

RESEARCH ARTICLE

Fusion Between TMPRSS2 and ETS Family Members (ERG, ETV1, ETV4) in Prostate Cancers from Northern China

Jian-Jiang Wang, Yue-Xin Liu*, Wei Wang, Wei Yan, Yu-Peng Zheng, Lu-Dong Qiao, Dan Liu, Shan Chen

Abstract

In this study we evaluated the frequency of fusion between TMPRSS2 and ETS family members (ERG, ETV1, ETV4) in prostate cancers in patients from northern China in order to explore differences in fusion rates among regions in northern and southern China, other parts of Asia, Europe, and North America. We examined 100 prostate cancer patients, diagnosed by means of prostate biopsy; fluorescence in situ hybridization (FISH) was used to detect the expression of TMPRSS2, ERG, ETV1 and ETV4 in cancer tissue. Differences in gene fusion rates among different ethnic groups were also analyzed. Of the 100 prostate cancer patients, 55 (55%) had the fusion gene. Among the patients with the fusion gene, 46 (83.6%) patients had the TMPRSS2:ERG fusion product, 8 (14.8%) patients had TMPRSS2:ETV1 fusion, 1 (1.6%) patient had TMPRSS2:ETV4.

Keywords: Prostate cancer - fluorescence - immunity hybrid - gene fusion

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Introduction

Prostate cancer is a malignant tumor of the urinary system, commonly occurring in elderly men and its incidence is increasing (Weir et al., 2003). Approximately 80% of prostate cancer patients are known to have the fusion proteins arising from the fusion of TMPRSS2 with members of the ETS family (ERG, ETV1, or ETV4); however, this phenomenon is not found in benign prostatic lesions. The most common fusion is that between TMPRSS2 and ERG or between ERG and other genes. The fusion products of TMPRSS2:ETV1 and TMPRSS2:ETV4 are less common (Huang et al., 2011). Different incidences of gene fusion have been reported in different regions of the world (Darnel et al., 2009; Lee et al., 2010; Mao et al., 2010; Rubio-Briones et al., 2010; Sun et al., 2010).

Recently, Chinese, Korean, and Japanese studies reported different incident rates of gene fusion in prostate cancer patients, even within Asia (Lee et al., 2010c; Mao et al., 2010b; Sun et al., 2010c). The relationship between the presence of the fusion product and PSA levels or cell classification is also not well defined (Tomlins et al., 2005; Tomlins et al., 2006; Darnel et al., 2009b; Lee et al., 2010b; Sun et al., 2010b). In this study, we used the fluorescence in situ hybridization (FISH, the fluorescence immunoassay, hybridization) technique to determine the relationships among the fusion gene, PSA, cell grade, and tumor stage in different ethnic, geographical groups. Further, prostate cancer detection kits containing TMPRSS2, ERG, ETV1, and ETV4 DNA-specific probes

were used for the detection of TMPRSS2:ETS fusion products in prostate biopsy specimens from northern Chinese cancer patients.

Materials and Methods

Materials

A total of 100 patients diagnosed with prostate cancer by prostate biopsy were selected. All specimens were obtained from patients during prostate biopsy or radical surgery.

We used the following reagents: xylene, deionized water, K protease, 2 × SSC (2 × sodium citrate buffer), 70% ethanol, 85% ethanol, 100% ethanol, 0.1% NP-40/2 × SSC, 70% ethanol, and 20-μl DAPI (4,6-diamidino-2-phenylindole dihydrochloride). TMPRSS2:ETV1, TMPRSS2:ERG, and TMPRSS2:ETV4 probes were obtained from GP Medical Technologies (Beijing).

