RESEARCH ARTICLE

TMPRSS2:ETS Fusions and Clinicopathologic Characteristics of Prostate Cancer Patients from Eastern China

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Abstract

TMPRSS2:ERG gene fusions in prostate cancer have a dominant prevalence of approximately 50.0%, but infomration is limited on differences among ethnic and geographical groups. Some studies focusing on Japanese and Korean patients reported a lower incidence. Investigations concerning Chinese revealed controversial results. We evaluated TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4 fusions in more than 100 Eastern Chinese prostate cancer patients. Paraffin blocks of needle biopsy and radical prostatectomy were collected from 91 and 18 patients respectively. All patients' clinicopathologic factors were gathered. TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4 fusions were tested by multi-probe fluorescence in situ hybridization (FISH) assay. TMPRSS2:ERG fusions was present in 14.3% biopsy specimens and 11.1% radical prostatectomy patients. Neither TMPRSS2:ETV1 nor TMPRSS2:ETV4 fusion was found in any case. Altogether, 13 (86.7%) TMPRSS2:ERG fusion positive cases possessed deletion pattern and 7 (46.6%) and insertion pattern. Some 5 cases had both deletion and insertion patterns. While 38.5% (5/13) patients with deletion pattern had distant metastasis, except for one metastatic case harboring both deletion and insertion, there were no patients with insertion pattern accompanied with metastasis. There were no differences between fusion positive and negative cases in the distribution of age, PSA, Gleason score and TNM stage. Eastern Chinese prostate cancer patients have a significantly low incidence of TMPRSS2:ERG fusion. They also lack TMPRSS2:ETV1 and TMPRSS2:ETV4 fusion. There are more deletion pattern than insertion pattern in TMPRSS2:ERG positive cases. Fusion positive and negative patients have no clinicopathologic factor differences.

Keywords: Prostate cancer - Chinese - gene fusion - TMPRSS2 - ETS

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Introduction

Since first observed gene fusions in prostate cancer of androgen-regulated trans-membrane-serine protease gene (TMPRSS2) and erythroblast transformation-specific (ETS) family members (Tomlins et al., 2005), much progress has been made not only in the understanding of fusion mechanism, but also in the transferring to clinical practice. Hessels detected TMPRSS2:ERG in post-DRE urine and reported the test a sensitivity of 37%, a specificity of 93%, and positive predictive value of 94% (Hessels et al., 2007). An observation was published that 83% of castration-independent prostate cancer patients with TMPRSS2:ERG fusion had a decrease in PSA following treatment of Abiraterone (Attard et al., 2008).

Up to date, more than 20 fusion types have been found. Among these, TMPRSS2: ERG fusion has a dominant prevalence of approximately 50%, compared with other fusion type less than 15% incidence. However, some studies focusing only on Asian people revealed a prevalence of TMPRSS2:ERG differed from these reports mainly concerned patients of western countries (Lee et al., 2010; Miyagi et al., 2010; Rawal et al., 2013). Six investigations studied Chinese cases, but the outcomes of which were quite dissimilar. The lowest incidence of TMPRSS2:ERG is 7.5%, while the highest is 90% (Dai et al., 2008; Mao et al., 2010; Sun et al., 2010; Xiang et al., 2011; Ren et al., 2012; Wang et al., 2012). Considering the prostate cancer prevalence and aggressiveness, as well as genomic alterations, vary in different ethnic origin and geographic locations (Grönberg et al., 2003; Sim et al., 2005), it is necessary to figure out the prevalence of fusion between TMPRSS2 and ETS family members in prostate cancers patients from eastern China and to explore differences in fusion rates in Different areas of China, other parts of Asia, Europe, and USA.

In this study, we evaluated TMPRSS2:ERG, TMPRSS2:ETV1, and TMPRSS2:ETV4 fusions in more than 100 eastern Chinese prostate cancer patients, using multi-probe fluorescence in situ hybridization (FISH) assay. The specimens were obtained by 12 core needle biopsies and radical prostatectomy, which were analyzed separately. The fusion result was studied with patients' clinicopathologic factors (age, PSA, Gleason score, TNM

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stage). And the fusion pattern was also investigated. All patients provided written, informed consent in accordance with the institutional guidelines.

