Clinical significance of ERG rearrangement subtype and its association with increased p53 expression in Japanese and German prostate cancer

J. NISHIJIMA[1], T. HARA[1], K. IKEMOTO[2], A. OGA[2], K. KOBAYASHI[1], Y. KAWAI[1], H. MATSUMOTO[1], K. NAGAO[1], K. SASAKI[2], V. GKOLEIZAKIS[3], J. FICHTNER[3], H. MATSUYAMA[1],*

[1]Department of Urology, Yamaguchi University School of Medicine, 1-1-1, Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan; [2]Department of Pathology, Yamaguchi University School of Medicine, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan; [3]Klinikum Niederrhein, Johanniter Krankenhaus, Steinbrinkstr. 96, 46145 Oberhausen, Germany

*Correspondence: hidde@yamaguchi-u.ac.jp

This study investigated differences in prevalence of the androgen-regulated transmembrane protease serine 2 (*TMPRSS2*) and ETS transcription factor family member, v-ets erythroblastosis virus E26 oncogene homolog (*ERG*) fusion gene (*TMPRSS2-ERG* fusions) in clinically localized prostate cancer Japanese and German patients. A total of 105 specimens, including 69 Japanese and 36 German patients, were collected. The status of *TMPRSS2-ERG* fusion was determined by fluorescence in situ hybridization, and correlations of the *TMPRSS2-ERG* fusion with clinicopathological characteristics and immunohistochemistry were studied. Gene fusions were identified in 20% (14/69) of Japanese and 53% (19/36) of German patients (*P* < 0.001). The difference in the type of gene fusion between the two ethnic groups was statistically significant (*P*=0.024). Overexpression of ERG protein was significantly associated with gene fusion. Biochemical recurrence was significantly higher in patients with ERG overexpression than in those without, and not related to *TMPRSS2-ERG* fusion status. Interestingly, two types of gene fusions (deletion and increase of copy number) were significantly associated with increased p53 expression (*P* = 0.005). Association of specific gene fusions harboring higher genomic alterations with p53 expression levels suggests that p53 mutation might drive more aggressive rearrangements of *TMPRSS2-ERG* fusion in prostate cancer.

Key words: ERG, p53, prostate cancer, TMPRSS2-ERG fusion

Fusions between the androgen-regulated transmembrane protease serine 2 (*TMPRSS2*, located on 21q22.3) and the ETS transcription factor family member v-ets erythroblastosis virus E26 oncogene homolog (*ERG*, located on 21q22.2) are observed in a substantial portion of patients with prostate cancer [1, 2]. These *TMPRSS2-ERG* fusions are present in approximately 15–80% of prostate cancers [3-6]. *TMPRSS2-ERG* fusions lead to high levels of ERG protein expression [7]. The association of the *TMPRSS2-ERG* fusions with prostate cancer prognosis has been evaluated in many studies, but with contradictory results. The fusions may alter the expression of the involved genes, but the clinical effect of this regulation is not fully understood [8-12].

Accumulating evidence has suggested that there are considerable ethnic variations in the incidence and clinical manifestation of prostate cancer, from the highest incidence in North American and European countries to the lowest in Asian populations [13]. Thus, it is reasonable to hypothesize that ethnic variation in the frequency of genetic mutations, including *TMPRSS2-ERG* fusion, might be associated with prostate cancer risk and progression.

The aims of this study were to investigate the difference in prevalence of the *TMPRSS2-ERG* fusion between Japanese and German patients, and to compare the status of the *TMPRSS2-ERG* fusion with clinicopathological characteristics and patient prognosis in clinically localized prostate cancer.