Experimental procedures

1. Pretreatment: For FISH hybridization, the prepared slides (3-μm thick) were pretreated at 56 °C overnight, immersed in xylene for dewaxing twice (10 minutes each time) at room temperature, then in 100% ethanol for 5 minutes, followed by rehydration in 100% ethanol, 85% ethanol, and 70% ethanol for 2 minutes each at room temperature. They were then immersed in deionized water at room temperature for 3 minutes, followed by 95 °C water treatment for 20 minutes. Then, 40 mg of pepsin was dissolved in 40 ml 0.01 HCL to obtain a working solution

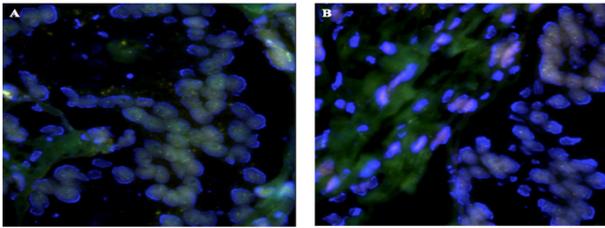


Figure 1. (A) Normal ERG Signal with 2 Fusion Signals; (B) Abnormal Fusion Signals, Comprising Red Signal, a Green Signal, and a Fusion Signal

of 1 mg/ml. The slides were treated with proteinase K solution at 37 °C for approximately 8 minutes, rinsed in 2 × SSC for 5 minutes, rehydrated in 70% ethanol, 85% ethanol, and 100% ethanol for 2 minutes each at room temperature, and air-dried for hybridization.

2. Denatured hybridization: For this process, 10 µl of the hybridization probe buffer (2 µl of the probe and 8 µl of the buffer) was centrifuged for 1–3 seconds and added dropwise to the slides, which were immediately covered with coverslips and sealed with rubber cement. The slides were then denatured at 86 °C for 10 minutes, and then placed in a wet box for hybridization at 42 °C, for 16 hours (Note that the humidity of the wet box was maintained).

3. Washing and staining: After removing the coverslips, the slides were placed at 46 °C, in 0.1% NP-40/0.4 × SSC solution, rinsed for 5 minutes, placed in 70% ethanol, rinsed for 3 minutes at room temperature and air-dried. The slides were put in the dark and 20 µl of DAPI were added for 10–20 minutes and then observed under a fluorescence microscope. The slides were stored at -20 °C in the dark.

Interpretation of FISH results

Gene fusion detection by FISH For TMPRSS2:ETV1, 2 red signals and 2 green signals were considered normal; the red signal indicated the presence of TMPRSS2; the green signal indicated the presence of ETV1. Further, 1 red, 1 green, and 2 fusion signals, indicated that TMPRSS2 was fused with ETV1. For TMPRSS2:ERG, 2 yellow signals (indicating 2 fusions) were considered normal; 1 yellow signal and 1 green signal indicated fusion; 1 yellow signal, 1 red signal, and 1 green signal indicated that fusion or reconstruction occurred on the ERG gene. For TMPRSS2:ETV4, 2 red signals and 2 green signals were considered normal. One red signal together with 1 green signal and 2 fusion signals, suggested TMPRSS2:ETV4 fusion.

Threshold definition

Ten paraffin-embedded specimens of benign prostatic hyperplasia were randomly chosen for FISH analysis. We analyzed 100 cells per sample to calculate the mean and standard deviation of the percentage of cells with abnormal signals. Abnormal threshold value was defined as the average plus 3 standard deviations (SDs), Exception threshold = mean (M) + 3 SDs. Thus, 100 cells were randomly counted for each sample and abnormal threshold was used to determine the test results. If the percentage of cells with abnormal signal was greater than the threshold value (3.9%), the test results for gene

