Modern Pathology (2010) 23, 1061–1067

ERG rearrangement is specific to prostate cancer and does not occur in any other common tumor

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Identification of specific somatic gene alterations is crucial for the insight into the development, progression, and clinical behavior of individual cancer types. The recently discovered recurrent *ERG* rearrangement in prostate cancer might represent a prostate cancer-specific alteration that has not been systematically assessed in tumors other than prostate cancer. Aim of this study was to assess, whether the *ERG* rearrangement and the distinct deletion site between *TMPRSS2* and *ERG*, both predominantly resulting in a *TMPRSS2–ERG* fusion, occur in tumors other than prostate cancer. We assessed 54 different tumor types (2942 samples in total) for their *ERG* rearrangement status by fluorescence *in situ* hybridization (FISH). To calibrate, we analyzed 285 prostate cancer samples for the *ERG* rearrangement frequency. Additionally, we interrogated a high-resolution single nucleotide polymorphism (SNP) data set across 3131 cancer specimens (26 tumor types) for copy number alterations. None of the 54 different tumor types assessed by FISH harbored an *ERG* rearrangement, whereas the prostate cancer samples revealed an *ERG* rearrangement in 49.5% of cases. Furthermore, within the 26 tumor types assessed for copy number alterations by SNP, the distinct deletion site between *TMPRSS2* and *ERG* (21q22.2–3) was detectable exclusively in prostate cancer. Although Ewing's sarcoma and AML have known rearrangements rarely involving *ERG*, we hypothesize that the *ERG* rearrangement as well as the distinct deletion site on 21q22.2–3 between *TMPRSS2* and *ERG* are prostate-cancer-specific genomic alterations. These observations provide further insight into the oncogenesis of prostate cancer and might be critical for the development of *ERG* rearrangement assessment as a clinical tool.
Modern Pathology (2010) **23,** 1061–1067; doi:10.1038/modpathol.2010.87; published online 14 May 2010

Keywords: *ERG* rearrangement; prostate cancer; carcinoma

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Received 6 January 2010; revised 12 March 2010; accepted 15 March 2010; published online 14 May 2010

Like other cancers, prostate cancer is driven by the acquisition of somatic genetic alterations resulting in oncogenesis. Among these, recurrent gene translocations have been supposed to be specific for hematological and mesenchymal malignancies (sarcomas) until recently.[1] Translocations have only

been observed in rare subtypes of common epithelial malignancies (carcinomas) such as breast, thyroid, and renal.[2,3] The recent discovery that the majority of prostate cancers harbor recurrent gene rearrangements suggests that translocations may occur more commonly than previously assumed in epithelial cancers.[4] The nature of recurrent gene rearrangements in prostate cancer involves androgen-regulated 5′ partners (eg, *TMPRSS2*, *SLC45A3*, and *NDRG1*) and ETS genes (eg, ETV1, ETV4, and ETV5), most commonly resulting in the *TMPRSS2–ERG* fusion.[5] Within this distinct subgroup of prostate cancer, the *TMPRSS2–ERG* fusion occurs through a deletion of the genomic material spanning from *TMPRSS2* to *ERG* in the majority of these cases.[6,7] It is intriguing to note that ETS genes have been detected earlier in translocations in Ewing's sarcoma, AML, and breast cancer. In these tumors the most frequent ETS genes involved differ from prostate cancer. For example, in Ewing's sarcoma, the ETS gene *FLI1* is fused to *EWS* most commonly, whereas *EWS–ERG* fusions occur rarely.[8] Given the high frequency of prostate cancers harboring *ERG* rearrangement, we undertook a large survey of common tumors to exclude the possibility that any of these harbor the *ERG* rearrangement. Additionally, to identify prostate-cancer-specific somatic copy number alterations, we interrogated a recently published and publicly available high-resolution data set of somatic copy number alterations across 3131 cancer specimens, derived from 26 different histological types.[9]

