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Technical Advance

Detection of *TMPRSS2-ERG* Translocations in Human Prostate Cancer by Expression Profiling Using GeneChip Human Exon 1.0 ST Arrays

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Translocation of *TMPRSS2* to the *ERG* gene, found in a high proportion of human prostate cancer, results in overexpression of the 3′-*ERG* sequences joined to the 5′-*TMPRSS2* promoter. The studies presented here were designed to test the ability of expression analysis on GeneChip Human Exon 1.0 ST arrays to detect 5′-*TMPRSS2-ERG*-3′ hybrid transcripts encoded by this translocation. Monitoring the relative expression of each *ERG* exon revealed altered transcription of the *ERG* gene in 15 of a series of 27 prostate cancer samples. In all cases, exons 4 to 11 exhibited enhanced expression compared with exons 2 and 3. This pattern of expression indicated that the most abundant hybrid transcripts involve fusions to *ERG* exon 4, and RT-PCR analyses confirmed the joining of *TMPRSS2* exon 1 to *ERG* exon 4 in all 15 cases. The exon expression patterns also indicated that *TMPRSS2-ERG* fusion transcripts commonly contain deletion of *ERG* exon 8. Analysis of gene-level data from the arrays allowed the identification of genes whose expression levels significantly correlated with the presence of the translocation. These studies demonstrate that expression analyses using exon arrays represent a valuable approach for detecting *ETS* gene translocation in prostate cancer, in parallel with analyses of gene expression profiles. ***(J Mol Diagn 2008, 10:50–57; DOI: 10.2353/jmoldx.2008.070085)***

Prostate cancer is the most common male cancer in the U.S. with 230,000 cases reported annually. Fusion of the *TMPRSS2* gene to the *ETS* transcription factor gene *ERG* has been reported in up to two-thirds of this disease.[1–8] Less frequently, *TMPRSS2* becomes fused to the related *ETS* genes *ETV1* and *ETV4*.[2–4] In RT-PCR-based studies using 5′-*TMPRSS2* primers and 3′-*ERG* primers, we and others[4–6] have shown that translocations of *TMPRSS2* to *ERG* can result in at least 17 distinctly structured fusion transcripts, which included fusions of exons 1, 2, and 3 of *TMPRSS2* to exons 2, 3, 4, 5 or 6, of *ERG*. However, the most commonly detected fusion transcripts involved joining of exon 1 of *TMPRSS2* to exons 4 or 5 of the *ERG* gene. As a consequence of this rearrangement, the 3′-exons of the *ERG* gene that become fused to 5′-*TMPRSS2* exons are expressed at very high levels in contrast to the untranslocated 5′-*ERG* exons that remain expressed at a low level.[4]

The GeneChip Human Exon 1.0 ST array consists of approximately 5.4 million 5-μm features (probes) grouped into 1.4 million probe sets examining more than 1 million exon clusters.[9] This array detects the levels of expression at both the gene and exon level. For each gene, the median number of probes is 30 to 40, usually distributed along the entire transcript length. For the *ERG* gene, exons 2 to 11 are represented by 19 probe sets. In

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principle, it should be possible to detect the presence and position of the *TMPRSS2-ERG* translocation in prostate cancer by monitoring the level of expression of each *ERG* exon using this array. Such information is potentially of considerable clinical interest because patients with cancers harboring *TMPRSS2-ERG* fusions appear to have a worse clinical outcome than those who lack this rearrangement.[5,7,10,11] Several expression signatures have been identified that appear to mark more aggressive disease,[12–14] so exon arrays additionally offer the potential for analysis of both translocation status and gene signature status in a single experiment. Through correlation of the expression levels of the translocated 3′-*ERG* exons to the levels of expression of other genes detected on the array, it should also be possible to find genes that represent downstream candidates for involvement in transformation after *ERG* overexpression. The study presented here is specifically designed to test the relative ability of expression analysis using GeneChip Human Exon 1.0 ST arrays compared with RT-PCR-based approaches to detect *TMPRSS2-ERG* translocations. Our results demonstrate the effectiveness of expression analysis using Exon 1.0 ST arrays for detecting *ERG* translocations and provide novel insights into the mechanism of development of human prostate cancer.