Materials and Methods

Study population and Specimens selection for FISH

Paraffin blocks needle biopsies were collected from 91 cases of eastern Chinese prostate cancer patients who were diagnosed in Huadong Hospital, Shanghai China from October 2010 to February 2012. Their age ranged from 55-90, with the median age of 75. The number of early stage (≤pT2b, without lymph node and bone metastasis) patients is 31 (34.1%), the local advanced (>pT2b, with or without regional lymph node but without bone metastasis) patients is 36 (39.6%) and metastatic patients is 24 (26.4%). The number of Gleason score<7, =7, >7 patients is 9, 43, 39, respectively. 14 cases PSA<10ng/ml, 16 cases PSA 10-20 ng/ml, and 61 cases PSA >20ng/ml. Additional, we observed 18 cases of Paraffin blocks gained from radical prostatectomy during the same period. Every case was diagnosed by two independent pathologists from our pathology department.

Tissue from paraffin blocks were obtained for the following procedure. For every fusion types, we selected at least three 3um tissue sections from each needle biopsy case. The sections came from different cores which contained cancer. We tried to choose scattered cores in order to reduce the influence of heterogeneity of prostate cancer. For radical prostatectomy paraffin blocks, sections were collected from different cancer foci.

FISH analysis

We used bacterial artificial chromosome clones as probes. TMPRSS2: ERG fusion was tested by a break-apart probe system, using two chromosomes, RP11-95I21 (5'ERG) and RP11-476D17 (3'ERG). The RP11-95I21 was labeled by nick translation with tetramethylrhodamine and RP11-476D17 with fluorescein. A dual-color dual-fusion model probes were introduced to identify TMPRSS2:ETV1 and TMPRSS2:ETV4 fusion. And chromosomes used were: TMPRSS2 (RP11-814F13, RP1-265B9, and CTD-2337B13), ETV1 (RP11-692G10, RP5-856O24, and CTD-2134C13), ETV4 (CTD-2326M16, RP11-100E5, and CTC-420I11). TMPRSS2 was attached to tetramethylrhodamine, ETV1 and ETV4 to fluorescein. All the probes were purchased from Beijing GP Medical Technologies, Inc., P.R. China. FISH was performed as previous described with minor modifications (Tomlins et al., 2005). We boiled sections in deionized water at 90°C for 20 minutes and found a 4 minutes digestion with Proteinase K (100μg/ml) at 38°C was enough. Posthybridization washing was done with 2XSSC/0.1%NP-40 for 5 minutes and then dehydrate in an ethanol series (70%, 85%, 100%) for 2 minutes each. Section slides were observed under X100 oil immersion lens on an Olympus BX-51TRE microscope (Olympus, Tokyo, Japan) equipped with DAPI, green, orange, aqua, and triple-pass (DAPI/Green/Orange) fil-ters (Abbott-Vysis) and imaged with a CCD camera using the IMSTAR software system (IMSTAR S.A., Paris, France).

Criteria for fusion positivity

For TMPRSS2:ERG fusion, the normal signal pattern is two yellow (red/green fusion) signals in a cell (Figure 1A). And the positive signal patterns are one yellow/one green in a cell, which revealed a deletion pattern of fusion (Figure 1B), or one yellow/one green/one red, which illustrated an insertion pattern (Figure 1C). The criteria to determine a prostate cancer case TMPRSS2:ERG fusion positive was that five or more cells with the positive signal pattern were found in a random count of 400 cells.

The normal signal pattern for TMPRSS2:ETV1 or TMPRSS2:ETV4 fusion is two red/two green in a cell (Figure 2). And the positive pattern is an appearance of at least one yellow signal. After a random count of 400 cells, if there were more than two cells with positive signal pattern, we decided the case was TMPRSS2:ETV1 fusion positive. And the criteria for TMPRSS2:ETV4 fusion positive was at least one.

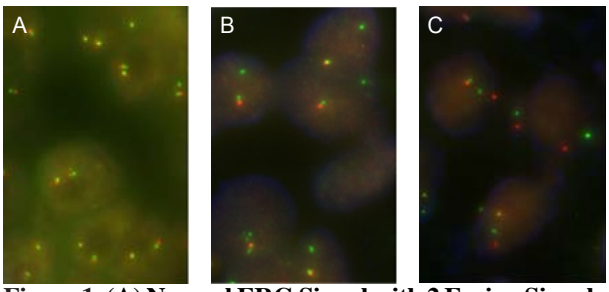

Figure 1. (A) Normal ERG Signal with 2 Fusion Signals (2 yellow); (B) Abnormal Fusion Signals, Deletion Pattern, Comprising a Green Signal, and a Fusion Signal (1 green and 1 yellow); (C) Abnormal Fusion Signals, Insertion Pattern, Comprising red Signal, a Green Signal, and a Fusion Signal (1 red, 1 green and 1 yellow)