Materials and methods

Samples

We assessed 54 different tumor types (2942 tumor samples in total) for their *ERG* rearrangement status using an *ERG* break-apart fluorescence *in situ* hybridization (FISH) assay as described earlier.[4,6,10] This assay can differentiate between the structural *ERG* rearrangement (ie, *ERG* rearrangement through insertion) and the distinct deletion on 21q22.2–3 associated with the *ERG* rearrangement (ie, *ERG* rearrangement through deletion), both most commonly resulting in a fusion with *TMPRSS2*.[6] In all, 2261 cases were assessable. These included 131 breast carcinomas, 36 colon carcinomas, 111 colon adenomas, 120 non-small cell lung carcinomas, 32 small cell lung cancers, 94 urinary bladder carcinomas, 85 kidney carcinomas, 77 thyroid carcinomas, 74 ovarian carcinomas, 68 endometrial carcinomas, 63 non-Hodgkin lymphomas, 60 malignant melanomas, 59 basal cell carcinomas, 58 hepatocellular carcinomas, 48 stomach carcinomas, 45 seminomas, 44 Schwann cell tumors, 44 non-seminomatous testicular carcinomas, 42 uterine leiomyomata, 41 pleomorphic adenomas of the salivary glands, 41 leiomyosarcomas, 40 oral cavity carcinomas, 40 meningiomas, 40 astrocytomas, 38 renal oncocytomas, 38 glioblastomas, 37 pancreatic carcinomas, 36 esophagus carcinomas, 36 Hodgkin lymphomas, 34 parathyroideal adenomas, 34 thymomas, 33 gallbladder carcinomas, 33 nevi, 33 carcinoid tumors, 32 neurofibromas, 31 laryngeal carcinomas, 29 thyroid adenomas, 29 malignant fibrous histiocytomas, 27 hemangiomas, 25 squamous cell skin cancers, 25 salivary gland cylindromas, 24 benign histiocytomas, 21 pheochromocytomas, 21 tendon sheath giant cell tumors, 21 liposarcomas, 19 mesotheliomas, 19 vulvar carcinomas, 19 oligodendrogliomas, 18 salivary gland adenolymphomas, 15 duodenal carcinomas, 13 benign skin tumors (NOS), 12 adrenal adenomas, 8 Kaposi's sarcomas, and 8 paragangliomas. All patients were diagnosed at the University Hospital of Basel, Switzerland. Of these samples, six tissue micro arrays were constructed with one core per case with a diameter of 0.6 mm.

To calibrate the frequency of *ERG* rearrangement prostate cancer, we assessed a well-defined cohort using a break-apart FISH assay. The cohort contains tumor material from 109 consecutive partially PSA-screened patients who underwent prostatectomy. Two cores were taken from the index tumor focus from the peripheral zone of each patient sample. Patients were treated at the University Hospital of Tuebingen.

Tissue Micro Array Construction

Formalin-fixed paraffin-embedded prostate cancer specimen were cut in 4 μm thick sections, mounted on slides, and stained with hematoxylin and eosin. Subsequently, the cancer region was marked. The cores, each 0.6 mm in diameter, were taken from the corresponding donor block and placed into a tissue micro array recipient block using a semiautomatic tissue arrayer (Beecher Instruments, Sun Prairie, WI, USA); 4 μm thick tissue sections were placed onto superfrost slides.

Fluorescence *In Situ* Hybridization

We used a FISH assay to detect the *ERG* rearrangement at the chromosomal level on formalin-fixed paraffin-embedded specimen. Hence, we performed a split-signal-approach, with two probes spanning the *ERG* locus as described earlier.[4,6] Deparaffinized sections were pretreated with a 100 mM Tris and 50 mM EDTA solution at 92.8°C for 15 min and digested with Digest-All III (dilution 1:2) at 37°C for 22 min; *ERG* FISH probes were denatured at 73°C for 5 min and immediately placed on ice. Subsequently, the tissue sections and *ERG* FISH probes were co-denatured at 94°C for 3 min and hybridized overnight at 37°C. We used BAC clones RP11-24A11 for centromeric labeling with biotin and RP11-372O17 for telomeric labeling with digoxigenin. Posthybridization washing was performed with 2 × SSC at 75°C for 7 min, and the fluorescence detection was

carried out using streptavidin-Alexa-594 conjugates (dilution 1:200) and anti-digoxigenin-FITC (dilution 1:200). Slides were then counterstained with 4′,6-diamidin-2′ phenylindoldihydrochlorid (DAPI) and mounted.