Materials and Methods

Clinical Samples

Fresh prostate cancer specimens were obtained from a systematic series of patients who had undergone a prostatectomy at the Royal Marsden Hospital NHS Foundation Trust and St Georges Hospital NHS Trust. Formalin-fixed, paraffin-embedded prostatic tissues from the same set of patients were also obtained from the pathology archives of these hospitals. None of these patients had evidence of metastasis at the time of diagnosis, and none received any previous treatment for prostate cancer. None of the patients had pathological stage >T3b, and all except one patient (PRC9) had prostate cancer with composite Gleason score ≤7. This study was approved by the Clinical Research and Ethics Committee at the Royal Marsden Hospital and Institute of Cancer Research.

Preparation of RNA

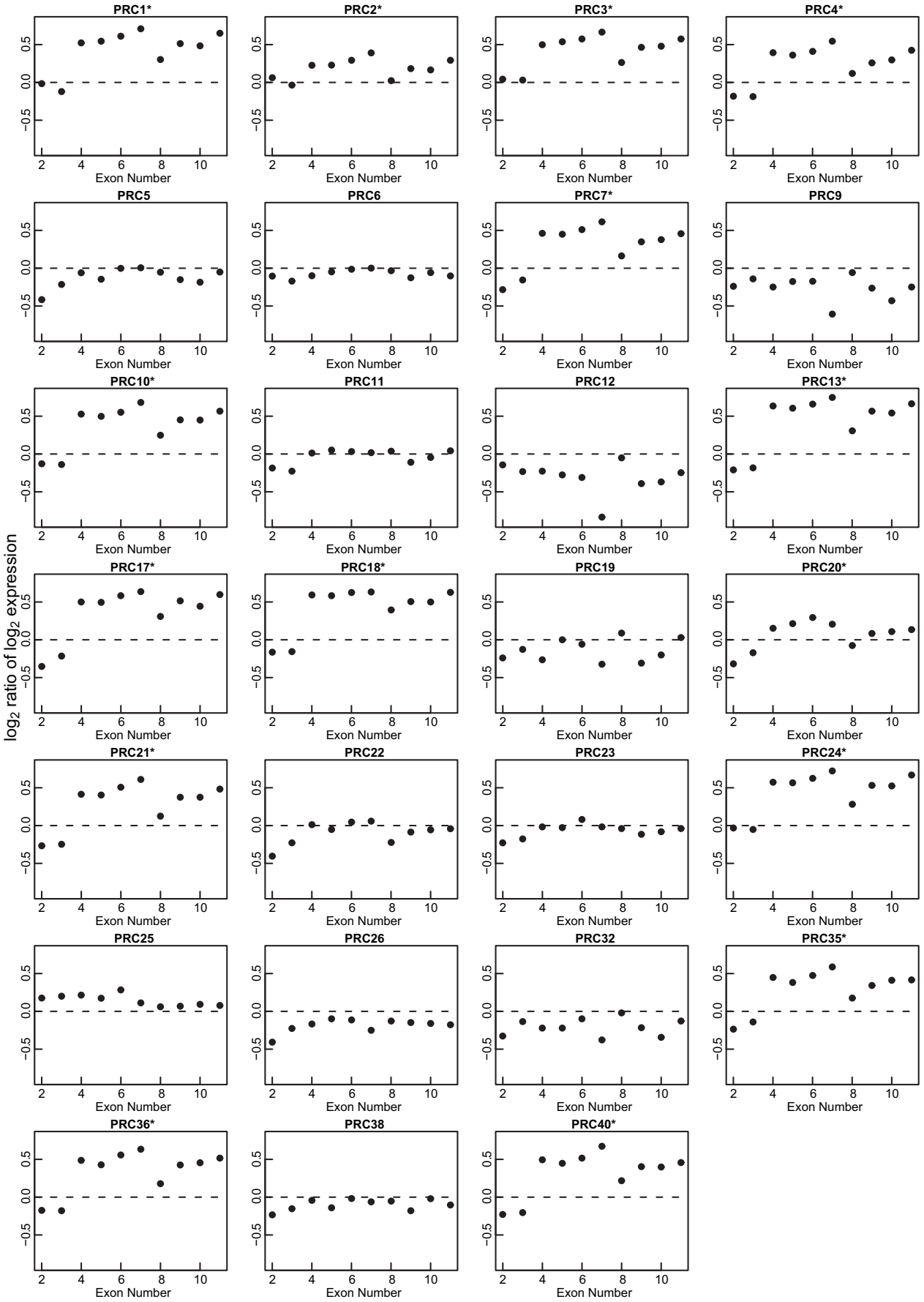
Frozen prostate slices were prepared and stored in RNA-*later* (Ambion Inc., Austin, TX) as described by Jhavar et al.[15] A small piece of tissue (~2 mm^3) containing areas of cancer and/or normal glands was identified within the frozen prostate slice with the help of H&E-stained whole-mount sections from the adjacent formalin-fixed slices. This piece of tissue was washed in ice-cold PBS for 2 minutes, embedded in OCT embedding medium (Raymond Lamb UK, Ltd., Eastbourne, East Sussex, UK) and frozen on dry ice. Frozen sections obtained from this tissue were stained with H&E and high molecular weight cytokeratin to confirm the presence and location of cancer and normal glands within it. Areas containing cancer and normal glands were then macrodissected from the OCT block using a fresh sterile scalpel blade and used for RNA extraction. Care was taken to avoid contamination by using a new scalpel blade for each macrodissected area. Care was also taken to keep the OCT block frozen during macrodissection. RNA was extracted from the tissue in TRIzol (Invitrogen Ltd., UK, Paisley, UK) and purified using the RNeasy MinEluteTM clean up kit (Qiagen Ltd., UK, Dorking, Surrey, UK) according to the manufacturer's instructions.