Patients and methods

Patients and specimens. A total of 105 specimens were obtained from patients who were diagnosed with clinically localized prostate cancer and underwent radical prostatectomy (RP). Of the 105 total specimens, 36 were from Johanniter Krankenhaus, Germany and 69 were from
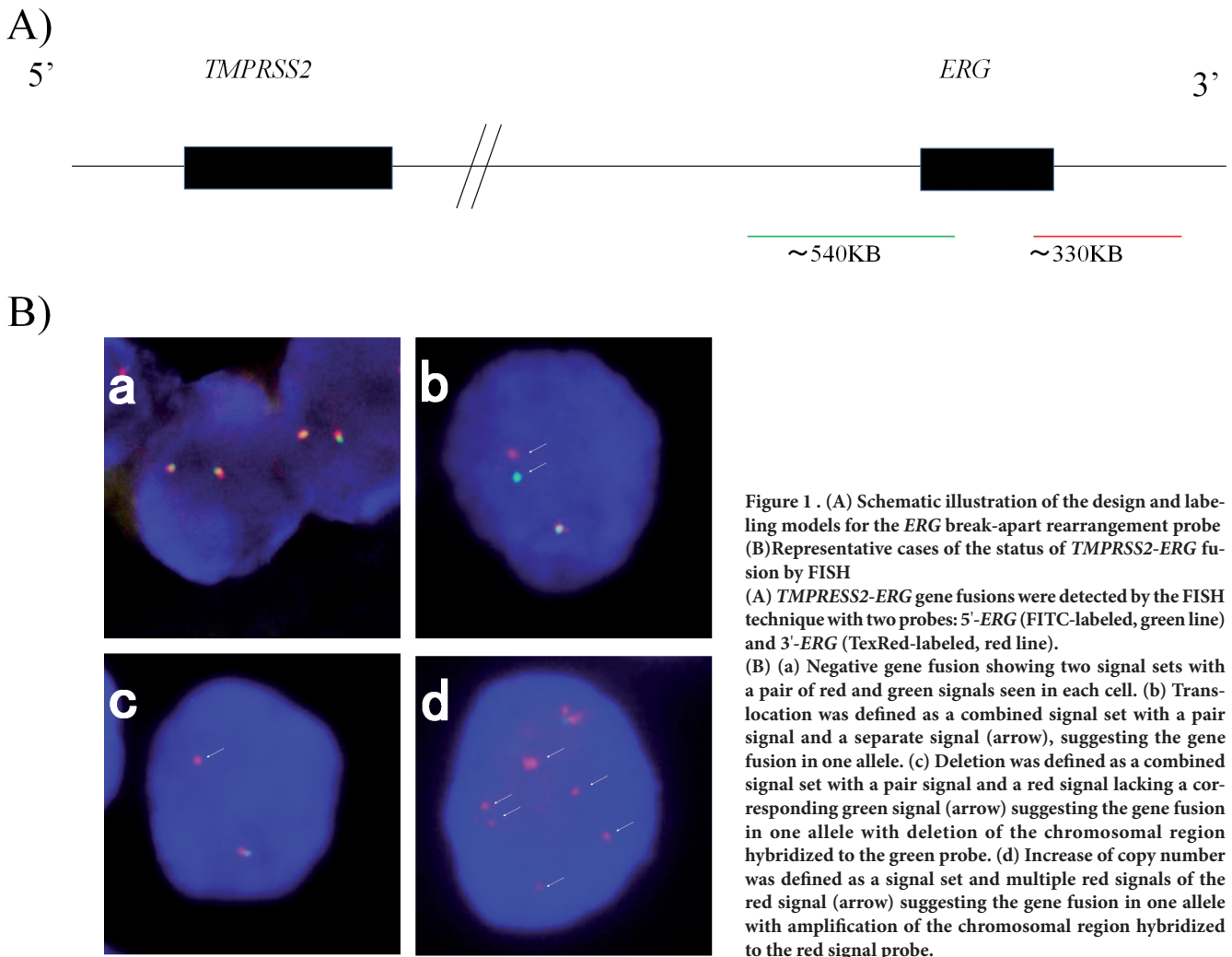
A)

B)

Figure 1 . (A) Schematic illustration of the design and labelling models for the *ERG* break-apart rearrangement probe (B)Representative cases of the status of *TMPRSS2-ERG* fusion by FISH

(A) *TMPRESS2-ERG* gene fusions were detected by the FISH technique with two probes: 5'-*ERG* (FITC-labeled, green line) and 3'-*ERG* (TexRed-labeled, red line).

(B) (a) Negative gene fusion showing two signal sets with a pair of red and green signals seen in each cell. (b) Translocation was defined as a combined signal set with a pair signal and a separate signal (arrow), suggesting the gene fusion in one allele. (c) Deletion was defined as a combined signal set with a pair signal and a red signal lacking a corresponding green signal (arrow) suggesting the gene fusion in one allele with deletion of the chromosomal region hybridized to the green probe. (d) Increase of copy number was defined as a signal set and multiple red signals of the red signal (arrow) suggesting the gene fusion in one allele with amplification of the chromosomal region hybridized to the red signal probe.

Yamaguchi University Hospital, Japan. Patients who received hormonal or radiation therapy before or after RP were excluded. Clinicopathological parameters, including age, preoperative prostate-specific antigen (PSA), Gleason score, pathological T and N stage, lymphovascular invasion, and status of resection margin, were collected from patient medical records. The Gleason score was assessed in accordance with the criteria outlined at the 2005 International Society of Urological Pathology Consensus Conference [14]. Biochemical recurrence (BCR) was defined as a PSA > 0.2 ng/mL, or a PSA rise in at least consecutive twice evaluations.

Tissue preparation. Slides of the prostatectomy specimens stained by hematoxylin and eosin were reviewed by two urologic pathologists (AO, KS). Slides containing the tumor were marked and paraffin blocks of the corresponding area were applied to construct the tissue microarray (TMA), as described elsewhere [15]. In brief, two representative core tumor tissue sections (2 mm in diameter) were taken from an individual paraffin block and arranged in a new TMA block.