The samples were analyzed under an $\times 63$ oil immersion objective using a fluorescence microscope (Zeiss, Jena, Germany) equipped with appropriate filters, a charge-coupled device camera, and the FISH imaging and capturing software Metafer 4 (Metasystems, Altlussheim, Germany). All cases were independently assessed by three experienced evaluators (MB, VS, and SP) At least 100 nuclei per case were evaluated.

This FISH assay allows for *ERG* rearrangement status (ie rearrangement *versus* no rearrangement of *ERG*) assessment. The assay is also capable of differentiating between two different mechanisms of *ERG* rearrangement.[6] These two mechanisms are *ERG* rearrangement through insertion and *ERG* rearrangement through deletion of DNA between *TMPRSS2* and *ERG* loci (interstitial deletion). A nucleus without an *ERG* rearrangement shows two pairs of juxtaposed red and green signals (mostly forming two yellow signals). A nucleus with an *ERG* rearrangement through insertion shows the split of a signal pair resulting in a single red and single green signal for the rearranged *ERG* allele and a still juxtaposed (yellow) signal pair for the non-rearranged *ERG* allele in each nucleus. A nucleus with an *ERG* rearrangement through deletion shows one juxtaposed red-green signal pair (yellow) for the non-rearranged allele and a single red signal for the allele involved in the rearrangement.

Analysis of Somatic Copy Number Alterations

Beroukhim *et al*[9] published a publicly available high-resolution single nucleotide polymorphism (SNP) data set of somatic copy number profiles across 3131 cancer specimens. We obtained average copy number profiles from 26 cancer types, including prostate cancer, each represented from at least 20 and up to 734 cancer specimens. Seventeen cancer types are represented by at least 40 specimens. In all, 2520 profiles were obtained from tissue specimens and 611 were obtained from cancer cell lines or short-term cultures. All copy number estimates were obtained using an SNP array (Affymetrix 250K Sty). Signal intensities from each cancer specimen were compared to SNP data obtained from 1480 specimens of normal tissue to identify regions of somatically generated copy number alterations. The raw data of this study were obtained from http://www.broadinstitute.org/tumorscape.

Results

By assessing 2942 tumor samples of 54 different common tumors for their *ERG*-rearrangement status using our *ERG* break-apart FISH assay, we could show that none of the 2261 assessable epithelial and non-epithelial tumors other than prostate cancer harbored a rearrangement of the *ERG* locus. For a detailed break down of the individual tumor entities assessed for the *ERG* rearrangement, see Table 1.

For calibration of the *ERG* rearrangement frequency we assessed a well-defined prostate cancer cohort by the same *ERG* break-apart FISH assay. We observed a frequency of the *ERG* rearrangement in 54/109 (49.5%) of cases in the partially PSA-screened prostatectomy cohort. Of these, 40/54 (74.1%) harbored the *ERG* rearrangement through deletion, whereas 14/54 (25.9%) harbored the *ERG* rearrangement through insertion.

The *ERG* break-apart FISH assay is capable of differentiating between the structural *ERG* rearrangement (ie *ERG* rearrangement through insertion) and the distinct numerical 21q22.2–3 alteration associated with the *ERG* rearrangement (ie *ERG* rearrangement through deletion). As indicated above, the *ERG* rearrangement through deletion is the prevalent mechanism in our prostate cancer cohort (ie 40 cases with *ERG* rearrangement through deletion *versus* 14 cases with *ERG* rearrangement through insertion). To determine whether the distinct deletion site between *TMPRSS2* and *ERG* is specific to prostate cancer, we examined copy number profiles from 3131 cancers across multiple cancer types.[9] Interestingly, we only observed the distinct deletion site between *TMPRSS2* and *ERG* in prostate cancer but in none of the other interrogated tumor types. A summary of these data can be seen in Figure 1, which shows the average copy number profiles on chromosome 21q for each of the 26 cancer types represented by at least 20 specimens.

