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Molecular Characterization of *TMPRSS2–ERG* Gene Fusion in the NCI-H660 Prostate Cancer Cell Line: A New Perspective for an Old Model[1*]

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Abstract

Recent studies have established that a significant fraction of prostate cancers harbor a signature gene fusion between the 5′ region of androgen-regulated *TMPRSS2* and an *ETS* family transcription factor, most commonly *ERG*. Studies on the molecular mechanisms and functional consequences of this important chromosomal rearrangement are currently limited to the VCaP cell line derived from a vertebral bone metastasis of a hormone-refractory prostate tumor. Here we report on the NCI-H660 cell line, derived from a metastatic site of an extrapulmonary small cell carcinoma arising from the prostate. NCI-H660 harbors *TMPRSS2–ERG* fusion with a homozygous intronic deletion between *TMPRSS2* and *ERG*. We demonstrate this by real-time quantitative polymerase chain reaction, a two-stage dual-color interphase fluorescence *in situ* hybridization (FISH) assay testing for *TMPRSS2* and *ERG* break-aparts, and single-nucleotide polymorphism oligonucleotide arrays. The deletion is consistent with the common intronic deletion found on chromosome 21q22.2–3 in human prostate cancer samples. We demonstrate the physical juxtaposition of *TMPRSS2* and *ERG* on the DNA level by fiber FISH. The androgen receptor–negative NCI-H660 cell line expresses *ERG* in an androgen-independent fashion. This *in vitro* model system has the potential to provide important pathobiologic insights into *TMPRSS2–ERG* fusion prostate cancer.

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Introduction

Recent work demonstrates that nearly half of prostate-specific antigen (*PSA*)–screened prostate cancers harbor *TMPRSS2-ETS* fusions [1–3]. *ERG* (21q22.3), *ETV1* (7p21.2), or *ETV4* (17q21) is activated by a genetic rearrangement that fuses the 3′ end of either gene to the 5′ end of androgen-regulated *TMPRSS2* (21q22.2). This generates an androgen-responsive fusion oncoprotein, leading to overexpression of the respective *ETS* family member [1,2]. *TMPRSS2-ETS* fusion is the first mechanistic explanation for dominant oncogene activation in a significant fraction of prostate cancers. *TMPRSS2–ERG* fusion is the most common rearrangement, which is significantly associated with prostate cancer–specific mortality [4]. Therefore, gene fusion status represents an important subclassification of prostate cancer from a biologic and a clinical standpoint.

Abbreviations: AR, androgen receptor; FISH, fluorescence *in situ* hybridization; PrSC, prostate stromal cell; *PSA*, prostate-specific antigen; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription–polymerase chain reaction; SNP, single-nucleotide polymorphism

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TMPRSS2–ERG fusion occurs most frequently through intronic deletion between *TMPRSS2* and *ERG* on 21q22.2–3 [3]. None of the classic prostate carcinoma cell lines (DU145, PC3, or LNCaP) harbors *TMPRSS2–ETS* fusions. Understanding the functional biology of underlying gene fusion is currently limited to the androgen-dependent VCaP cell line that was established from the vertebral bone metastasis of a hormone-refractory prostate tumor [5]. The VCaP cell line represents an *in vitro* model for *TMPRSS2–ERG* gene fusion but has unusual genomic features in that it shows copy number gain on chromosome 21q, which has not been observed in > 300 prostate cancer samples studied to date [1,3,4]. In addition, VCaP has at least one normal *TMPRSS2* and *ERG* gene, which makes it difficult to study *TMPRSS2–ERG* rearrangement *in vitro* in the presence of wild-type copies of both fusion partners. In VCaP, fusion transcripts encode *TMPRSS2* fused to exon 4 of *ERG*, corresponding to the *ERGa* described by Tomlin et al. [1].

Here we present the androgen-independent NCI-H660 cell line as a novel *in vitro* model for prostate cancer. NCI-H660 cells harbor *TMPRSS2–ERG* gene fusion through a common intronic deletion on chromosome 21 in homozygous form. We propose it as a potential cell culture system to study and manipulate *TMPRSS2–ERG* fusion *in vitro*.

Materials and Methods

Cell Lines and Xenografts

The prostate cancer cell lines NCI-H660, PC3, DU145, LNCaP, 22Rv1, and CA-HPV-10 were purchased from the American Type Culture Collection (Manassas, VA) and maintained according to the provider's instructions. The VCaP cell line is derived from a vertebral metastatic lesion as part of the Rapid Autopsy Program at the University of Michigan [5]. Normal prostate stromal cells (PrSCs) were obtained from Cambrex Bio Science (East Rutherford, NJ).

LuCaP 23.1, 35, 49, 58, 73, 81, 86.2, 92.1, 93, 96, and 115 have been established by R.L.V. and have been previously described [3]. Of note, LuCaP 49 (established from an omental fat metastasis) and LuCaP 93 are hormone-insensitive [androgen receptor (AR)–negative] small cell prostate cancers with a neuroendocrine phenotype.