Expression Analysis Using Affymetrix GeneChip Exon 1.0 ST Arrays

Expression profiles were determined using Affymetrix 1.0 Human Exon ST arrays according to the manufacturer's instructions. The Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay was used to generate amplified and biotinylated sense-strand DNA targets from the entire expressed genome (1.5 μg of total RNA) without bias. Manufacturer's instructions were followed for the hybridization, washing, and scanning steps. Arrays were hybridized by rotating them at 60 rpm in the Affymetrix Gene Chip hybridization oven at 45°C for 16 hours. After hybridization, the arrays were washed in the Affymetrix GeneChip Fluidics station FS 450. The arrays were scanned using the Affymetrix Gene Chip scanner 3000 7G system. Gene- and exon-level expression signal estimates were derived from CEL files generated from Affymetrix GeneChip Exon 1.0 ST arrays using the multiarray analysis algorithm[16] implemented from the Affymetrix Power Tools software. Gene-level estimates were obtained using the "core" metaprobe list annotation release 21. Exon-level data were filtered to include only those probe sets in the core metaprobe list. All probe sets (25) that mapped within *ERG* were found and assigned to the appropriate exon identified from Ensembl. Those probe sets (six in all) that did not lie entirely within an exon were removed. For each probe set within an exon, the $\log_2$ ratio of expression level in the test sample to that observed in control non-neoplastic prostate epithelium was calculated. Where more than one value was available for a single exon, the median value was taken as the representative ratio for that exon. The control normal prostate expression signal was defined as the mean of the three non-neoplastic prostate epithelium samples.

Detection of TMPRSS2-ERG Fusions by RT-PCR

RT-PCRs were performed using the conditions described by Clark et al[6] using a forward *TMPRSS2* exon 1 primer, 5′-CAGGAGGCGGAGGCGGA-3′, and a reverse *ERG* exon 4 primer, 5′-GTAGGCACACTCAAACAACGACTGG-3′. The quality of all cDNAs that failed to give *TMPRSS2-ERG* PCR products was verified by PCR using *ETV1* primers (results not shown). Nested PCR was performed using 0.25 μl of primary PCR product and the following primers: *TMPRSS2*-Ex1-nest, 5′-GGAGCGC-


PRC1*
PRC2*
PRC3*
PRC4*
PRC5
PRC6
PRC7*
PRC9
PRC10*
PRC11
PRC12
PRC13*
PRC17*
PRC18*
PRC19
PRC20*
PRC21*
PRC22
PRC23
PRC24*
PRC25
PRC26
PRC32
PRC35*
PRC36*
PRC38
PRC40*
log2 ratio of log2 expression
Exon Number
−0.5
0.0
0.5
2
4
6
8
10

CGCCTGGAG-3′; and *ERG*-Ex4-nest, 5′-GTGAGGAC-CAGTCGTTGTTTGAGTG-3′. RT-PCR products were separated by electrophoresis in agarose gels and identified by DNA sequencing exactly as described previously.[6]

Statistical Analysis

Differential expression analysis was applied to the gene-level data with two groups divided based on whether an *ERG* break was detected. Linear models were determined for each transcript cluster (gene), and an estimate for the global variance was calculated by an empirical Bayes approach.[17] A moderated *t*-statistic was computed for each transcript cluster with the resulting *P* values adjusted for multiple testing using Benjamini and Hochberg's method to control the false discovery rate.[18] Those transcript clusters with an adjusted *P* value less than 0.05 were considered significantly differentially expressed between the two groups.

