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Case Report

Prostatic adenocarcinoma with novel *NTRK3* gene fusion: a case report

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Abstract: *TMPRSS2-ERG* gene fusion occurs in approximately 50% of prostatic adenocarcinoma and their expression is associated with aggressive phenotype, higher tumor stage, and tumor metastasis. A case of prostatic adenocarcinoma with *IRF2BP2-NTRK1* translocation was previously reported. We report a prostatic adenocarcinoma with novel *NTRK3* gene fusion that occurs in a 71-year-old male patient with aggressive histologic phenotype and multiple bony metastases. Prostatic biopsy revealed that there is a prostatic adenocarcinoma with a Gleason score of 9 (4+5), grade group 5, and multiple sites of perineural and ganglional invasion. Fluorescence in-situ hybridization (FISH) and next-generation sequencing were performed. FISH studies showed a breakage within the *NTRK3* gene in prostatic adenocarcinoma cells. Next-generation sequencing confirmed that there is a *PRPSAP1-NTRK3* translocation in the prostatic adenocarcinoma. In addition, *ASXL1, KIF5B, MED12, PIK3CA* mutations were found. *NTRK* alterations or dysregulation of PI3K signaling pathway were found in many types of cancers. TRK inhibitors including larotrectinib and entrectinib were approved by the US Food and Drug Administration for treating TRK fusion-positive malignant tumors and PI3K/AKT/mTOR pathway inhibitors were under clinical studies on various cancers including prostate cancer. In our current case, both *NTRK3* and *PIK3CA* may serve as biomarkers for precision targeted therapy.

Keywords: Prostatic adenocarcinoma, *PRPSAP1-NTRK3*, translocations, *TMPRSS2-ERG*, *NTRK3*, *PIK3CA, PI3K*

Introduction

Prostate cancer is the most common non-cutaneous cancer in men and the second leading cause of cancer-related deaths in American men. Over 174,650 new cases and 31,620 deaths are reported in 2019 [1]. Tomlins *et al.* discovered the first *TMPRSS2-ERG* translocation in prostatic adenocarcinoma [2]. Subsequently, other fusions of ETV family genes including *TMPRSS2-ETV4*, *TMPRSS2-ETV5* and *SLC45A3-ETV5* have been characterized in prostatic cancer samples [3-5]. *TMPRSS2-ERG* gene fusion occurs in approximately 50% of prostatic adenocarcinoma and their expression is associated with aggressive phenotype, higher tumor stage, and tumor metastasis [6, 7]. The *TMPRSS2-ERG* fusion gene could serve as molecular markers for progression of prostate cancer [7].

A case of prostatic adenocarcinoma with *IRF2BP2-NTRK1* translocation was previously reported [8]. In this study, we report a prostatic adenocarcinoma with novel *PRPSAP1-NTRK3* translocation that occurs in a patient with aggressive histologic phenotype and multiple bony metastases.

Case report

A 71-year-old Caucasian man presented to the Urology Clinic at the Overton Brooks VA Medical Center, Shreveport, Louisiana. The patient had a past medical history of peripheral vascular disease, hypertension, and type 2 diabetes. Digital rectal examination showed an abnormal prostate in April 2017. However, the prostatic serum antigen (PSA) was 0.48 ng/ml. Six-part prostatic biopsy including right and left base, mid, and apex was performed and prostatic