Tissue Samples

Lymph node specimens were collected from the radical prostatectomy series at the University of Ulm (Ulm, Germany) [6]. Hormone-refractory metastatic samples were part of the Rapid Autopsy Program for prostate cancer (University of Michigan Specialized Program of Research Excellence) and consist of histologically confirmed prostatic tumors involving solid organs (e.g., liver and lung) or distant lymph nodes [7,8]. All samples were collected as part of institutional review board protocols at each respective institution.

Polymerase Chain Reaction (PCR) and Real-Time Quantitative PCR (qPCR) for TMPRSS2–ERG *Fusion Transcripts*

We used the following primers for the detection of *TMPRSS2–ERG*, as described by Tomlins et al. [1]: *TMPRSS2:ERG*_f and *TMPRSS2:ERG*_r as reverse primers in *ERG* exon 4 for real-time qPCR, and exon 5–6_r as reverse primer in *ERG* exon 6 for reverse transcription (RT) PCR. Exon 5–6_f and exon 5–6_r primers were used for the detection of *ERG*. Total RNA was isolated with Trizol (Invitrogen, Carlsbad, CA) and reverse-transcribed using *TaqMan* reverse transcription reagent in the presence of random hexamers and oligo dT primers (Applied Biosystems, Foster City, CA).

RT-PCR amplifications were performed using 30 ng of cDNA as template in a final volume of 50 μl using the Platinum *Taq* DNA Polymerase kit (Invitrogen) at an annealing temperature of 63°C. Amplified PCR fragments were cloned and sequenced as described [9].

For qPCR, the amount of each target gene relative to *GAPDH* was determined for each sample using the comparative threshold cycle method (Applied Biosystems), as described [1]. For androgen stimulation experiments, 2× Power SYBR Green Master Mix (Applied Biosystems) and 25 ng of both forward and reverse primers were used for *ERG*, *PSA*, and *HMBS* [10]. A 2× *TaqMan* Universal PCR Master Mix, a final concentration of 900 nM forward and reverse primers, and a 250-nM probe were used for *TaqMan TMPRSS2–ERGa* detection using the following primers and probe: TM_ERGa forward, CTGGAGCGCGGCAGGAA; TM_ERGa reverse, CCGTAGGCACACTCAAACAACGA; TM_ERGa probe, TTATCAGTTGTGAGTGAGGAC.

Determining TMPRSS2–ERG *Fusion and Deletion Status Using a Two-Stage Dual-Color Interphase Fluorescence* In Situ *Hybridization (FISH) Assay*

To assess the *TMPRSS2–ETS* fusion status, we applied a two-stage dual-color break-apart FISH assay. First, we identified the rearrangement of *TMPRSS2*. Second, we tested for *ERG* break-apart to determine if *ERG* was the fusion partner for *TMPRSS2*. The *ERG* break-apart assay has been described [3]. The *TMPRSS2* break-apart assay consisted of the biotin-16-dUTP–labeled BAC clone RP11-662D5 and the digoxigenin-11-dUTP–labeled BAC clone RP11-260O11. These probes span the neighboring centromeric and telomeric regions of the *TMPRSS2* locus, respectively. Samples were mounted on Prolong Gold Antifade Reagent with DAPI (Invitrogen) and analyzed under a 60× oil immersion objective using an Olympus BX-51 (Olympus America Inc., Center Valley, PA) fluorescence microscope equipped with appropriate filters, a charge-coupled device camera, and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA).

Fiber FISH

DNA was isolated from ~ 5×10^6 cells followed by 24 hours of incubation with 300 μl of Puregene Cell Lysis Solution (Gentra Systems, Minneapolis, MN). About 10 μl of

lysed DNA solution was stretched on a poly-L-lysine–coated slide with a coverslip, followed by fixation in ethanol [11]. The slides were hybridized for 16 hours at 37°C with probes specific to *TMPRSS2* (RP11-120C17 labeled with biotin-16-dUTP; red) and *ERG* (RP11-476D17 labeled with digoxigenin-11-dUTP; green).

Single-Nucleotide Polymorphism (SNP) Array Analysis

Genomic DNA was isolated from a total of 25 samples (cell lines, punch biopsies of xenografts, lymph nodes, and visceral organ metastases) according to standard procedures. DNA was hybridized according to the manufacturer's protocol (Affymetrix, Santa Clara, CA) [12], either to Affymetrix 10K or 50K Xba SNP arrays. Arrays were scanned with a GeneChip Scanner 3000 (Affymetrix). Data were analyzed using the informatics platform dChipSNP [13]. For each array type, preprocessing included array data normalization to a baseline array using a set of invariant probes and subsequent processing to obtain single intensity values for each SNP on each sample using a model-based (PM/MM) method [14]. We then merged the datasets on common SNPs (both represented on the 10K and the 50K arrays), ending with a set of ~ 8K SNPs.

Androgen Stimulation

Treatments with the synthetic androgen R1881 and the AR antagonist flutamide were performed as described [1].