In independent tumor samples, a significant subset of tumor entities was both assessed for the *ERG* rearrangement status by the break-apart FISH assay and for copy number alterations by SNP analysis. As expected, we found losses and gains of the FISH signals at different levels in subsets of the tumors highly corresponding to the copy number assessments by SNP analysis (data not shown). To emphasize, FISH signal patterns specific to *ERG* rearrangement through deletion or *ERG* rearrangement through insertion only appeared in prostate cancer samples.

Discussion

Important implications for the diagnosis, prediction, prognosis, and development of therapeutic targets in cancers can be obtained from systemic efforts to identify somatic genetic alterations. Of these, cancer-type-specific alterations are most promising. Surprisingly, the recurrent *ERG* gene rearrangement was recently discovered in the majority of prostate cancers.[5,6,11–22] By assessing 2295 samples comprising a broad spectrum of tumor entities, we were the

Table 1 *ERG* rearrangement status assessment by FISH on different tumors types

			ERG rearrangement status	
Tumor type	*No. of cases*	*Assessable cases*	*Positive*	*Negative*
Breast carcinomas	190	131	0	131
Colon carcinomas	49	36	0	36
Colon adenomas	143	111	0	111
Non-small cell lung carcinomas	159	120	0	120
Small cell lung carcinomas	49	32	0	32
Urinary bladder carcinomas	115	94	0	94
Kidney carcinoma	107	85	0	85
Thyroid carcinomas	92	77	0	77
Ovarian carcinomas	111	74	0	74
Endometrial carcinomas	81	68	0	68
Non-Hodgkin lymphomas	66	63	0	63
Malignant melanomas	78	60	0	60
Basal cell carcinomas	73	59	0	59
Hepatocellular carcinomas	97	58	0	58
Stomach carcinomas	76	48	0	48
Esophagus carcinomas	43	36	0	36
Seminomas	49	45	0	45
Non-seminomatous testicular carcinomas	48	44	0	44
Uterine leiomyomata	57	42	0	42
Leiomyosarcomas	47	41	0	41
Pleomorphic adenomas of the salivary gland	49	41	0	41
Oral cavity carcinomas	49	40	0	40
Schwann cell tumors	49	44	0	44
Meningiomas	48	40	0	40
Astrocytomas	48	40	0	40
Glioblastomas	44	38	0	38
Renal oncocytomas	48	38	0	38
Pancreatic carcinomas	49	37	0	37
Hodgkin lymphomas	42	36	0	36
Parathyroideal adenomas	43	34	0	34
Thymomas	37	34	0	34
Gallbladder carcinomas	45	33	0	33
Nevi	44	33	0	33
Carcinoid tumors	37	33	0	33
Neurofibromas	41	32	0	32
Laryngeal carcinomas	57	31	0	31
Thyroid adenomas	39	29	0	29
Malignant fibrous histiocytomas	30	29	0	29
Hemangiomas	34	27	0	27
Squamous cell skin cancer	38	25	0	25
Salivary gland cylindromas	32	25	0	25
Benign histiocytomas	28	24	0	24
Pheochromocytomas	28	21	0	21
Tendon sheath giant cell tumors	28	21	0	21
Liposarcomas	28	21	0	21
Mesotheliomas	31	19	0	19
Vulvar carcinomas	28	19	0	19
Oligodendrogliomas	25	19	0	19
Salivary gland adenolymphomas	25	18	0	18
Duodenal carcinomas	22	15	0	15
Benign skin tumors, NOS	19	13	0	13
Adrenal adenomas	14	12	0	12
Kaposi's sarcomas	25	8	0	8
Paragangliomas	8	8	0	8

first to prove that the *ERG* rearrangement does not occur in other common epithelial and non-epithelial tumors. Although Ewing's sarcoma and AML have known rearrangements sporadically involving *ERG* (ie *EWS-ERG* and *FUS-ERG,* respectively), the current finding supports the hypothesis that the mentioned mechanisms of *ERG* rearrangement are specific to prostate cancer. These findings are essential for the application of *ERG* rearrangement as a clinical marker and might have further implications for the development of targeted therapies. In addition, by interrogating the Beroukhim data set of somatic copy number profiles across 3131 cancer specimens, we found that the distinct deletion site on 21q22.2–3 spanning from *TMPRSS2* to *ERG* is a copy number alteration specific to