ERG break-apart assay. We analyzed the rearrangement of the *TMPRSS2-ERG* fusion with break-apart fluorescence in situ hybridization (FISH) [10, 16, 17]. The break-apart probes, a FITC-labeled 5' ERG probe (BAC GSP1581G08, GSP1485E03, GSP1097C10) and a TexRed-labeled 3' ERG probe (BAC GSP0083F06, GSP1266F04) were obtained from the GSP laboratory (Kawasaki, Japan) (Fig 1A). Procedures, including hybridization, washing, and fluorescence detection, were performed according to the manufacturer's protocol, described in brief as follows. Sections of the paraffin block (4 μm each) were deparaffinized and dehydrated. The slides were incubated with 0.05% pepsin at 37°C for 10 min and washed twice with phosphate-buffered saline (PBS), pH 7.4, at room temperature (RT) for 5 min. The slides were then fixed in 1% formaldehyde solution for 3 min, washed twice in PBS at RT for 5 min, re-fixed in Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) for 5 min, dehydrated in 70%, 85%, and 100% ethanol solution for 2 min each, and air dried. The probe set was applied onto the slides,

and the slides were covered with a coverslip and sealed with rubber cement. The slides were incubated at 75°C for 5 min to denature the probe and then at 37°C for 72 h in a humidified chamber to allow hybridization. Post-hybridization washes were performed in 2X standard saline citrate containing 0.3% Nonidet P-40 (NP-40) for 5 min at RT and at 73°C for 2 min twice, followed by air drying and counterstaining with a DAPI-II antifade solution (Abbott Molecular, Downers Grove, IL, USA). At least 100 nuclei with FISH signals were analyzed under a BX60 fluorescence microscope (Olympus, Tokyo, Japan) with triple band-pass filter equipment (Chroma, Bellows Falls, VT, USA). In the quantification of FISH signals, overlapping cells and cells with indistinct and blurry signals were avoided, as evaluation of these cells was difficult.

To establish normal cutoffs for scoring, seven specimens of non-neoplastic prostate tissue were analyzed, and the mean and SD for *TMPRSS2-ERG* fusion signal ratios were calculated. A tumor was considered to have gene fusion if the percentage of cells showing a 0 or 1 ERG probe signal was greater than 3 SD (1.96±7.83%) above the mean established from the normal tissue.

Signals were evaluated as: [1] negative: the two combined red and green signals (Fig 1Ba); [2] translocation: one combined signal and one separated red and green signal (Fig 1Bb); [3] deletion: one combined signal and a single copy of the

red signal (Fig 1Bc); and [4] increase of copy number: one combined signal and many copies of the red signal (Fig 1Bd), according to the previous report [4, 10]. FISH interpretation was performed by two urologic pathologists (AO, KS).

Immunohistochemistry. The corresponding specimens of FISH slides from TMA were analyzed by immunohistochemistry (IHC). Antibodies against the following proteins were used: ERG (rabbit polyclonal, 1:500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA), PTEN (mouse monoclonal, 1:100 dilution; Dako, Carpinteria, CA, USA), NKX3.1 (rabbit polyclonal, 1:100 dilution; Abcam, Cambridge, MA, USA), Ki-67 (rabbit polyclonal, 1:500 dilution; Abcam), AR (mouse monoclonal, 1:75 dilution; Dako), and p53 (clone DO-7, mouse monoclonal, 1:50 dilution; Dako). The selection of these antibodies was based on cell-cycle-related proteins, androgen-responsible gene products, and gene products frequently reported to be closely associated with *TMPRSS2*-ERG fusion.

After rehydration, endogenous peroxidase was inactivated for 10 min in 0.3% H_2O_2 in methanol. Slides were incubated with 10mM sodium citrate buffer, pH 6.0. Nonspecific binding sites were blocked with 1% bovine serum albumin and 0.1% Tween 20 (Sigma, Saint Louis, MO, USA) in PBS. The slides were incubated with a primary antibody in a controlled dilution overnight at 4°C. The sections were incubated with Histofine Simple Stain Rat MAX-PO (MULTI) (Nichirei Biosciences Inc., Tokyo, Japan) for 30 min at RT. Immunoreactive species were detected with DAB (Nichirei Biosciences Inc.). The slides were counterstained with hematoxylin.

Samples were assigned a mean score (0, no staining, 1, weak staining, 2, moderate staining, 3, strong staining) and a staining score (H-score) was calculated by multiplying the mean intensity score and the percent of positive cells stained, with a value in the range of 0–300. For categorical dichotomization, the H-score of 100–150 (median score of each antibody) was used as a cut-off to categorize high and low. P53 mutation was also determined based on the H-score described above.

Statistics. The Chi-square test or the Fisher's exact test was used to compare the results of the ERG break-apart assay in accordance with the clinicopathological variables and histological pattern. Univariate analysis of a risk factor for biochemical recurrence used the log-rank test of the Kaplan–Meier analysis and the Cox's logistic regression analysis. Multivariate analysis was based on the Cox's proportional hazard model and the forward likelihood ratio was used. Statistical tests were two sided and p-values < 0.05 were considered to be statistically significant. To estimate correlations among expression levels of PTEN, NKX3.1, Ki-67, AR and p53, linear regression analysis was used. All statistical analyses were conducted using JMP®, version 10 (SAS Institute, Cary, NC, USA).