Results

The NCI-H660 cell line was originally described as a small cell lung carcinoma [15]. Subsequently, it was correctly identified as an extrapulmonary small cell carcinoma originating from the prostate gland [16,17]. The line is derived from a lymph node metastasis taken from a patient before therapy. The patient was a 63-year-old white male who was diagnosed with extrapulmonary small cell carcinoma in 1983. He died from this cancer 18 days after the initial diagnosis and was autopsied with multiple metastatic sites (Bruce E. Johnson, personal communication, Dana Farber Cancer Institute, Boston, MA). NCI-H660 has been reported to be AR-negative by Western blot analysis, and its growth has been reported to be androgen-independent [18]. When we tested the NCI-H660 cell line for *TMPRSS2–ERG* fusion, we detected two RT-PCR products (Figure 1*A*). Cloning and sequencing identified the transcripts as *ERGa*, a previously reported fusion type [1], plus an additional isoform containing the first two exons of *TMPRSS2*, juxtaposed to exon 4 of *ERG* (fusion type VI according to Wang et al. [9]) (Figure 1*B*). The two-sequence verified *TMPRSS2–ERG* fusion transcripts in NCI-H660 are identical to the ones found in human tumor samples [9]. Real-time qPCR demonstrated expression of *TMPRSS2–ERG* gene fusion in the NCI-H660 cell line, leading to about a 6000-fold elevated *ERG* expression compared to normal PrSCs (Figure 1*C*). For comparison, the VCaP cell line shows about 50,000 times higher

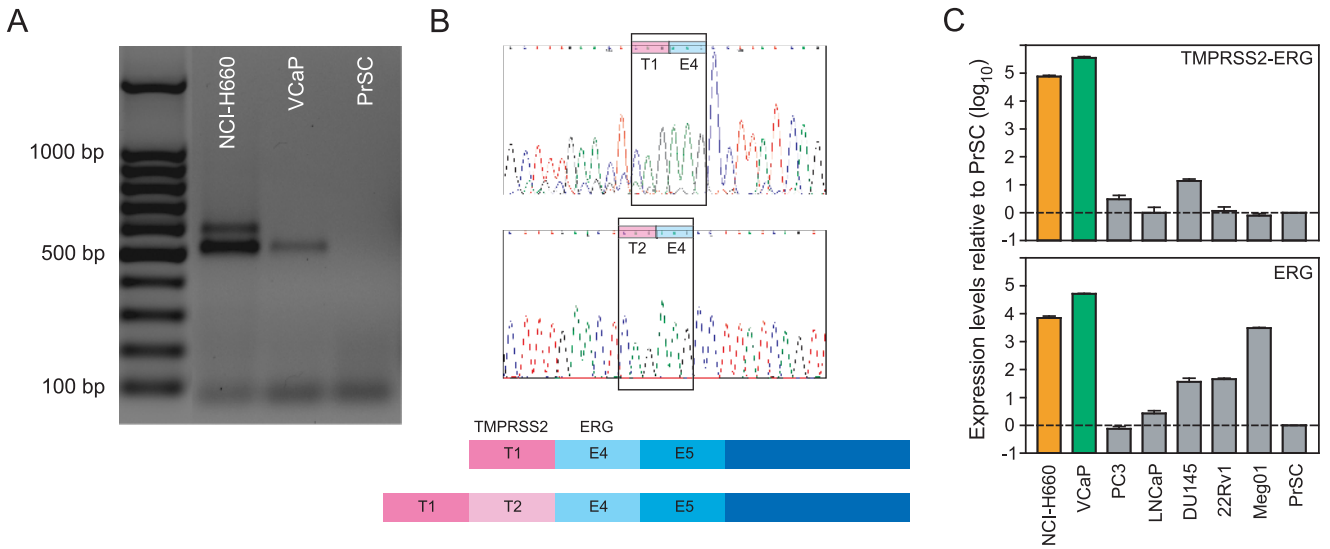

Figure 1. *Characterization of* TMPRSS2–ERG *fusion and* ERG *expression in the NCI-H660 prostate cancer cell line. (A) RT-PCR using a forward primer in exon 1 of* TMPRSS2 *and a reverse primer in exon 6 of* ERG *revealed two transcripts of* TMPRSS2–ERG *fusion in NCI-H660 (first lane). For comparison, VCaP expressed only one fusion transcript (second lane), whereas normal PrSCs did not harbor* TMPRSS2–ERG *fusion (third lane). (B) The identity of the fusion transcripts was verified by sequencing. The shorter transcript consisted of* TMPRSS2 *exon 1 (T1) fused to* ERG *exon 4 (E4). This transcript was also found in the VCaP cell line. The longer transcript was identified as* TMPRSS2 *exons 1 and 2 (T1 and T2), fused to* ERG *exon 4 (E4). Upper panel: sequencing profiles of T1-E4 and T2-E4 transcripts. Lower panel: corresponding schemes of the two fusion types. (C) Real-time qPCR for* TMPRSS2–ERG *expression (upper panel) and* ERG *expression (lower panel) on several cancer cell lines. The standard (PrSCs) was expressed as 1 (dashed line). Orange bars correspond to* TMPRSS2–ERG *fusion through deletion in NCI-H660, leading to a ~ 6 × 10^3–fold overexpression of* ERG. *Green bars correspond to* TMPRSS2–ERG *fusion in VCaP, leading to a ~ 5 × 10^4–fold overexpression of* ERG. *Grey bars correspond to the other prostate cancer cell lines tested (PC3, LNCaP, DU145, and 22Rv1) that do not harbor* TMPRSS2–ERG *fusion and show baseline or moderately elevated* ERG *expression. Meg01 is a leukemia cell line without* TMPRSS2–ERG *fusion, but with overexpression of* ERG *(~ 3 × 10^3–fold), which served as a positive control for* ERG *expression.*

