOH) and 100 nM DHT treatments in both PC-3 and PC-3(NKX3.1) cells. D. Quantitative PCR analysis for *TMPRSS2-ERG* fusion transcripts in cells exposed to DHT, etoposide, and doxorubicin as shown. E. PC-3 cells transfected with pcDNA3 empty expression vector or the vector with the indicated constructs were cultured in IMEM with CCS and exposed to 100 nM DHT +/- 10 μ M etoposide. After 24 hr cells were harvested for immunoblotting and FISH analysis (shown on histogram). For each experimental condition the protein level by immunoblotting is shown directly below the quantitation of *TMPRSS2-ERG* rearrangement by FISH.

Figure S4. NKX3.1 expression and *TMPRSS2-ERG* rearrangement are related to histologic grade.

A. Eighty-six primary prostate cancer samples described in Figure 4 were stained for NKX3.1 and histone H1 expression. The relationship between NKX3.1 expression measured by Image J and Gleason is shown. B. Mean NKX3.1 levels are shown for tissue sections with Gleason grades 3, 4, and 5. Red labels show mean NKX3.1 expression levels. Differences between values by t-test are shown with brackets. C. Correlation of Gleason grade with incidence of *TMPRSS2-ERG* is shown. Chi squared test for independence was significant, $p=0.00026$. Differences between the incidence of gene rearrangement for each Gleason grade was significant by t-test ($p<0.001$).

Figure S5. AR and NKX3.1 are recruited to break sites on *TMPRSS2* and *ERG* genes. A. LNCaP cells in 100 nM DHT were exposed to 10 μ M etoposide for 4 hours. Cells were then

pulsed with BrdU for 1 hr. Cells were processed for chromatin immunoprecipitation with the antibodies indicated. The presence of BrdU at the target sites indicates the presence of DNA breakage that was augmented by etoposide. Recruitment of AR and NKX3.1 to various sites was also enhanced by the effect of etoposide.

The map at the top shows candidate sites in *TMPRSS2* and *ERG* for involvement in gene fusion. These sites were targeted for ChIP analysis. Site II in *TMPRSS2* and sites II, III, and IV of *ERG* corresponding respectively to regions F, C, and A as described by Lin et al., 2009. B. Specificity of breakpoint choice was tested by identifying adjacent candidate ARE and NKE sites for ChIP-reChIP analysis. ChIP-reChIP was performed with DNA obtained from Fig. 5B by first round immunoprecipitation with AR antibody and second round immunoprecipitation with either NKX3.1 antibody or IgG. In the histogram fold change shows the relative induction of quantitative PCR from immunoprecipitation with NKX3.1 antibody over IgG control. Real-time PCR was done with primers shown in the table. C. LNCaP cells treated as shown were subjected to ChIP-reChIP assay with primers flanking ERG II,III and ERG IV break sites. Cell extracts went through two rounds of chromosome immunoprecipitation and real time PCR quantification. Fold change was calculated relative to the EtOH control.

Figure S6. NKX3.1 facilitates assembly of OGG1 at *ERG* gene break site

Top two panels show ChIP assays done at time points after addition of 10nM DHT to derivative LNCaP cells that had been treated with etoposide for four hours or irradiated 4 hours prior to DHT exposure. The lower panel shows the effect of 25 μ M enzalutamide added at $t = -30$ minutes on OGG1 assembly in LNCaP(siLuc) cells in comparison to the effect of NKX3.1 knockdown. See Table S5 for p-values of ANOVA comparing data for LNCaP(siLuc) and LNCaP(si471) for each antibody.

Figure S7. Transcription is not required for *TMPRSS2-ERG* juxtaposition or rearrangement.

A. LNCaP(siLuc) and LNCaP(si471) cells were pretreated with 5 μ M α -amanitin for 2 hour, followed by addition of 100 nM DHT for the indicated times. mRNA was isolated with the RNeasy mini kit (QIAGEN) and cDNA was generated with Superscript TM First-Strand Synthesis System for RT-PCR (Invitrogen). QPCR was performed with primers for *TMPRSS2*, forward: 5' AAT CGG TGT GTT CGC CTC TAC3'; reverse: 5' GCG GCT GTC ACG ATC C 3'. B. LNCaP(siLuc) and LNCaP(si471) cells cultured in medium with 10% CCS for 3 days were exposed to 5 μ M α -amanitin for 2 hour for synchronization, followed by subsequent cultured in medium with 10% CCS for 1 hour, and additional exposure to 5 μ M α -amanitin for 30 min. Cells were then exposed to 100 nM DHT. To assay gene rearrangement cells were treated with 100 μ M etoposide after DHT addition. FISH juxtaposition and rearrangement assays were performed as described above.

Reference List

- (1) Tilley WD, Bentel JM, Aspinall JO, Hall RE, Horsfall DJ. Evidence for a novel mechanism of androgen resistance in the human prostate cancer cell line, PC-3. *Steroids* 1995;60:180-6.