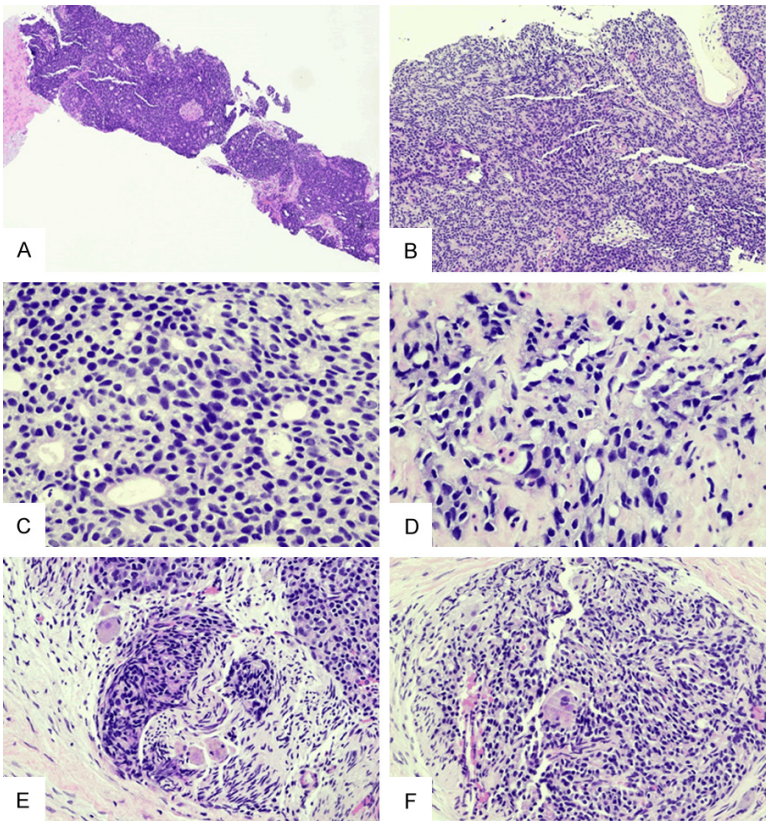

Figure 1. Histopathological features of prostatic adenocarcinoma. A. The prostatic core shows 80% involvement by prostatic adenocarcinoma, Gleason score 9 (4+5), grade group 5 (H&E, 40×). B. The prostatic carcinomas are composed of fusion glands arranged in a cribriform pattern (H&E, 100×). C. The adenocarcinoma cells are characterized by hyperchromatic nuclei containing one or more nucleoli, consistent with Gleason pattern 4 (H&E, 400×). D. Focal areas of adenocarcinoma show dispersed carcinoma cells with hyperchromatic nuclei and scanty cytoplasm, consistent with Gleason pattern 5 (H&E, 400×). E. Multiple areas demonstrate perineural invasion (H&E, 200×). F. Invasion of ganglional cells is evident (H&E, 400×).

adenocarcinoma was found bilaterally. A diagnostic bone scan demonstrated multiple metastatic lesions in the frontal skull, left occipital portion of skull, right maxilla, bilateral ribs, bilateral scapula, and most of the cervical, thoracic, and lumbar spines. Metastatic lesions were also noted throughout the pelvic girdle, right greater trochanter as well as the right femoral shaft. The patient was treated with leuprolide acetate since 2017. Clinical follow-up of the patient revealed persistent multiple metastatic lesions.

Histopathological features

The prostate biopsy showed proliferative and infiltrating malignant prostatic glands involving all the biopsy cores of the prostate (**Figure 1A**). Fusion of prostatic glands forming large cribriform structures was seen (**Figure 1B** and **1C**). The lining epithelial cells were characterized by hyperchromatic nuclei, occasional one or more prominent nucleoli and abundant amphophilic cytoplasm. Mucin-like secretions were noted in the glandular lumens. This Gleason pattern 4 involved approximately 90% of the carcinoma glands (**Figure 1C**). In addition, there were focal areas of dispersed infiltrating cells with hyperchromatic nuclei and scanty cytoplasm. This pattern of Gleason pattern 5 involved approximately 10% of the malignant glandular components (**Figure 1D**). Occasional signet ring-like carcinoma cells were present in other areas. Multiple perineural and ganglional invasions were evident and possible lymphovascular invasion was shown (**Figure 1E** and **1F**). Immunohistochemical stains including synaptophysin, chromogranin A, and CD56 for neuroendocrine differentiation were negative in the cancer cells. In summary, the prostate adenocarcinoma was graded with a Gleason score of 9 (4+5) and a grade group of 5.

Molecular features

Fluorescence in-situ hybridization (FISH) using *NTRK* "break-apart" probe was performed at Creative Bioarray, Inc., Shirley, New York. Two probes flanking 3' and 5' terminal regions of *NTRK3* were labeled with red and green fluorophores, respectively.

FISH techniques were performed in formalin-fixed paraffin-embedded tissue sections. *NTRK3* with no gene rearrangements showed closely located red and green signals. Of the 20 cells evaluated, 12 cells demonstrated separately located red and green signals, indicating a breakage of the *NTRK3* (**Figure 2**).