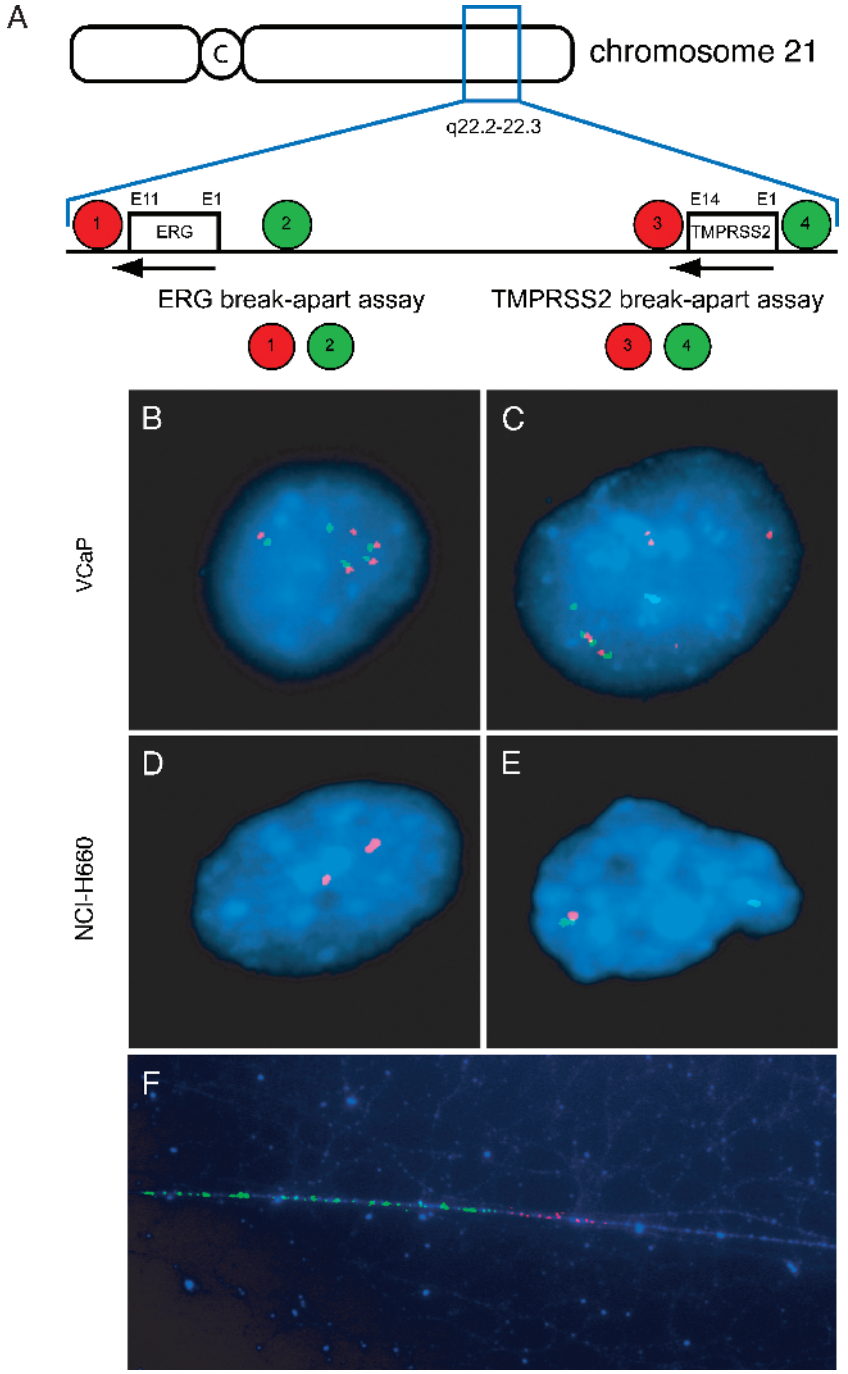

Figure 2. *Dual-color* TMPRSS2 *and* ERG *break-apart FISH assays for the detection of* TMPRSS2–ERG *fusion in NCI-H660 and VCaP. (A) The scheme of the dual-color* TMPRSS2 *and* ERG *break-apart FISH assays explains the detection of* TMPRSS2–ERG *fusion. The location of the genes is indicated relative to the chromosome (boxes); the orientation of the genes is indicated by arrows. C = centromere; E = exon. For both assays, the relative location of differentially labeled telomeric and centromeric BAC probes is indicated by colored circles, with the color indicating the probe color in (B), (C), (D), and (E), and with the number identifying the BACs as follows: 1 = RP11-24A11; 2 = RP11-372O17; 3 = RP11-662D5; 4 = RP11-260O11. (B) FISH image of a VCaP interphase nucleus assessed by dual-color* ERG *break-apart assay (as illustrated in A). The nucleus contains several juxtaposed red and green signals for amplified wild-type alleles, and separated red and green signals indicating* ERG *insertion. (C) FISH image of a VCaP interphase nucleus assessed by dual-color* TMPRSS2 *break-apart assay (as illustrated in A). The nucleus contains several juxtaposed red and green signals for amplified wild-type alleles, and separated red and green signals indicating* TMPRSS2 *insertion. (D) FISH image of an NCI-H660 interphase nucleus assessed by dual-color* ERG *break-apart assay (as illustrated in A). The nucleus shows only two single red centromeric signals but no telomeric green signals. This is indicative of a fusion of* TMPRSS2 *with* ERG *through homozygous deletion of the intergenic region. (E) FISH image of an NCI-H660 interphase nucleus assessed by dual-color* TMPRSS2 *break-apart assay (as illustrated in A). The nucleus contains a juxtaposed red and green signal for the wild-type allele, and a single green signal indicating the deletion of one centromeric red probe. This is indicative of the fusion of* TMPRSS2 *with* ERG *through deletion of the intergenic region. (F) High-resolution FISH on chromatin fibers of NCI-H660 assessed by a* TMPRSS2*-specific probe (RP11-121A5; red) and an* ERG*-specific probe (RP11-476D17; green) (as illustrated in Figure W1).*