Results

Exon-Level Expression Profiling at the ERG Locus

GeneChip Human Exon 1.0 ST arrays were used to obtain genome-wide expression profiles from RNA samples prepared from 27 prostate cancer specimens and from 3 nonmalignant epithelial samples. A 3- to 5-mm-thick "research slice" was taken from individual prostatectomy specimens as described by Jhavar et al.[15] H&E-stained whole-mount sections from above and below this slice were subject to histopathological examination. When cancer was detected at a particular location in both of these sections, it was inferred that cancer was also present in the intervening research slice at the same position. For the *ERG* gene, expression levels could be determined for exons 2 to 11 that were represented by 19 probe sets. GeneChip Human Exon 1.0 ST arrays do not contain a 'core' probe set for *ERG* exon 1. For every cancer, we determined the ratio of the level of expression of each individual *ERG* exon probe set relative to its expression in the samples of non-neoplastic prostate epithelium.

Many cancer samples appeared to exhibit both increased expression of exons 4 to 11 relative to the expression observed for exons 2 and 3 (Figure 1) and increased expression of exons 4 to 11 relative to those observed in control non-neoplastic prostate epithelium. This pattern of expression is precisely what would be expected if the *TMPRSS2* sequences had become fused to *ERG* exon 4, causing increased expression of exon 4 and of the further of 3′- *ERG* exons. Increased expression invariably started at exon 4 in contrast to the diversity of possible *ERG* exon fusion positions previously detected in RT-PCR-based studies.[5,6] To confirm these alterations, we performed two statistical tests: to qualify as "altered," the expression profile needed to reach statistical significance in both tests. First, a two-sample *t*-test was used to determine whether the $\log_2$ expression ratio of the probe sets representing exons 4 to 11 were greater than the $\log_2$ expression ratios of probe sets representing exon 2 and 3. Second, a one-sample *t*-test was used to assess whether the $\log_2$ ratios of the probe sets representing exons 4 to 11 were greater than 0. Overall, 15 cancers gave a significant result ($P < 0.05$) in both tests (Table 1). Notably in all of the 15 cancers exhibiting this expression pattern, the 72-bp exon 8 of *ERG* appeared to be expressed at a lower level compared with exons 4 to 7 and 9 to 11, indicating that this exon can be spliced out in *ERG* fusion transcripts: the predicted structures of this alternatively spliced transcript is shown in Figure 2. For a single additional cancer, PRC25, a statistically significant increase in expression was observed across all *ERG* exons 2 to 11 (Figure 1; Table 1).

The remaining 11 samples failed to show any evidence of altered or increased expression (Figure 1). Within this category expression levels for exon 7 could be divided into two groups (for example, compare PRC6 and PRC9 in Figure 1). In one group, the expression of exon 7 was low compared with that observed for exons 2 to 6 and exons 8 to 11, suggesting that in some cancers, the predominant *ERG* transcripts had spliced out this 81-bp exon: the predicted structures of the alternatively spliced normal *ERG* transcripts are shown in Figure 2. *ERG* transcripts lacking exon 7 have previously been reported in several tissues including prostate (see annotated mRNA record at NCBI AceView; *http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?db=35g&c=MRNA&q=ERG.bAug05&h=_1_2007*).

RT-PCR Detection of the TMPRSS2:ERG Fusion Transcripts

RT-PCR protocols were also used to detect *TMPRSS2:ERG* fusions in the same 27 cancer RNA samples. First round PCR was performed using a 5′ primer from exon 1 of the *TMPRSS2* gene and a 3′ primer from exon 4 of the *ERG* gene. Samples that failed to yield PCR products in a first round of amplification were subject to nested PCR to see whether lower levels of hybrid transcript were present. Samples that failed to yield a visible PCR product after either a first-round or nested PCR reaction were