Table 1. Clinical and pathological characteristics of patients

Parameter		Japanese	German	P-value
No. of patients		69	36	
Age (years)		66.7±5.6	65.4±5.3	0.23[†]
Preoperative PSA (ng/ml)		17.1±16.4	–	–
Gleason score	4–6	19	26	<0.001[§]
	7	35	10	
	8–10	15	0	
Tumor stage	≤pT2	49	36	<0.001[‡]
	≥pT3	17	0	
LVI	Positive	29	–	–
	Negative	35	–	
SM	Positive	42	–	–
	Negative	23	–	
Biochemical recurrence	Yes	13	4	0.41[‡]
	No	53	32	

Abbreviations: LVI, lymphovascular invasion; PSA, prostate-specific antigen; SM, surgical margin
Data are presented as mean±SD.
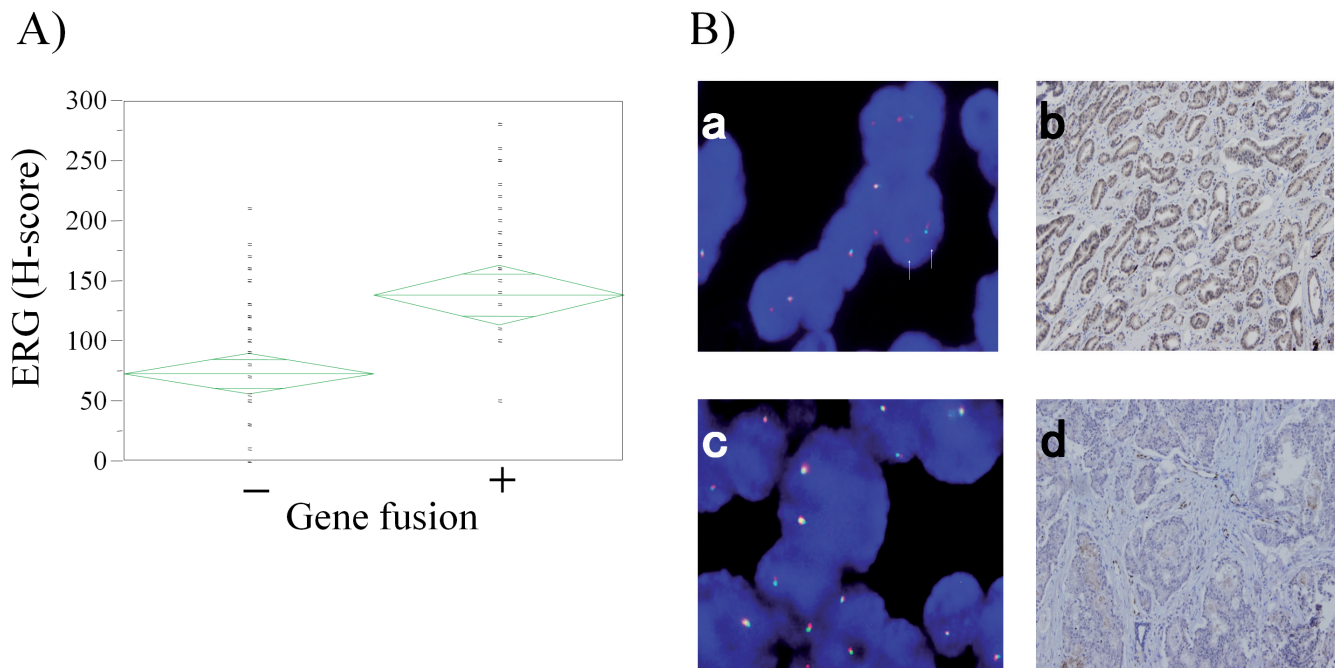Statistical analyses were used as follows: †Wilcoxon rank sum test, ‡Fisher's exact test and §Chi-square test.

Results

Patient characteristics. The clinicopathological variables for the Japanese and German patients are summarized in Table 1. Japanese patients had higher Gleason scores (>7)

A)

B)

Figure 2. Relationship of gene fusion status with ERG expression and gene fusion subtype
(A) ERG expression by immunohistochemical analysis (H-score) was significantly correlated with presence of gene fusion ($P < 0.001$). (B) Status of gene fusions and ERG staining by FISH (a, c) or immunohistochemical expression of ERG (b, d). Note the blue signal that was associated with a red signal in another allele was deleted (arrow) in B-a. (a) Case with positive gene fusion (subtype: deletion); (b) positive ERG staining in the same case as in (a); (c) case with negative gene fusion; (d) negative ERG staining in the corresponding case of (c).

and a higher pathological stage compared with the German patient group ($P < 0.001$ and $P < 0.001$, respectively). The median follow-up durations were 53.6 years (range 6.3–106.6) in Japanese patients and 25.6 months (range 0.2–33.3) in German patients.