ERG expression, and Meg01, a leukemia cell line with known overexpression of the *ERG* oncogene, expresses about 3000 times more *ERG* than PrSCs (Figure 1*C*). The prostate carcinoma cell lines PC3, LNCaP, and DU145, and the xenograft cell line 22Rv1 neither harbor *TMPRSS2–ERG* fusion nor show high *ERG* expression levels (Figure 1*C*).

To confirm *TMPRSS2–ERG* rearrangement in the NCI-H660 cell line, we used a two-stage dual-color break-apart FISH assay testing first for *TMPRSS2* and then for *ERG* break-apart as indirect evidence for *TMPRSS2–ERG* fusion. The indirect two-stage break-apart assessment is necessary because both genes are located so close to each other (a distance of only ~ 3 megabases) that a fusion cannot be observed directly by a conventional FISH fusion assay. Fusion through deletion of the region between *TMPRSS2* and *ERG* is indicated by loss of the probes located between the two genes [3]. FISH assays for *TMPRSS2* and *ERG* break-apart are presented schematically in Figure 2*A*. These assays demonstrated that NCI-H660 cells have biallelic *TMPRSS2–ERG* fusion through genomic deletion between *TMPRSS2* and *ERG* on chromosome 21q22.2–3 (Figure 2, *D* and *E*). The *ERG* break-apart assay showed that on the centromeric side of the deletion, both telomeric signals (probe 2, green; Figure 2*A*) are lost. The *TMPRSS2* break-apart assay showed one centromeric signal (probe 3, red; Figure 2*A*), consistent with one deletion reaching this area and one deletion occurring at a more centromeric region. This observation of a homozygous genomic loss between *TMPRSS2* and *ERG* on chromosome 21q22.2–3 has important implications for using this cell line as a prostate cancer model system, as no wild-type *ERG* allele is expressed. To date, we have observed one homozygous deletion in over 300 fusion-positive prostate cancers (unpublished observation). The fact that genomic deletions between *ERG* and *TMPRSS2* show different borders is in concordance with our earlier observation that the deleted region is variable in length [3]. For the first time, we were able to visualize the fusion of *TMPRSS2* and *ERG* on chromatin fibers of NCI-H660 cells by a high-resolution fiber FISH approach [11] using probes spanning specifically both loci (Figures 2*F* and W1).

We then used oligonucleotide SNP arrays to further characterize the NCI-H660 cell line and to compare it with other cell lines, xenografts, and prostate cancer samples, including