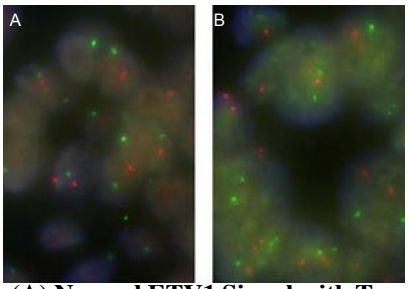

Figure 2. (A) Normal ETV1 Signal with Two Red and Two Green Signals; (B) Normal ETV4 Signal with Two Red and Two Green Signals

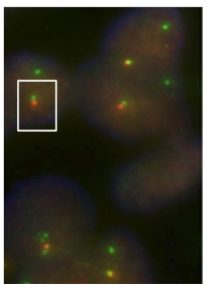

Figure 3. Normal ERG Signal, The Green and Red Signals in the White Rectangle Can be Distinguished, but it cannot be Considered as Fusion Positive Pattern

Statistical analysis

The chi-square test or Fisher's exact test were used to compare the results of TMPRSS2:ERG fusion in accordance with the patients' clinicopathologic factors (age, PSA, Gleason score, TNM stage). A P value of< 0.05 was considered to be statistically significant. All statistical analyses were conducted using SPSS v. 20.0.

Results

Among the 91 cases of needle biopsies, 13 (14.3%, 95% confidence interval, 7.1%-21.5%) patients were TMPRSS2:ERG fusion positive. There was neither TMPRSS2:ETV1 nor TMPRSS2:ETV4 fusion positive case. Two (11.1%, 95% confidence interval, 0%-25.6%) cases were found TMPRSS2:ERG fusion positive in the 18 radical prostatectomy patients. TMPRSS2:ETV1 or TMPRSS2:ETV4 fusion still lacked positive case. 5 patients' biopsies and radical prostatectomy specimens were both underwent FISH test. The biopsies showed no fusion positive, which corresponded with the outcomes of counter radical prostatectomy specimens.

Table 1. TMPRSS2: ERG Fusion Pattern

	Insertion Pattern (%)	Deletion Pattern(%)†	TNM Stage
1	-	+(49)	$T_{2C}N_0M$
2	-	+(69)	$T_{2C}N_OM_{1b}$
3	-	+(43)	$T_{2b}N_0M_0$
4	-	+(58)	$T_3N_1M_{1C}$
5	+(24)	+(36)	$T_{2a}N_0M_0$
6	-	+(41)	$T_{2a}N_XM_0$
7	-	+(58)	$T_3N_0M_{1b}$
8	-	+(22)	$T_{2C}N_XM_{1b}$
9	+(15)	+ (6)	$T_3N_0M_{1b}$
10	+(24)	+(15)	$T_{2C}N_0M_0$
11	+(42)	-	$T_4N_1M_0$
12	+(42)	-	$T_{2a}N_0M_0$
13	+(12)	+(31)	$T_2CN_0M_0$
14	+(18)	+(35)	$T_{4a}N_0M_0$
15	-	+(40)	$T_{2b}N_0M_0$

†Percentage in the random count of 400 cells

Table 2. Clinicopathologic Factors of Different TMPRSS2:ERG Fusion Outcomes

		TMPRSS2/ERG fusion(+)(%)	TMPRSS2/ERG fusion(-) (%)	*p* value
Age	<65	1 (7.7)	10 (12.8)	1
	65-75	7 (53.8)	30 (38.5)	
	>75	5 (38.5)	38 (48.7)	
Stage	Early Stage†	5 (38.5)	28 (35.9)	2.052
	Local Advanced‡	3 (23.1)	32 (41.0)	
	Metastasis†‡	5 (38.5)	18 (23.1)	
Primary	3	6 (46.2)	25 (32.1)	2.186
Gleason	4	6 (46.2)	50 (64.1)	
pattern	5	1 (7.7)	3 (3.8)	
Sum of	6	0 (0)	9 (11.5)	2.107
Gleason	7	8 (61.5)	35 (44.9)	
Score	8	4 (30.8)	21 (26.9)	
	9	1 (7.7)	13 (16.7)	
PSA (ng/ml)	<10	2 (15.4)	12 (15.4)	1.138
	10-20	2 (15.4)	14 (17.9)	
	>20,<100	4 (30.8)	32 (41.0)	
	≥100	5 (38.5)	20 (25.6)	

*†≤pT2b, without lymph-node and bone metastasis; ‡>*p*T2b, with or without regional lymph-node but without bone metastasis; †‡M1a-M1c