Table 2. Prevalence and type of *TMPRSS2-ERG* fusion in prostate cancer

	Japanese	German	P-value
All cases			
Number of positive gene fusion (%)	14 (20%)	19 (53%)	<0.001[†]
Gene fusion type			
Translocation	10/14 (71%)	6/19 (32%)	0.024[‡]
Deletion/increase of copy number	4/14 (29%)	13/19 (68%)	
Cases with ≤pT2 and Gleason Score ≤6			
Number of positive gene fusion (%)	1 (6%)	13 (50%)	0.003[†]
Gene fusion type			
Translocation	1/1 (100%)	5/13 (38%)	0.009[‡]
Deletion/increase of copy number	0/1(0%)	8/13 (62%)	
Cases of ≤ pT2 and Gleason Score >7			
Number of positive gene fusion (%)	7 (22%)	6 (60%)	0.050[†]
Gene fusion type			
Translocation	4/7 (57%)	1/6 (17%)	0.013[‡]
Deletion/increase of copy number	3/7 (43%)	5/6 (83%)	

[†]Fisher's exact test and [‡]Chi-square test were used for statistical analyses.

***TMPRSS2-ERG* fusions in Japanese and German patients.** The status of the *TMPRSS2-ERG* fusion in both groups is summarized in Table 2. Overall, gene fusion was identified in 31% (33/105) of prostate cancer cases. A significantly higher frequency of gene fusion was observed in German patients (19/36, 53%) than in Japanese patients (14/69, 20%) ($P < 0.001$). In several cases, cancer cells contained a heterogeneous combination of different types of gene fusion events, with translocation, deletion, and an increase of copy number observed. Among 14 gene fusion cases in Japanese patients, 71% (10/14) had translocation, 21% (3/14) had deletion, and 7% (1/14) had an increase in copy number. In German patients, 32% (6/19) had translocation, 68% (13/19) had deletion, and no cases showed an increase in copy number. Only one case with an increase in copy number was detected in both groups.

Table 3. Univariate and multivariate analysis for *TMPRSS2-ERG* fusion in prostate cancer

	Univarate	Multivariate	
Variables	P-value	OR(95% CI)	P-value
Race (Japanese vs German)	<0.001	3.91(1.59-10.03)	0.003
pT stage (≤pT2 vs ≥pT3)	0.22		
Gleason score (≤6 vs ≥7)	0.082	0.54(0.07-2.32)	0.43

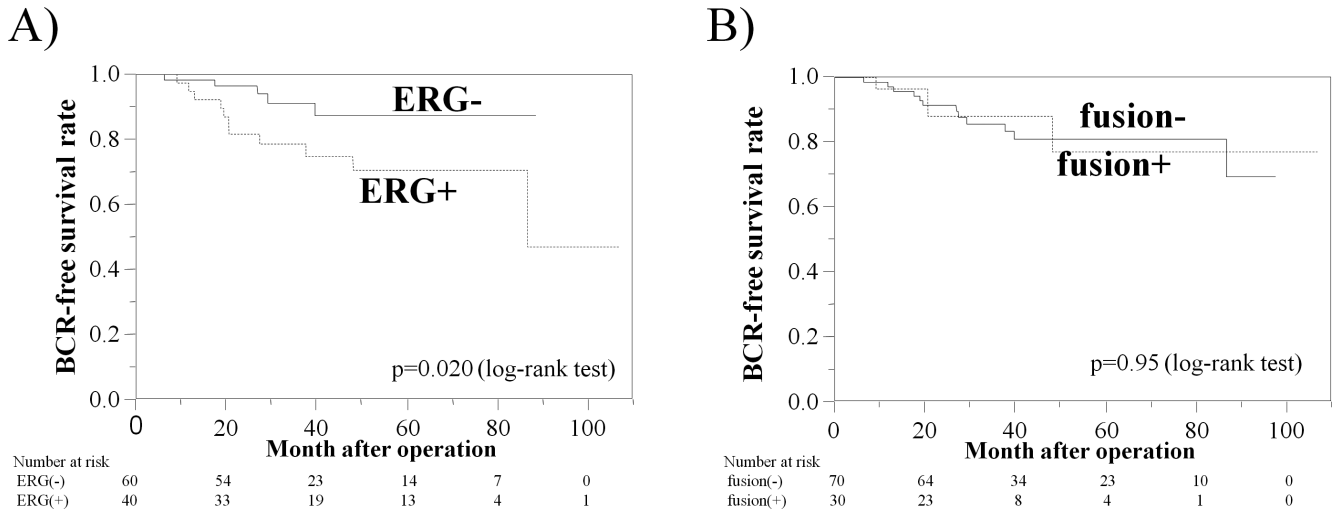Logistic regression were used for statistical analyses.