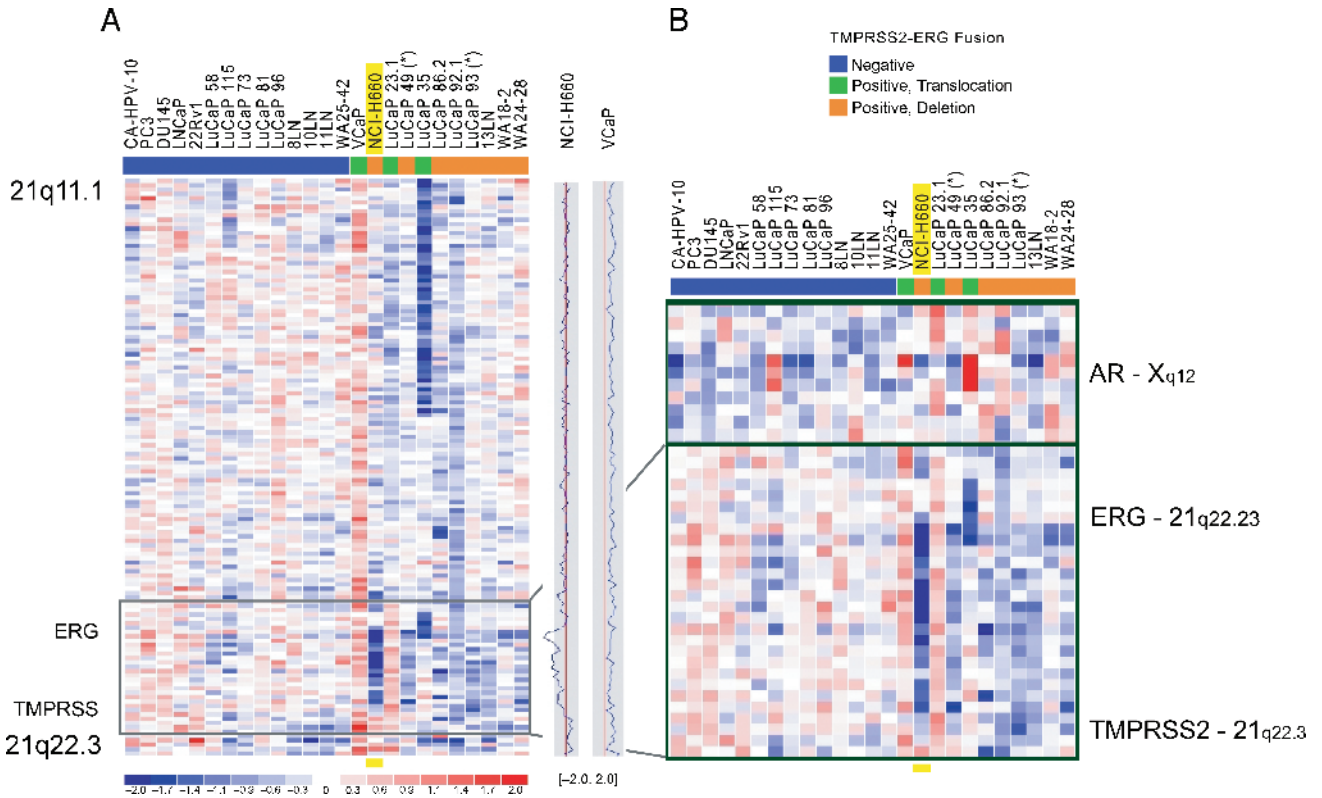

Figure 3. *Genomic loss between* TMPRSS2 *and* ERG *on chromosome 21q22.2–3 in prostate cancer cell lines, xenografts, and metastatic prostate cancer samples. 8K SNP data on a panel of 25 prostate cancer samples revealed genomic deletion between* TMPRSS2 *and* ERG *(21q22.2–3) in a subset of samples. (A) Twenty-five prostate cancer samples, including 7 cell lines, 11 xenografts, and 7 prostate cancer metastases, were analyzed for their* TMPRSS2–ERG *fusion status by qPCR and/or FISH and color-coded as described before [3] (blue, fusion-negative; green, fusion-positive through translocation; orange, fusion-positive through deletion). The plots on the right side of the panel represent the copy number ratio of the NCI-H660 and VCaP cell lines (vertical red lines represent baseline; no copy number variation). It is evident that VCaP shows copy number gain throughout the whole q arm of chromosome 21. (B) Magnification of the black framed box in (A), and status of the AR on chromosome X in these 25 samples. The blue signal in NCI-H660 corresponds to genomic copy number loss between* TMPRSS2 *and* ERG. *The strong intensity of this signal is consistent with homozygous loss, as demonstrated by FISH. The boundaries of the intronic deletion of NCI-H660 are very similar to the deletions seen in xenografts and tissue samples. Toward the telomeric side of the deletion in NCI-H660, the signal intensity is weaker, confirming different lengths of the deletions seen by FISH. The androgen-independent NCI-H660 cell line shows a loss in the region of the AR, whereas VCaP, which is known to be androgen-responsive, shows genomic gain in this area.*

hormone-naïve and hormone-refractory metastases (Figure 3, *A* and *B*). We identified the loss of about three megabases of genomic material on SNPs located between *TMPRSS2* and *ERG* in the NCI-H660 cell line with a strong loss of signal intensity, corresponding to the homozygous deletion in these cells that was detected by FISH (Figure 2, *D* and *E*). Toward the telomeric side of the deletion in NCI-H660, the copy number ratio increases, suggesting that one allele is present in that area. This confirms the different lengths of the deletions that were detected by FISH. For comparison, the VCaP cell line showed significant copy number gain on chromosome 21q (Figure 3*A*). In agreement with SNP data, we found multiple signals for both wild-type *TMPRSS2* and wild-type *ERG* in VCaP nuclei using independent *TMPRSS2* and *ERG* break-apart FISH assays (Figure 2, *B* and *C*). Therefore, we conclude that the copy number gain on chromosome 21 leads to copy number gains of wild-type *TMPRSS2* and *ERG*. Interestingly, the two small cell prostate cancer xenografts included in this study (LuCaP 49 and LuCaP 93) both showed *TMPRSS2–ERG* rearrangement through genomic deletion (denoted by * in Figure 3, *A* and *B*).