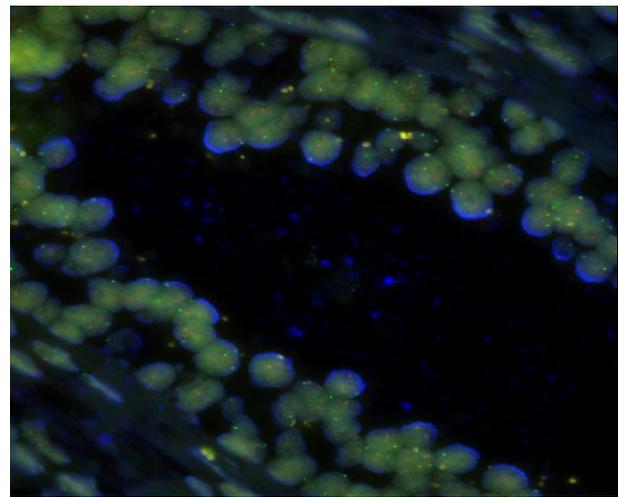


Figure 2. Abnormal ETV1 Signal, Showing 1 Red and 1 Green Normal Signals and 2 Fusion Signals (1 yellow and 1 red)

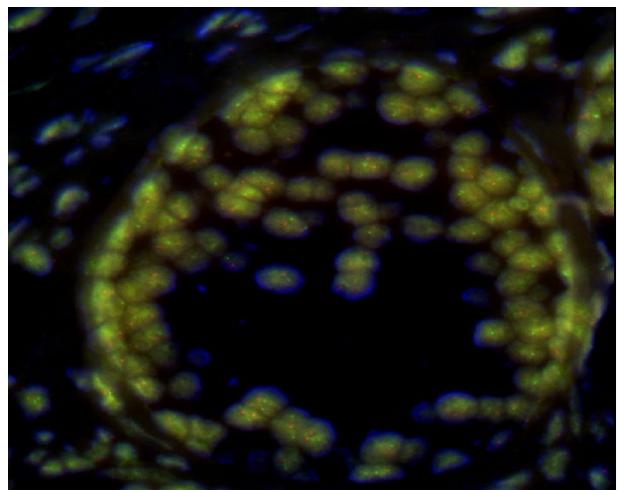


Figure 3. Abnormal ETV4 Signal, Showing 1 Red, 1 Green Normal Signals, and 2 Fusion Signals, of Which 1 is Yellow, 1 Red, and 1 Green

fusion were considered positive, whereas if the percentage of cells with abnormal signal was below the threshold value, the test result for gene fusion was considered negative. If the percentage of cells with abnormal signal was equal to the threshold, the cell number of the sample was increased to determine the final outcome. FISH was performed to detect TMPRSS2:ETV1, TMPRSS2:ETV4 and TMPRSS2:ERG gene fusion in HGPIN and prostate cancer sections.

Statistical analysis

All data were analyzed with SPSS13.0 statistical software. Chi-square test was used in the gene fusion study of low-risk group and high-risk group patients.

Results

Figure 1. (A) Normal ERG signal with 2 fusion signals; (B) Abnormal fusion signals, comprising red signal, a green signal, and a fusion signal. Figure 2. Abnormal ETV1 signal, showing 1 red and 1 green normal signals and 2 fusion signals (1 yellow and 1 red). Figure 3. Abnormal ETV4 signal, showing 1 red, 1 green normal

signals, and 2 fusion signals, of which 1 is yellow, 1 red, and 1 green.

In all, 55% of the prostate cancer patients contained the gene fusion products. In 51 (83.6%) of these patients, the fusion involved the ERG gene, in 9 (14.8%) patients the ETV1 gene, and in 1 (1.6%) patient the ETV4 gene.

In conclusions, cancer patients from northern China had a lower rate of TMPRSS2:ETS gene fusion than patients from southern China ($P=0.000$). TMPRSS2:ETS gene fusion may be related to geography, dietary habits, and environmental pollution.