A)

B)

Figure 3. Biochemical recurrence-free survival rate according to presence of ERG expression (A) and gene fusion (B)

Therefore, we categorized fusion subtypes into two groups for analysis: translocation or deletion/increase in copy number. The *TMPRSS2-ERG* fusion subtype also differed between both ethnic groups (*P* = 0.024). Between the two groups, there was a statistically significant difference in the Gleason score and

pT stage, thus we matched these different characteristics. After adjusting for the Gleason score and pT stage, the German group had a significantly higher prevalence of *TMPRSS2-ERG* fusion and the Japanese group had more translocations (Table 2). We also analyzed factors including races, pT stage

Table 4. Relationship between type of *TMPRSS2-ERG* fusion and other clinicopathological parameters

Characteristic	Gene fusion (−)	Gene fusion (+)	P-value[1]	Gene fusion (+)		P-value[2]
				Translocation	Deletion/increase of copy number	
Age (years)						
	66.4±5.5	65.8±5.4	0.81[†]	65.8±5.7	65.8±5.3	0.96[†]
Preoperative PSA (ng/mL)						
	18.1±17.6	12.0±6.9	0.62[†]	11.3±7.2	13.0±8.3	0.57[†]
Gleason score						
4–6	31	14	0.80[†]	6	8	0.61[†]
7	28	17		9	8	
8–10	13	2		1	1	
Tumor stage						
≤pT2	58	27	0.40[‡]	11	16	0.54[‡]
≥pT3	14	3		2	1	
LVI						
Positive	24	5	0.41[‡]	4	1	0.20[‡]
Negative	30	5		2	3	
SM						
Positive	37	5	0.58[‡]	3	2	1.00[‡]
Negative	18	5		3	2	
Biochemical recurrence						
Yes	13	4	0.65[‡]	1	3	0.43[‡]
No	59	26		12	14	

Abbreviations: LVI, lymphovascular invasion; SM, surgical margin
Data are presented as mean±SD.
P[1] shows the P-values from the comparison of normal cases and cases with any gene fusions. P[2] shows the P-values from the statistical test of any differences between cases with translocation and deletion/increase of copy number. Statistical analyses were used as follows: [†]Wilcoxon rank sum test and [‡]Chi-square test.

and Gleason score against *TMPRESS2-ERG* gene fusion using multivariate analysis (Table 3). Race was independent factor for *TMPRESS2-ERG* gene fusion.

Relationships between *TMPRSS2-ERG* fusion and clinicopathological parameters. The comparisons of the class of *TMPRSS2-ERG* fusion and several clinicopathological variables are summarized in Table 4. No significant association was observed between clinicopathological parameters and status of *TMPRSS2-ERG* fusion or fusion subtype.

Correlation of *TMPRSS2-ERG* gene fusion with ERG expression. Overexpression of ERG protein was significantly associated with gene fusion ($P < 0.001$) (Fig 2A), while no fusion-negative tumors showed ERG overexpression (Fig 2A, B). No association was found between ERG expression and the subtype of gene fusion (data not shown).

Patient prognosis according to *TMPRSS2-ERG* fusion status and ERG expression level. BCR was observed in 17 patients (16.2%) at a median of 20.5 months (range: 6.2–86.5). No patient died of prostate cancer. Cases with ERG overexpression had a significantly shorter time to progression than those without overexpression (Fig 3A), while *TMPRSS2-ERG* fusion was not related to BCR in our series (Fig 3B). Among cases with gene fusion, the incidence of BCR was higher in cases with a deletion/increase in copy number than in translocation cases, although the difference was not statistically significant (Fig 4).

Correlations with type of *TMPRSS2-ERG* fusion and p53 overexpression. We next examined p53 overexpression by immunohistochemistry to elucidate the possible relationship between p53 status and gene fusion. Cases with gene fusion were significantly associated with increased p53 expression ($P = 0.011$) (Table 5). In regard to the type of gene fusion, cases with a deletion or increase in copy number had marginal association with p53 overexpression ($P = 0.054$, Table 5).

To examine the relationship between *TMPRSS2-ERG* fusion with proteins known to play important roles in prostate cancer, we next examined the expression levels of PTEN, NKX3.1, Ki-67, and AR (Table 6). Results showed that the expression levels of these four proteins were not associated with *TMPRSS2-ERG* fusion. However, NKX3.1 expression was significantly correlated with AR and Ki-67 expression ($P < 0.001$ and $P < 0.001$, respectively) as well as p53 expression ($P = 0.003$). The expression level of PTEN was significantly
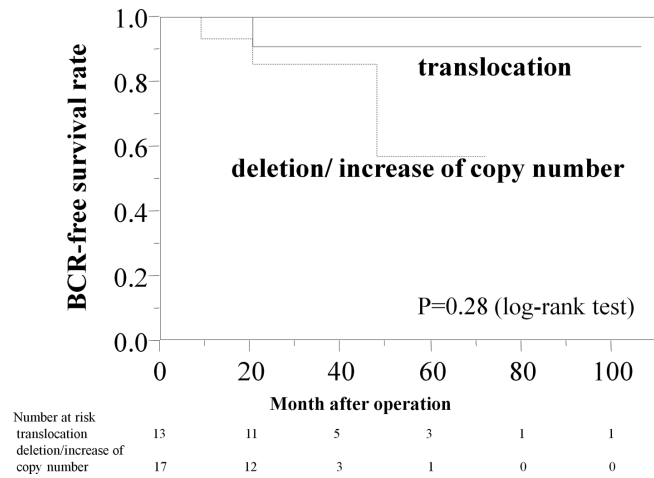
associated with ERG expression ($P = 0.002$). AR expression was also correlated with p53 expression ($P = 0.003$).