Because NCI-H660 is derived from an androgen-independent small cell prostate tumor and was shown to be negative for AR on both transcript and protein levels [18], we also analyzed the AR locus on chromosome X for all samples (Figure 3*B*). NCI-H660 had a loss in that region, as most of the other xenografts and tissue samples in that panel. SNP data confirmed the negative AR status of this cell line.

To investigate whether *TMPRSS2–ERG* fusion results in the androgen regulation of *ERG* in AR-negative NCI-H660 cells, we assessed the expression of *ERG* by qPCR in androgen-treated NCI-H660 and VCaP cells. The androgen-sensitive VCaP cell line has been shown to respond to androgen stimulation with increased *ERG* expression sensitive to bicalutamide and flutamide [1] (Figure 4*A*). As expected, NCI-H660 does not respond to androgen stimulation with increased expression of *PSA* or *ERG*, nor is it sensitive to the AR antagonist flutamide (Figure 4*B*).

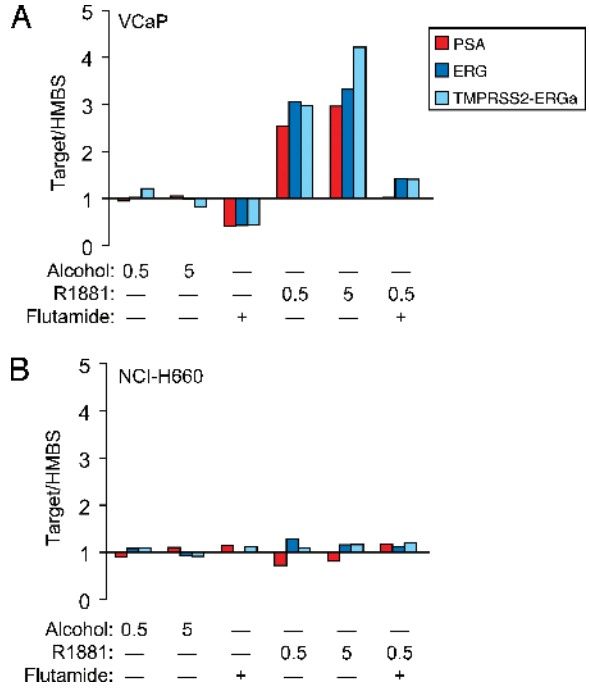

Figure 4. *The androgen stimulation of the VCaP and NCI-H660 prostate cancer cell lines carrying* TMPRSS2–ERG *fusion.* PSA *(red bars),* ERG *(exons 5 and 6; dark blue bars), and* TMPRSS–ERG *(*TMPRSS2 *exon 1 to* ERG *exon 4; light blue bars) expression relative to* HMBS *in androgen-sensitive VCaP cells (A) and androgen-insensitive NCI-H660 cells (B) was assessed by qPCR. Cell lines were incubated with vehicle or 10 μM of the AR antagonist flutamide for 2 hours before treatment for 24 hours with 0.5 or 5 nM of the synthetic androgen R1881 or vehicle, as indicated. Relative amounts of* PSA, ERG, *or* TMPRSS2–ERG *per* HMBS *were compared for VCaP and NCI-H660.*

Discussion

A significant percentage of prostate cancers express a signature gene fusion of the 5′ region of androgen-regulated *TMPRSS2* gene to an *ETS* family transcription factor, most commonly the *ERG* gene. This is the first demonstration of constitutive oncogene activation in prostate cancer. However, the functional consequences of this chromosomal rearrangement are difficult to predict and study. Reliable preclinical *in vitro* models, which use homogenous human cellular material that can be studied in large quantities over time, are needed. The NCI-H660 cell line is a novel *in vitro* model for prostate cancer that closely relates to the molecular signature of clinical cases. We demonstrate that NCI-H660 has an intronic deletion on chromosome 21 that was found to be the most common mechanism underlying *TMPRSS2–ERG* fusion [3]. This deletion is observed on both alleles as a homozygous loss. Therefore, NCI-H660 expresses neither *TMPRSS2* nor *ERG* in the native genomic context.