Discussion

In recent years, with the development of medical genomic studies, the understanding of the occurrence and development of prostate cancer has improved greatly. The diagnosis of prostate cancer has become more accurate owing to the use of molecular cytogenetic techniques. Approximately 80% of prostate cancers are characterized by the presence of the fusion products, produced by the fusion between the TMPRSS2 gene and the ETS gene family members (ERG, ETV1, ETV4). Fusion between TMPRSS2 and ERG, or ERG and other loci is the most commonly occurring fusion, followed by the fusion between TMPRSS2 and ETV1, and then TMPRSS2 and ETV4.

However, no such genetic changes have been found in benign prostatic tissues. The fusion gene TMPRSS2:ETS is found to be an early event in prostate cancer (Huang et al., 2011b). The incidence of prostate cancer shows geographical and ethnic differences, with the highest incidence in Australia, New Zealand, Caribbean, and Scandinavian regions and a lower incidence in Asia and North Africa. Prostate cancer ranks second among all common malignant tumors in males worldwide, but it ranks first in the United States. In China, the incidence of prostate cancer is relatively low, but is rising rapidly (Song et al., 2010). It was recently reported that in Shanghai, in 2007, prostate cancer was the fifth most common cancer in males (Sun et al., 2004). TMPRSS2:ERG and TMPRSS2:ETV1 fusion genes have been found in prostate cancer patients with a total incidence rate of 79.3% (23/29 patients, 16/29 patients with TMPRSS2:ERG, and 7/29 patients with TMPRSS2:ETV1) as reported by Chaohong He and Tomlins (Tomlins et al., 2006d, Tomlins et al., 2005d). The FISH technique was used to detect the fusion gene in prostate cancer tissues from 254 patients who underwent radical prostatectomy in Korea, and 20.9% (53/254) of the patients were found to have the fusion gene, with the majority of them with a Gleason score of less than 7 (Lee et al., 2010d). The rate of gene fusion was 50% (21/42) in Caucasians, 31.3% (20/64) in African Americans and 15.9% in Japanese populations, suggesting a possible relationship with race, but not with age, preoperative PSA levels, or pathological Gleason score. In the literature, the data available for the Chinese population is very limited. The gene fusion rate was found to be 90% in 50 Chinese prostate cancer patients (Sun et al., 2010e), which was higher than that reported in other countries; this may be due to several factors, such as the

samples selected, patients' age, preoperative PSA levels, tumor stage, or Gleason scores. However, this high incidence rate may also suggest a higher gene fusion rate in the Chinese population, although this is inconsistent with previous reports. Indeed, there is evidence that the rate of TMPRSS2:ETS gene fusions in prostate cancer patients was lower in China than in the Western countries (Mao et al., 2010d). Considering the limited number of reports in China and the limited number of cases studied by Xin Gao et al., the accurate gene fusion rate in Chinese prostate cancer patients is still unclear. Moreover, no report has shown any difference in the gene fusion rate between high-risk and low-risk prostate cancer patients.

In order to detect the fusion products in Chinese prostate cancer patients and to compare the gene fusion rate between low-risk and high-risk patients, we used the FISH technique. A total of 100 prostate cancer specimens from patients diagnosed by prostate biopsy 3–5 years ago were selected. We carried out analysis for detection of gene fusion between TMPRSS2 (21q22.2) and ERG (21q22.3), TMPRSS2 (21q22.2) and ETV1 (7p21.2), and TMPRSS2 (21q22.2) and ETV4 (17q21).