Figure 1. Expression data for *ERG* exons 2 to 11 in prostate cancers determined using GeneChip Human Exon 1.0 ST arrays. Cancers scored as having altered *ERG* expression profiles based on the statistical analyses presented in Table 1 are marked with an **asterisk**. In cancers designated as having altered *ERG* expression profiles, exon 8 of *ERG* was always expressed at lower levels than exons 4 to 7 and exons 9 to 11, indicating that this exon can be spliced out in rearranged *ERG* transcripts. In many of the cancers without altered *ERG* expression profiles, exon 7 was expressed at lower levels than exons 2 to 6 and exons 8 to 11, consistent with the presence of splice variants containing deletion of this 81-bp exon. The apparently depressed level of expression observed in some cancers (eg, PRC9) reflects the variation in observed level of ERG expression across the 27 cancers observed in this study, as illustrated in Figure 3, and was not believed to reflect an abnormal *ERG* expression pattern. For each probe set within an exon, the $\log_2$ ratio of the expression observed in the cancer to that observed in control non-neoplastic prostate epithelium was calculated. For each exon, the black dot represents the median of these values.

Table 1. Assessment of *ERG* Gene Alterations

Cancer	Discontinuous *P* value*	Up-regulation *P* value†	Expression on exon arrays‡	*TMPRSS2-ERG* by RT-PCR§	Fusion structure¶
PRC1	0.0029	1.17×10^{-10}	Y	Y	T1-E4
PRC2	0.0334	2.64×10^{-6}	Y	Y	T1-E4 T1-E2
PRC3	1.63×10^{-10}	7.73×10^{-11}	Y	Y	T1-E4
PRC4	5.58×10^{-10}	1.93×10^{-7}	Y	Y	T1-E4
PRC5	0.1204	0.9985	N	N	
PRC6	0.1587	1.0000	N	N	
PRC7	0.0077	1.06×10^{-8}	Y	Y	T1-E4 T2-E4 T1-E3
PRC9	0.8866	1.0000	N	N	
PRC10	4.99×10^{-12}	2.17×10^{-10}	Y	Y	T1-E4
PRC11	0.0026	0.0905	N	Y	*T1-E4*‖
PRC12	0.9257	1.0000	N	N	
PRC13	3.64×10^{-13}	1.07×10^{-11}	Y	Y	T1-E4 T2-E4 T1-E3
PRC17	0.0109	8.22×10^{-12}	Y	Y	T1-E4
PRC18	2.61×10^{-13}	1.49×10^{-11}	Y	Y	T1-E4 T2-E4 T1-E3
PRC19	0.1995	0.9931	N	N	
PRC20	0.0430	1.47×10^{-05}	Y	Y	T1-E4
PRC21	5.66×10^{-12}	9.38×10^{-09}	Y	Y	T1-E4
PRC22	0.0895	0.9495	N	N	
PRC23	0.0192	0.9943	N	N	
PRC24	8.74×10^{-12}	4.01×10^{-11}	Y	Y	T1-E4
PRC25**	0.9908	1.78×10^{-05}	N**	Y	T1-E2
PRC26	0.1692	1.0000	N	N	
PRC32	0.3233	1.0000	N	N	
PRC35	0.0040	8.68×10^{-10}	Y	Y	T1-E4
PRC36	6.14×10^{-12}	7.12×10^{-10}	Y	Y	*T1-E4*‖
PRC38	0.0820	0.9995	N	N	
PRC40	4.81×10^{-12}	9.26×10^{-10}	Y	Y	T1-E4 T2-E4 T1-E3

*The *P* value obtained in a one-sided Welch two-sample *t*-test that examines whether the $\log_2$ ratios of probe sets within exon 4 to 11 are greater than the $\log_2$ ratio of probe sets within exon 2 and 3.
†The *P* value obtained in a one-sample *t*-test that examines whether the $\log_2$ ratios of probe sets within exon 4 to 11 are greater than 0.
‡*ERG* status assessed based on discontinuous and unregulated *P* values. Both *P* values needed to be significant for the sample to be considered to have an altered expression pattern and designated Y.
§Presence of *TMPRSS2*-ERG fusions assessed by RT-PCR.
¶Structure of *TMPRSS2*-ERG fusions detected in RT-PCR analyses.
‖Italics indicates that *TMPRSS2-ERG* fusions were detected only after nested RT-PCR.
**Cancer PRC25 exhibited overexpression of exons 2 to 11.