Discussion

The frequency of incidence of the *TMPRSS2-ERG* fusion in prostate cancer was significantly different between Japanese and German patients (20% vs. 53%, respectively). This result is in accordance with previous studies in Caucasian cohorts,

Figure 4. Biochemical recurrence-free survival rate according to gene fusion types

Table 5. Relationship between p53 mutations and type of *TMPRSS2-ERG* fusions

	p53 mutations		
	Negative	Positive	P-value
TMPRESS2-ERG fusions			
Gene fusion (+)	13	20	0.011[†]
(−)	48	24	
Gene fusion type			
Translocation	9	7	0.054[‡]
Deletion/increase of copy number	4	13	

Table 6. Correlation between immunohistochmeical expression in prostate cancer

	NKX3.1	PTEN	Ki-67	AR	p53
ERG	0.13 (*P*=0.17)	0.31 (*P*=0.002)	0.18 (*P*=0.070)	0.17 (*P*=0.087)	0.03 (*P*=0.73)
NKX3.1	–	0.11 (*P*=0.27)	0.37 (*P*<0.001)	0.43 (*P*<0.001)	0.28 (*P*=0.003)
PTEN		–	0.07 (*P*=0.48)	0.17 (*P*=0.080)	0.14 (*P*=0.17)
Ki-67			–	0.12 (*P*=0.21)	0.09 (*P*=0.31)
AR				–	0.26 (*P*=0.003)
p53					–

Linear regression analysis were used for statistical analysis. Correlation coefficient (r) and P-value were shown in the Table.

which reported frequencies of 46–80% [3-6], and in Asian cohorts, which reported frequencies of 16–28% [4, 9, 18]. Several studies have described the different incidence of the *TMPRSS2-ERG* fusion in Caucasians and non-Caucasians. Magi-Galluzzi et al. reported a significant difference in the prevalence of *TMPRSS2-ERG* in Caucasian, African-American, and Japanese prostate cancer patients [4].

The genes for *TMPRSS2* and *ERG* are located approximately 3 Mb apart in the same orientation on chromosome 21q22.2. The rearrangement between the two genes occurs via intra-chromosomal deletion or translocation of the intervening region to another chromosome [19, 20]. The gene fusion subtypes (translocations, deletions, and increase in copy number) were significantly different among ethnic groups. Magi-Galluzzi et al. reported that translocation was the predominant subtype in Caucasians and Japanese patients (62% and 71%, respectively), while deletion was dominant in African-American patients (80%) [4]. The higher frequency of gene fusion deletion events in African-Americans may be one of the explanations for the worse prognosis of prostate cancer in African-Americans compared with other ethnic groups. These data partially support our result showing that translocation was dominant in the Japanese patients (71%), whereas deletion was dominant in the German patients. The discrepancy may be attributable to the different patient characteristics. Lower incidence and different subtype of *TMPRSS2-ERG* fusions may imply different pathways of prostate carcinogenesis between Caucasians and non-Caucasians.

The deletion and increase in copy number subtypes may confer more aggressive biological behavior to the *TMPRSS2-ERG* fusion [10, 22]. These reports may support our data that the incidence of BCR had a higher tendency of deletion/increase in copy number than translocation. Whether the *TMPRSS2-ERG* fusion affects patient prognosis remains controversial [3, 9, 12, 23]. Meticulous observation of gene fusion subtype seems to be important for the prediction of prostate cancer with gene fusion. Presumably, gene fusion is one of the genetic steps in the oncogenesis of prostate cancer, particularly the dominant form in Caucasians.

Although ERG overexpression is a rare event in non-neoplastic prostate tissue, most prostate cancer patients with *TMPRSS2-ERG* fusion have ERG overexpression [8, 16, 24, 25]. The incidence of ERG overexpression and gene fusion was 42% and 31%, respectively. The concordance rate between two methods was 66.7%. As regards race, the rates in Japanese and German were 78.6% and 57.9%, respectively (p=0.273). The discrepancy of IHC and FISH analysis results may be explained by several possibilities. Heterogeneity of genomic fusion has been previously reported in prostate cancer [11, 17, 26]. The *TMPRSS2-ERG* fusion might not be the only factor involved in ERG overexpression. Consistent with our findings, Spencer et al. suggested that *TMPRSS2-ERG* fusion itself does not increase the proliferation and progression of prostate cancer cells [7]. Alternatively, the discrepancy may be attributed to the difference in detection power between the different meth-

odologies. A previous study clearly demonstrated that ERG is a key player of prostate cancer migration and invasion [27]. Minner et al. suggested that intrafocal heterogeneity challenges the concept of *TMPRSS2-ERG* fusion always representing an early step in prostate cancer development [26]. Taken together with the previous reports and our data, ERG overexpression seems to be critical for the estimation of aggressive behavior of prostate cancer and may become an important prognostic marker reflecting *TMPRSS2-ERG* fusion.