NCI-H660 does not express AR and is representative of *TMPRSS2–ERG*–positive androgen-independent prostate cancer. It has been shown that both *TMPRSS2–ERG* fusion and the associated deletion occur in androgen-independent prostate cancers and metastases that have no functional AR [3]. The membrane-bound serine protease *TMPRSS2* can be expressed in both androgen-dependent and androgen-independent tumors (i.e., this usually androgen-responsive gene can be uncoupled from androgen control) [19]. Androgen-independent expression of *TMPRSS2–ERG* in the NCI-H660 cell line is in line with this observation. NCI-H660 as an androgen-independent model system might facilitate the search for androgen-independent factors and pathways influencing *TMPRSS2–ERG* fusion. In contrast to a recent report showing that *TMPRSS2–ERG* gene fusion is not expressed in AR-negative prostate cancer specimens [20], we and others [21] have found *TMPRSS2–ERG* and *ERG* expression in the AR-negative NCI-H660 cell line. This implies that *TMPRSS2–ERG* fusion is constitutively stimulated in this androgen-independent prostate cancer cell line. Further efforts will be directed at investigating which factors can activate *TMPRSS2–ERG* fusion with subsequent *ERG* overexpression, using the NCI-H660 cell line as an *in vitro* model for *TMPRSS2–ERG* fusion through deletion.

In summary, the NCI-H660 cell line is a potent *in vitro* tool with a genotypic profile that reflects the *TMPRSS2–ERG*

rearrangement found in human prostate cancer. The NCI-H660 cell line should be a valuable model for prostate cancer and has the potential to provide important pathogenic insight and guidance for therapeutic strategies.

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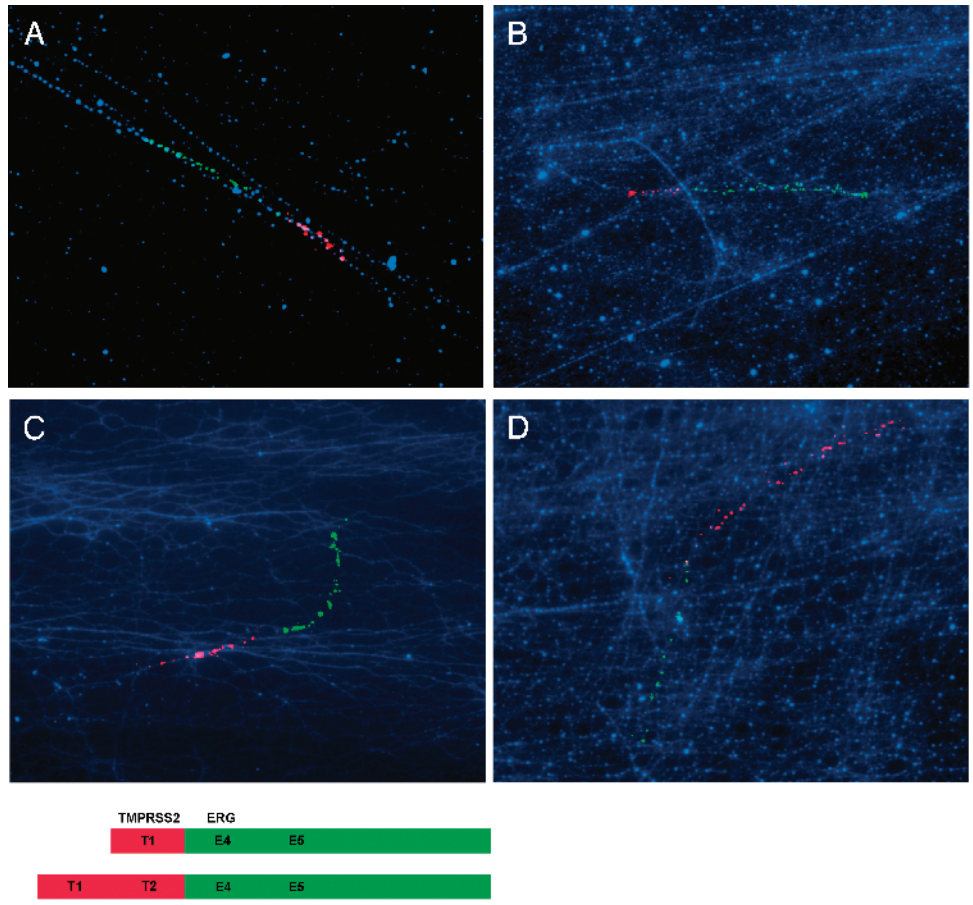

Figure W1. *FISH on DNA fibers to map* TMPRSS2–ERG *fusion in the NCI-H660 cell line. FISH was conducted on preparations of extended chromatin fibers of NCI-H660, resulting in a superior mapping resolution compared to interphase FISH. This is useful in mapping and assessing the relative length of adjacent DNA fragments. (A–D) Stretched NCI-H660 DNA fibers were hybridized with probes specific to* TMPRSS2 *(RP11-120C17; red) and* ERG *(RP11-476D17; green). The* TMPRSS2 *fusion partner is shorter relative to the* ERG *one (as seen from the illustration). Four representative pictures are shown. Immediately after exposure to light, DNA fibers start breaking at many locations. From each break, the two free ends of DNA spring back to the nearest attachment point and coil, leaving a gap between them. These ends resemble little beads and are thicker than the rest of the fiber. Therefore, DNA probes hybridize like arrays of dots ("beads on a string").*