scored as negative for *TMPRSS2-ERG* transcripts. PCR products were then subject to DNA sequencing to confirm their identity. An excellent correlation was observed between the detection of *TMPRSS2-ERG* fusions by RT-PCR and the altered *ERG* exon expression profiles obtained using GeneChip Human Exon 1.0 ST arrays (Table 1). All 15 cancers that scored as having an altered *ERG* expression pattern contained hybrid transcripts where *TMPRSS2* exon 1 was fused to *ERG* exon 4 (called T1-E4 transcripts), consistent with each of these cancers demonstrating increased levels of expression of exons 4 to 11 relative to exons 2 and 3 (Table 1). Five of the cancer samples additionally contained T1-E3, and T2-E4 transcripts, and one cancer (PRC2) also contained T1-E2 transcripts (Table 1). Interestingly, despite the frequent presence of T1-E3 transcripts, only fusions to *ERG* exon 4 (T1-E4 and T2-E4) appeared to be present at sufficiently high levels to be detected by expression analysis using GeneChip Human Exon 1.0 ST arrays.

For two cancers (PRC25 and PRC11), discrepancies were found between the results based on analysis of expression data from GeneChip Human Exon 1.0 ST arrays and those obtained in RT-PCR analyses. For PRC11, nested RT-PCR studies identified a T1-E4 fusion transcript, but no altered pattern of expression was observed (Figure 1; Table 1). This result is explained if the fusion transcript is only present at low abundance within sample PRC11. RT-PCR studies identified a T1-E2 fusion transcript in cancer PRC25. This cancer demonstrated statistically significantly increased levels of expression across all exons 2 to 11 (Figure 1; Table 1). However, the GeneChip Human Exon 1.0 ST array does not contain probe sets corresponding to ERG exon 1, so a differential expression between 5′-*ERG* and 3′-*ERG* exons for this sample could not be scored.

The ETV1, ETV4, and TMPRSS2 Genes

GeneChip Human Exon 1.0 ST arrays contain probe sets that detect exons 1 to 13 of *ETV1*, exons 2 to 13 of *ETV4*, and exons 2 to 14 of *TMPRSS2*. Because *TMPRSS2* has been found fused to exons 4 and 5 of *ETV1* and within the intron immediately 5′ to exon 3 at *ETV4*, it should also be possible to detect increased expression of translocated *ETV1* and *ETV4* sequences that result from fusion to *TMPRSS2* by expression analysis using exon arrays. However, in this series of 27 cancer samples, we failed to observe alteration and patterns of expression at these loci (results not shown) consistent with the observation that rearrangements of the *ETV1* and *ETV4* loci only occur at low frequency in human prostate cancers.[2–4] Most

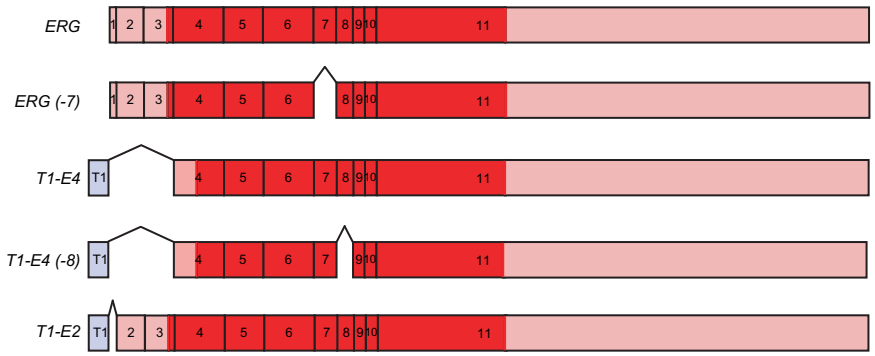


Figure 2. Structure of *ERG* and *TMPRSS2:ERG* transcripts. Sequences derived from *TMPRSS2* are shown in blue; sequences derived from *ERG* are shown in red. Light color, untranslated regions; heavy color, open reading frames predicted by tools available at *http://www.dnalc.org/bioinformatics/dnalc_nucleotide_analyzer.htm#translator*. The figure shows alternatively spliced variants of the normal *ERG* transcripts [*ERG* and *ERG* (−7)] and of the *TMPRSS2:ERG* hybrid transcripts [*T1-E4* and *T1-E4*(−8)]. The spicing out of the 81-bp exon 7 (−7) in normal *ERG* transcripts and of the 72-bp exon 8 (−8) in *TMPRSS2:ERG* hybrid transcripts is predicted to occur in some prostate cancers based on the results of exon expression profiling (Figure 1). The figure also shows the predicted structure of the T1-E2 *TMPRSS2:ERG* hybrid transcript. Expression data were only available for *ERG* exons 2 to 11; exon 1 was assumed to be present in the normal *ERG* transcripts.