Table 3. TMPRSS2: ERG Fusion Results in Different Cancer Stage†

	TMPRSS2/ERG(+)	TMPRSS2/ERG(-)	All(%)
T1	0	8	8 (8.8)
T2a	3	8	11 (12.1)
T2b	2	11	13 (14.3)
T2c	4	25	29 (31.9)
T3	3	19	22 (24.2)
T4	1	4	5 (5.5)
M1a-1c	5	18	23 (25.3)

†: Only biopsy cases concluded

Altogether, there were 15 TMPRSS2:ERG fusion positive cases. 13 (86.7%) hold deletion pattern, 7 (46.7%) hold insertion pattern. And 5 cases had both deletion and insertion pattern (Table 1). 38.5% (5/13) patients with deletion pattern had metastasis. By contrast, 14.2% (1/7) patients with insertion pattern had metastasis. And the only metastatic case also had deletion pattern.

In the 91 biopsy cases, the median age of TMPRSS2:ERG fusion positive cases was 72, and TMPRSS2: ERG fusion negative cases was 75. There were no significant differences between TMPRSS2:ERG fusion positive and negative cases in the distribution of age, PSA, Gleason score and TNM stage (Table 2). TMPRSS2:ERG fusion seemed to appear at any stage of cancer (Table 3). Among the positive patients, there was no low risk prostate cancer (pSA<10ng/ml, Gleason score<7, ≤T2a), but 2 (15.4%) medial risk (10≤PSA≤20ng/ml, Gleason score=7, T2b) and 11 (84.6%) high risk (pSA>20ng/ml, Gleason score>7, ≥T2c). The number of negative cases was 5 (6.4%), 7 (9.0%), 66 (84.6%), respectively. There was also no significant differences in the distribution (p=1.036). There are two cases of TMPRSS2:ERG fusion positive in the 18 radical prostatectomy samples. Their clinicopathologic characteristics were Gleason score=6, T2N0M0, PSA=5.0ng/ml and Gleason score=9, T4aN0M0, PSA=40.1ng/ml, respectively. They both hold insertion pattern and deletion pattern.

Discussion

8 years after the first discovery of gene fusions in prostate cancer, relevant studies had already covered a large amount of samples, although mostly the western populations. Methods of PCR, FISH and immunohistochemistry have been introduced to investigate the types, prevalence and mechanism of gene fusions. TMPRSS2:ERG fusion has a prevalence ranging from 40%-70% depending on the clinical cohorts observed. Next to TMPRSS2:ERG, SPINK1-positive fusion and fusion including ETV1 have incidence of 10%-15%, respectively (Rubin et al., 2011). Other fusion types have been reported less than 5%. But studies focused on Asian patients observed different frequencies of gene fusions, especially TMPRSS2:ERG. Miyagi tested transcripts of TMPRSS2:ERG, TMPRSS2:ETV1, SLC45A3:ETV1, HERV-K:ETV1, C15ORF21:ETV1, HNRPA2B1:ETV1 by RT-PCR and found only 54 cases (28%) of TMPRSS2: ERG fusion positive and 2 cases (1%) of HNRPA2B1:ETV1 fusion positive

. Additionally, they also reported 5 cases (2.6%) of SLC45A3:ELK4 fusion positive (Miyagi et al., 2010). In Lee's investigation, only TMPRSS2:ERG was examined by FISH and the fusion positive was 20.9% (Lee et al., 2010). Furthermore, Magi-Galluzzi conducted a research compared the TMPRSS2:ERG fusion differences among Caucasian, African-American and Japanese Patients and reported that TMPRSS2:ERG fusion was present in 50.0% (21/42) of Caucasian, 31.3% (20/64) of African-American, and 15.9% (7/44) of Japanese, with largest difference between Caucasian and Japanese patients (p=0.001) (Magi-Galluzzi et al., 2011). Furusato analyzed the expression of the ERG oncoprotein in Japanese prostate cancer tissues and reported 20.1% (42/209) of expressing rate (Furusato et al., 2011). The observation of Japanese and Korean patients showed a significant lower incidence of fusion including ERG and ETV1.

Whether Chinese patients have a similar frequency like Japanese and Korean is still unknown. In present study, we found eastern Chinese prostate cancer patients had a lower incidence of TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4 fusion similar to Japanese and Korean, TMPRSS2:ERG incidence is 14.3% (13/91) (95% confidence interval, 7.1%-21.5%) by biopsy specimens or 11.1% (2/18) (95% confidence interval, 0%-25.6) by radical prostatectomy samples and TMPRSS2:ETV1 and TMPRSS2:ETV4 were found in none of the cases. However, the frequency of gene fusion varies among different studies of China, and the reasons maybe complex, probably owing to the sensitivity of the technique used, the number of samples included in the study, the criteria be used to determined a positive signal and the patients from different areas (northern, southern, eastern and western) of China (Dai et al., 2008; Mao et al., 2010; Sun et al., 2010; Xiang et al., 2011; Ren et al., 2012; Wang et al., 2012).