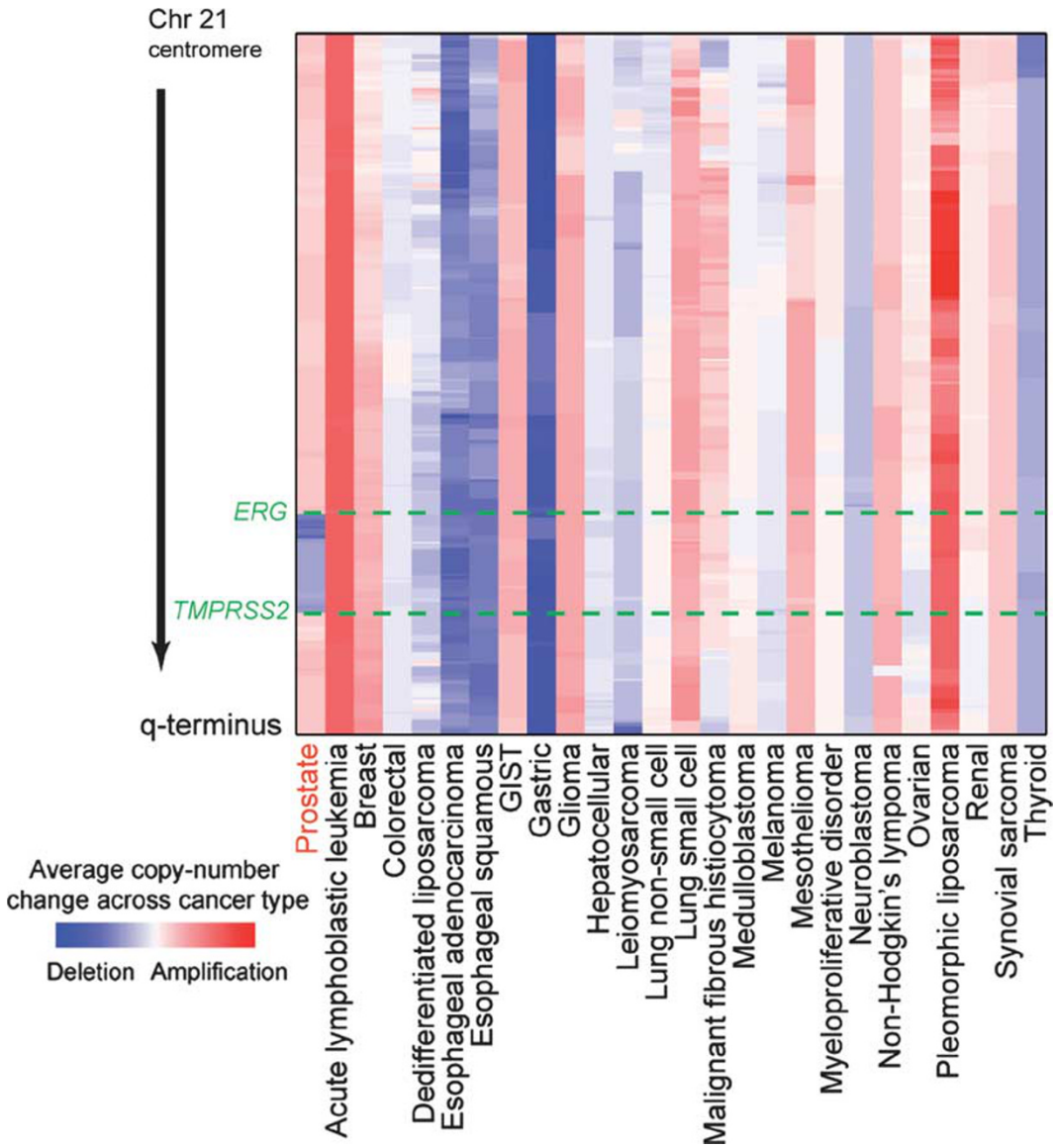

Figure 1 Average copy number changes across chromosome 21q for 26 different cancer types. Each cancer type (arranged along the *x* axis) represents average log2 ratios from SNP array data obtained from at least 20 and up to 734 cancer specimens. Only prostate cancer exhibits characteristic deletions between *ERG* and *TMPRSS2*.