To explore the influence by which *TMPRSS-ERG* fusion might affect the development and progression of prostate tumorigenesis, we examined expression levels of several proteins that are known to be important for prostate carcinogenesis. Previous studies discussed that the influence of ERG rearrangements cooperating with genetic loss or alteration mutation of PTEN and p53. [22, 27-29]. NKX3.1 expression level was positively correlated with AR expression level, which was consistent with previous studies [30, 31]. We found that the level of PTEN expression was positively associated with ERG expression. Prostate cancers with *PTEN* deletion are well known to have more aggressive behavior [28]. On the other hand, ERG rearrangements have not found an increase in frequency in relation to disease stage [32]. Therefore, ERG rearrangements seem to occur prior PTEN loss. Probably, PTEN loss and ERG rearrangements are independent events each other.

In this study, immunohistochemical detection of p53 expression was used to detect p53 mutations. The p53 antibody (clone DO-7) recognizes an epitope located within amino acids 35-45 of the N-terminus of both wild type and mutant types of p53. Wild-type p53 protein has a very short half-life, whereas mutated p53 is stable and accumulated at high concentration in the nuclei of cells. As consequence, immunohistochemical staining with the specific antibody can be used to detect mutant p53 proteins. Our previous study demonstrated that the concordance rate between immunohistochemistry and p53 point mutation was 80% [33]. P53 mutations have been reported in 3–20% of prostate cancers and are often correlated with BCR and grade of the tumor with an aggressive clinical course [22, 34-36]. We could not find the relationship of Gleason score with expression level of p53, which might result from the different characteristics of our specimen which were collected from localized prostate cancers. The strong correlation of p53 overexpression with alteration of *p53 gene* detected by polymerase chain reaction-single strand conformation polymorphism [37]. We observed that overexpression of p53 protein was associated with *TMPRSS2-ERG* fusion. Moreover, p53 overexpression were found more frequently in the deletion and increase in copy number cases than in translocation *TMPRSS2-ERG* fusion cases. A prolonged exposure of androgen to non-malignant prostate epithelial cells induces the *TMPRSS2-ERG* fusion transcript [38]. Inactivation of DNA damage checkpoint proteins, such as ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) protein, is a crucial step in promoting androgen-induced

genomic instability. Cdc25A and p53 are major downstream effectors of the ATM/ATR DNA damage checkpoints. Chiu et al. demonstrated that androgen regulates the protein level of Cdc25A, but not p53, through ATM in LNCaP cells. Although androgen treatment did not alter the protein level of p53 in their experiments, p53 mutations may play an important role in the escape from the DNA damage checkpoint in prostate cancer cells [39]. Moreover, p53 overexpression represses androgen-induced transactivation of NKX3.1 by inhibiting the promoter of the AR gene and blocking AR DNA binding activity [40]. Because p53 negatively regulated androgen-induced events, p53 mutations might enhance androgen-related phenomenon including genomic instability. Indeed, we observed that expression of NKX3.1, which was upregulated by AR [31], was associated with p53 overexpression in this study. Hence, our results might indicate that p53 mutations might drive more aggressive arrangements of *TMPRSS2-ERG* fusion in prostate cancer. In contrast, Yang Zonga et al. reported to determine whether loss of p53 can collaborate with ERG overexpression during the development of PCa, prostate cells from adult p53-null mice were transduced with either ERG or control lentivirus and implanted in the prostate regeneration assay. They found no evidence to support cooperation between loss of p53 and increased expression of ERG, suggesting that two oncogenic influences do not always collaborate to advance the disease [41]. Carcinogenesis involves multiple genetic alterations and further accumulation of genetic alteration occurs during tumor progression. Our cohorts contain different phases of tumor progression. Therefore we believe our logic that p53 mutations drive more aggressive arrangements of *TMPRSS2-ERG* fusion in prostate cancer is suitable. Further studies are needed to determine whether p53 mutations may affect the occurrence of *TMPRSS2-ERG* fusion.

The present study has some limitations. First, we used a FISH break-apart assay, so called two-color FISH assay for detection of TMPRESS2-ERG gene fusion. Since the specific 5'-partner is not identified by the break-apart assay, the frequency of TMPRESS2-ERG fusion might be overestimated comparing with three-color FISH analysis. Second, our cohorts limited the cases with radical prostatectomy. So we could not estimate more aggressive prostate cancer in this study.

In conclusion, our study showed that the prevalence and type of *TMPRSS2-ERG* fusions are significantly different in Japanese and German prostate cancer cases. ERG expression, but not the presence of the *TMPRSS2-ERG* fusion, was associated with biochemical recurrence after radical prostatectomy. Association of specific gene fusions harboring higher genomic alterations with p53 mutations suggests that p53 mutations might drive more aggressive rearrangements of *TMPRSS2-ERG* fusion in prostate cancer.

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