Our results showed that the total gene fusion rate in these patients was 55% (55/100). In 46 cases (83.6%) TMPRSS2 was fused with ERG, in 8 (14.5%) with ETV1, and in 1 (1.8%) with ETV4. This result was consistent with those of previous reports (Tomlins et al., 2006d, Tomlins et al., 2005d, Huang et al., 2011c). The rate of gene fusion reported in our study was lower than that reported by Xin Gao et al. in Southern China, but was significantly higher than those reported in Japanese and Korean studies. Furthermore, our data showed that the gene fusion rate in prostate cancer patients in northern China was different from that in the prostate cancer patients in southern China and other parts of Asia, Europe, and the United States of America ($P = 0.000$), further validating the association between race the presence of gene fusion. The gene fusion rate in prostate cancer patients in northern China was lower than that in prostate cancer patients in southern China, but higher than those in other regions of Asia. Interestingly, the gene fusion rate in southern China was also higher than those in European, American, Caucasian, and African American prostate cancer patients. This was inconsistent with previous reports stating that the gene fusion rate was low in Asian prostate cancer patients (Mao et al., 2010c). Further studies are needed to validate these results because of the small size of the samples used. It is already known that the incidence of prostate cancer is correlated with age, race, diet, and environmental pollution. Dietary habits and environmental pollution and other factors may lead to a higher rate of gene fusion in China than in Japan and Korea. Plant foods are the major kinds of food in Japan and Korea, and majority of the production industry employs high-technology with low pollution (Sun et al., 2004).

In China, especially in Southern China, in recent years, high-fat animal foods have gained popularity, and most of these food industries cause high pollution (Sun et al., 2004), which may be one of the reasons for the high fusion rates. Studies with large sample sizes are needed for further verify this hypothesis. However, our data suggest

that TMPRSS2:ETS gene fusion, PSA levels, Gleason score, or tumor stage are related to the progression and invasion of prostate cancer, possibly providing new clues for the pathogenesis of this tumor.

Overexpression of ERG was found in most prostate cancer patients with TMPRSS2-ERG fusion (Tomlins et al., 2005b; Tomlins et al., 2006b). Over expression of ERG in itself does not increase the proliferation of prostate cancer cells, but may promote invasion (Takai et al., 2000), which may be one of the reasons for the poor prognosis in patients with this fusion product. The fusion was present in 114 (50.4%) cases out of 226 prostate cancer patients with radical resection and no difference was found in survival rate and prognosis between patients with or without the fusion gene (Rubio-Briones et al., 2010b). This suggested that no significant correlation exists between TMPRSS2:ETS gene fusion and aggressiveness or prognosis of prostate cancer. In a study involving 196 Canadian patients who underwent radical resection, the fusion product was found to be more common in prostate cancer patients with a Gleason score of 6 or 7 (82%)(Darnel et al., 2009c). Our study also showed that the majority of the patients with gene fusion had a Gleason score of less than 8, which is consistent with previous reports (Takai et al., 2000; Tomlins et al., 2005c; Tomlins et al., 2006c; Rubio-Briones et al., 2010b). Further validation is needed to assess the relationship between gene fusion and Gleason score or PSA.

We also analyzed the effects of the technical factors of the FISH procedure on the experimental results. We found that the thickness, uniformity, cleanness of the slices had the greatest impact on the results. Uniform slices with a thickness of 3 μm were most suitable for FISH. Moreover, digestion times also had a strong impact; incubation times that were either too short or too long compared to the standard 8 minutes caused inadequate or excessive digestion of the nuclei, respectively. If the slices were thick like in the case of surgical specimens, the digestion times could be extended to 9 minutes. In addition, moderate humidity and DNA denaturation temperature were critical parameters. In this study, 2 cases of non-prostate cancer patients (one with HGPIN, one with benign prostatic hyperplasia) had suspicious positive gene fusion, which could have been caused by the thickness or the impurity of the slices.

In conclusion, our study has detected the 3 subtypes of gene fusion, namely ERG, ETV1, and ETV4 found in prostate cancer patients. Most fusions involved the ERG gene, followed by ETV1 and ETV4, as was previously reported. The gene fusion rate in prostate cancer patients in northern China was 55%, consistent with other results (Tomlins et al., 2005b; Tomlins et al., 2006b; Sun et al., 2010d). The rates of gene fusion differed between different races, which suggested a potential relationship between TMPRSS2:ETS and race, providing new clues for the pathogenesis of prostate cancer. However, since no significant correlation was found to date, further studies are required to confirm our results.

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