prostate cancer and is not shared by other human cancers.[9] Furthermore, we could verify that the distinct numerical 21q22.2–3 alteration associated with the *ERG* rearrangement (ie *ERG* rearrangement through deletion) is the prevalent mechanism resulting in the *TMPRSS2–ERG* gene fusion in prostate cancer.[6,7] It is of particular interest that this specific deletion most frequently results in the *TMPRSS2–ERG* fusion. Beroukhim *et al* compared the significant somatic copy number alterations in each of the individual cancer types to the remaining samples and found that the majority of somatic copy number alterations is shared by different cancers. This indicates that the major genomic alterations on 21q22.2–3, ie the *ERG* rearrangement and the distinct deletion site spanning from *TMPRSS2* to *ERG*, both resulting in the *TMPRSS2–ERG* gene fusion, are specific to prostate cancer. Of note, in our previous study, we could identify significantly downregulated genes with tumor suppressor potential located in the area of the distinct deletion site on 21q22.2–3.[6] The additional loss of these genes might have important biological and clinical effects specific to prostate cancer.

Until now, the reason for the occurrence of cancer-type-specific gene fusions is largely unknown. Recently, Mani *et al*[23] could provide evidence that androgen stimulation causes a physical approximation of *TMPRSS2* and *ERG* in androgen-sensitive prostate cancer cell lines. Subsequent irradiation—representing a DNA-double-strand-breaking event—resulted in accumulated *TMPRSS2–ERG* fusions. As prostate cancer is the only androgen-sensitive malignancy, these findings could help to better

understand why the *TMPRSS2–ERG* gene fusion is a prostate-cancer-specific genomic alteration. In this context it is worth mentioning that complex interactions between chromosomes and folding patterns represent a broad field that is poorly elucidated. Lieberman-Aiden *et al*[24] described a method that allows identification and visualization of chromatin interactions across a whole genome. Using this technique, complex relationships between chromatin structure, gene activity, and the functional state of the cell might be assessed in cancers with specific genomic alterations. Thus, new aspects of the genesis of genomic alterations might be provided.

In summary, we were the first to assess a large collection of common epithelial and non-epithelial tumors and found that the distinct genomic alterations on 21q22.2–3, ie the *ERG* rearrangement and the deletion site spanning from *TMPRSS2* to *ERG*, both resulting in the *TMPRSS2–ERG* gene fusion, are specific to prostate cancer and do not occur in any other common tumor. Despite the broad spectrum of tumors and the large number of cases for each tumor entity we evaluated, a sporadic appearance of the *ERG* rearrangement in rare tumors not assessed by us or in an extremely low frequency in the assessed tumor entities cannot be excluded.

We believe that a genetic alteration, which is specific to malignant cells, might be targeted by modern therapies. In recent studies by Wang *et al*[25] and Sun *et al*[26], a knock down of TMPRSS–ERG in fusion-positive cells has been shown to inhibit tumor growth in xenograft assays. Additionally, as the *ERG* rearrangement is assumed to drive prostate cancer development through downstream target genes, downstream genes might be promising candidates for new therapeutic strategies.[5]

Furthermore, the *ERG* rearrangement might gain relevance in diagnostic usage as this alteration can be detected in urine samples and biopsies. Confirming prostate cancer by the detection of the prostate-cancer-specific *ERG* rearrangement can be helpful in malignancies of unknown primary such as bone metastasis or small cell cancers of unclear origin.[27] In these cases, early therapy can dramatically slow down the progress of the disease. The diagnostic step forward is already concrete, therapeutic impact in terms of rational therapy has to be investigated.

Acknowledgements

This work was supported by a grant of the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, Emmy-Noether-Program, PE1179/2-1) and the University Hospital of Tuebingen (fortuene Program, No. 1809-1-0) to SP.

Disclosure/conflict of interest

The Brigham and Women's Hospital and the University of Michigan have filed a patent on ETS gene rearrangements in prostate cancer, on which SP and MAR are co-inventors and the diagnostic field of use has been licensed to GenProbe. GenProbe has neither played a role in the design and conduct of the study, nor in the collection, analysis, or interpretation of the data and no involvement in the preparation, review, or approval of the manuscript.

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