fusions to *ETS* genes involve exon 1 of the *TMPRSS2* gene. The absence of probe sets corresponding to *TMPRSS2* exon 1 means that it is not possible to use alterations in the relative levels of expression of individual exons in this particular gene to detect the *TMPRSS2-ETS* translocations.

Gene Signature Associated with the Presence of the ERG Gene Translocation

To identify gene signatures associated with *ERG* gene translocation, we compared gene-level expression data in the 15 cancers that contained the *ERG* translocations, as judged by expression analysis onto GeneChip Human Exon 1.0 ST arrays, with the 11 cancers that were judged not to contain the translocation. For these analyses, we additionally included cancer PRC25, which exhibited overexpression across all *ERG* exons 2 to 11, within the ERG-rearrangement-positive group. A moderated *t*-statistic was computed for each gene, with the resulting *P* values adjusted for multiple testing. Sixteen genes were identified as significantly overexpressed in cancers containing *ERG* translocations (Table 2): the top 3 ranked genes were *ERG*, *TDRD1* (*tudor domain containing 1*), and *KCNH8*. In addition to *KCNH8*, two other genes encoding potassium channels (*KCNN4* and *KCNS3*) were also present in this list.

Discussion

We have demonstrated that expression profiling onto GeneChip Human Exon 1.0 ST arrays can provide an accurate method for monitoring the *ERG* gene translocation in human prostate cancer. To our knowledge, this represents the first demonstration that exon arrays can be accurately used for this purpose. Potentially, however, the same approach could be used to detect other translocations in which the gene fusion is associated with overexpression of the translocated sequence, for example, in synovial sarcomas in which fusion of *SYT* to *SSX1* results in abnormal expression of *3′-SSX* sequences.[19,20] Our statistical analyses of the exon array data failed to correctly predict the presence of the translocation in two cases. In one cancer, PRC11, the *TMPRS2-ERG* fusion transcript was only detected in nested PCR studies, in-

Table 2. Genes Identified as Significantly Overexpressed in Cancers Containing *ERG* Translocations

Adjusted *P* value	Gene	Description
1.37×10^{-6}	*ERG*	*v-ets-erythroblastosis virus E26 oncogene like (avian)*
4.73×10^{-5}	*TDRD1*	*Tudor domain containing 1*
2.38×10^{-4}	*KCNH8*	*Potassium voltage-gated channel, subfamily H (eag-related), member 8*
7.25×10^{-3}	*LOC643036*	*Similar to proteoglycan 3*
7.25×10^{-3}	*ChGn*	*Chondroitin β 1,4 N-acetylgalactosaminyltransferase*
1.42×10^{-2}	*HES1*	*Hairy and enhancer of split 1*
1.42×10^{-2}	*C8ORF4*	*Chromosome 8 open reading frame 4*
1.42×10^{-2}	*AMPD3*	*Adenosine monophosphate deaminase (isoform E)*
1.42×10^{-2}	*KCNN4*	*Potassium intermediate/small conductance calcium-activated channel subfamily N*
2.38×10^{-2}	*FLJ10246*	*FLJ10246*
2.85×10^{-2}	*NR4A2*	*Nuclear receptor subfamily 4, group A member 2*
3.05×10^{-2}	*NCALD*	*Neurocalcin δ*
3.26×10^{-2}	*KCNS3*	*Potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3*
4.36×10^{-2}	*F3*	*Coagulation factor III (thromboplastin, tissue factor)*
4.36×10^{-2}	*FAM77C*	*Family with sequence similarity 77, member C*
4.36×10^{-2}	*LOC651278*	*Similar to serine hydrolase-like 2*