It has already been reported that smaller studies used PCR revealed higher incidence of ERG fusion than large samples (Cerveira et al., 2006; Soller et al., 2006; Wang et al., 2006). Two investigators from China reported TMPRSS2:ERG fusion rate were 50.3% (16/30) and 53.10% (17/32) (Dai et al., 2008; Xiang et al., 2011). But a relatively small amount of samples decreasing each one's conviction. The criteria determining a positive signal for TMPRSS2:ERG fusion influenced greatly on the outcomes. There were situations that the green and red signal could be distinguished but were very close in position (Figure 3). Actually, when the green and red signal was not separated for more than two signal space, it could not be considered as a fusion positive signal pattern. What's more, only when the signal between TMPRSS2 and ERG was missing, it can be considered as fusion of deletion pattern. The lost of the other signal only means the lost of other sequence on the chromosome rather than the key one. So in Sun study, the criteria 'one yellow/one green (or one red) represented abnormal signal patterns indicative of partial deletion' may include cases which were not fusion positive (Sun et al., 2010). Using FISH technique, Wang and Sun identified TMPRSS2:ERG fusion rate of prostate cancer patients from northern and southern China was 46% and 90%, respectively (Sun et al., 2010; Wang et al., 2012). Ren used RT-PCR to detect TMPRSS2:ERG 10 out of 54 (18.5%) cases of prostate adenocarcinoma from eastern China (Ren et al., 2012). The frequency was similar to our finding (14.3%) .

Collecting biopsy specimens as study object may underestimate the incidence of fusion positivity when prostate cancer posses multifocality with heterogeneity. Report had shown about 75% prostatectomy specimens had multiple cancer foci (Meiers et al., 2007). And 41% to 67% cases may differed among individual foci in the presence of gene fusion or the mechanism of fusion (Barry et al., 2007; Mehra et al., 2007; Clark et al., 2008; Furusato et al., 2008). Miyagi found 11 cases with multi foci, 6 of which is TMPRSS2:ERG fusion positive. And 5 of the positive cases showed heterogeneity among foci (Miyagi et al., 2010). The two fusion positive radical prostatectomy cases in our study also hold multi-foci with different fusion mechanisms. Heterogeneity did exist in Asian prostate cancer patients. However, compared with an investigation of 12 core needle biopsies in USA, Whose TMPRSS2:ERG fusion prevalence was 46% (46/100), we still hold a lower incidence (Mosquera et al., 2009).

Our study also concerned different characteristics related to TMPRSS2:ERG fusion pattern. We found deletion pattern count for 86.7% (13/15) of positive cases. While insertion pattern incidence was 46.7% (7/15). Correspondingly, Mao report 5 cases of deletion pattern and 2 cases insertion pattern (Mao et al., 2010). What Lee found was 64.2% (34/53) of deletion pattern. We also observed that 5 of 13 deletion pattern cases had metastasis. Except one metastatic case harbored both deletion and insertion pattern, there was no insertion pattern accompanied with metastasis. Tomlins had suggested that only one focus in a prostate cancer case is seeding metastatic deposits, based on the observation that all metastatic foci in an individual patient are uniformly positive or negative for ETS fusion (Tomlins et al., 2009). Because our small sample and lack of study on the metastatic foci, we could not think deletion pattern related to metastasis in Chinese cases. But it is worth further study.

There were no differences between fusion positive and negative cases in the distribution of age, PSA, Gleason score and TNM stage in our study. A majority of studies suggested that age did not correlate with TMPRSS2:ERG fusion (Magi-Galluzzi et al., 2011). In some studies, TMPRSS2:ERG fusion correlated with a more aggressive clinical outcomes, but others reported the opposite results (Cerveira et al., 2006). Large population-based investigation is needed and fusion types and fusion-transcript isoforms should be included in the analysis.

In conclusion, our study reveal eastern Chinese prostate cancer patients have a significant lower incidence of TMPRSS2:ETS fusion rate consistent with other results from eastern Chinese patients and other Asian countries patients. Our study also find TMPRSS2: ERG fusion positive cases harbor more deletion pattern than insertion pattern. There is a possibility that deletion pattern may correlate with distant metastasis in eastern Chinese patients. As low incidence, it seems that the application of TMPRSS2:ERG fusion in diagnosis and treatment will be limited in eastern Chinese. But, we still need larger

samples to support our view and discover the relation between fusion pattern and clinical outcomes.

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