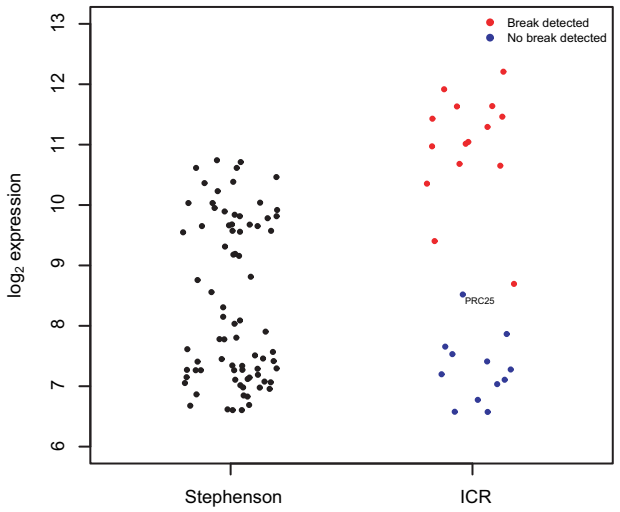


Figure 3. Expression levels of the *ERG* gene. **Right:** Institute of Cancer Research: expression data from exons 4 to 11 of the *ERG* gene from 27 cancers determined in the current study using GeneChip Human Exon 1.0 ST arrays. Samples were designated as *ERG* rearrangement-positive (red dots) or *ERG* rearrangement-negative (blue dots) based on the presence of an altered *ERG* expression profile (see Figure 1). Cancer PRC25, which had a statistically significant elevated expression of exons 2 to 11, is marked. **Left:** Stephenson et al[12]: expression data for the *ERG* gene determined using Affymetrix U133A human gene array as described previously. Data from each separate cancer sample is presented a black dot. The Stephenson dataset was normalized to our data by altering the complete expression distribution (including all genes and samples) such that the mean and variance were the same as those of the Institute of Cancer Research dataset.

dicating that it was only present at low levels. A second cancer, PRC25, containing a T1-E2 fusion transcript, exhibited overexpression of all *ERG* exons 2 to 11. However, for this particular fusion, differential expression between 3′-*ERG* exons and 5′-*ERG* exons could not be scored because the GeneChip Human Exon 1.0 ST array does not contain sequences representing *ERG* exon 1.

Overexpression of the *ERG* gene alone has been proposed as a surrogate for the presence of the translocation.[4] This is illustrated in Figure 3 by the expression levels of *ERG* from individual cancers in the dataset of Stephenson et al.[12] The cancers exhibiting high levels of *ERG* expression would be expected to contain *ERG* gene rearrangements, whereas those with low levels of *ERG* expression would be expected to lack such rearrangements. However, for cancers with an intermediate level of expression, it is not clear which cancers should be considered to harbor *ERG* gene fusions and which should be considered to lack the fusion. In the current study, we show that measurement of *ERG* expression using GeneChip Human Exon 1.0 ST arrays additionally provides data on the differential expression of 5′-*ERG* exons and 3′-*ERG* exons that for most prostate cancers allows a firm assignment of *ERG*-fusion status to be made. For comparison, the *ERG* gene expression levels obtained in the current study using GeneChip Human Exon 1.0 ST arrays together with the designation of *ERG* status based on analyses of the exon-level expression data are also shown in Figure 3.

Our results have also provided important new insights into the mechanism of prostate cancer development. Previous analyses of the *TMPRSS2-ERG* fusions, confirmed by RT-PCR results presented here, have revealed a diverse variety of possible fusion structures with several fusion transcripts commonly occurring within a single sample.[4–6] Specifically, it has been found that T1-E4 and T1-E5 fusion transcripts occur together in many cancer samples. Expression analyses using exon arrays reveal that the most abundant transcript almost always involves fusion to exon 4 of *ERG,* suggesting that *TMPRSS2* fusions to this exon may represent the biologically important fusion transcript. Analysis of the exon expression array data indicated that exon 7 of *ERG* was spliced out in some prostate cancers that lacked *ERG* alterations. It has been established that *ERG* gene transcripts with and without exon 7 have diverse functions during chondrocyte differentiation: transcripts containing exon 7 stimulate differentiation, whereas those lacking this exon block differentiation.[21] Our results also suggest the possible importance of the splicing out of *ERG* exon 8 in *TMPRSS2-ERG* hybrid transcripts. However, the function of exon 8 in *ERG* is currently unknown. We conclude that expression analysis onto exon microarrays not only represents a valuable approach for scoring *ETS* gene translocations in parallel with determining gene expression profiles but also provides additional insights into the mechanism of prostate cancer development.

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