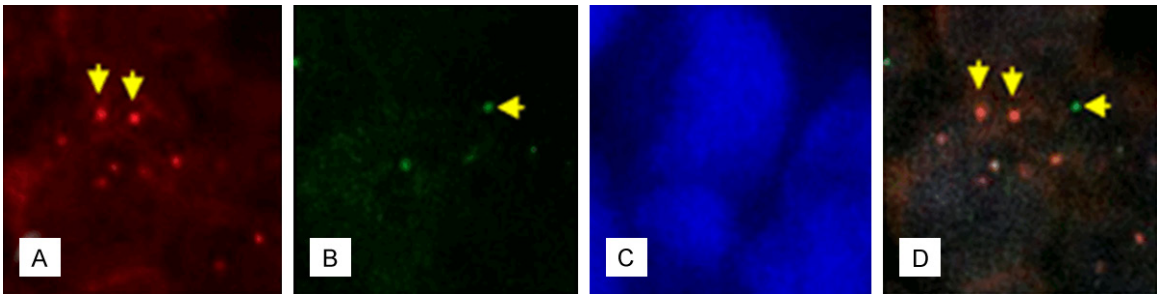

Figure 2. Fluorescence in-situ hybridization (FISH) of *NTRK3* "break-apart" probe. A. The red signal (yellow arrows) shows that the *NTRK3* "break-apart" FISH probe targeting the 3' terminal region. B. The green signal (yellow arrow) reveals that the NTRK3 "break-apart" FISH probe targeting the 5' terminal region. C. The blue staining represents DAPI nuclear stain. D. The red and green signals (yellow arrows) demonstrate the two separately located *NTRK3* "break-apart" FISH probes. These results indicate a breakage of the *NTRK3*.

Table 1. Results of next-generation sequencing

ASXL1 NM_015338.5: c.1772 dup (p.Y591fs)	
KIF5B NM_004521.2: c.1231G>C (p.G411R)	
MED12 NM_005120.2: c.3670C>G (p.L1224V)	
PIK3CA NM_006218.2: c.817A>G (p.I273V)	
PRPSAP1-NTRK3 fusion	
Breakpoints:	17:74340752 (*PRPSAP1*) 15:88727530 (*NTRK3*)
Fusion:	exons 1-3 of the *PRPSAP1* gene with exons 4-19 of the *NTRK3* gene
The actual fusion sequence:	CTGTTCGTGGCCAAGATATTTTCATTATACAGA CAATACCCAG*ACACATAGAGAACTGGCGCAG TCTTCACACGCTCAACGCCGTG

DNA and RNA using Illumina next-generation sequencing was performed at Personalis, Inc., Menlo Park, California. ACE CancerPlus test included 181 genes in coding and relevant non-coding regions were analyzed. The results showed that the prostatic adenocarcinoma harbored *PRPSAP1-NTRK3* fusion (**Table 1**). These findings confirmed the breakage of *NTRK3* on FISH study. In addition, *ASXL1, KIF5B, MED12, PIK3CA* mutations were found (**Table 1**). The breakpoints were at nucleotide 74340752 of chromosome 17 (*PRPSAP1*) and nucleotide 88727530 of chromosome 15 (*NTRK3*). As a result, the fusion occurred between exons 1-3 of the *PRPSAP1* gene and exons 4-19 of the *NTRK3* gene (**Table 1**). Therefore, the tyrosine kinase domain of the *NTRK3* gene located in exons 13-18 was preserved. The actual fusion sequence was shown in **Table 1**.

Discussion

Since the initiation of the precision oncology program, the application of next-generation sequencing has identified multiple oncogenic mutations in malignant neoplasms. Many of these genetic alterations could serve as biomarkers for oncogenic-targeted chemotherapies. *NTRK1, 2, and 3* gene rearrangements leading to novel gene fusions have been detected in more than twenty different types of tumors including lung adenocarcinoma, colon adenocarcinoma, secretory breast carcinoma, and malignant melanoma [9-12].

The majority of translocations which occurred in prostate cancer involved fusions of *TMPRSS2* and *ETS* gene family [2-5]. *NTRK1* gene rearrangement with fusion of exon 8 of *NTRK1* and exon 1 of *IRF2BP2*, was previously reported in a case of prostate cancer [8]. In this study, we report a novel *NTRK3* gene fusion with *PRPSAP1*. The *NTRK3* gene fusion was most likely to be pathogenic since the tyrosine kinase domain of this gene was preserved in the fusion products. Pathological examination of the prostatic tissue revealed high-grade adenocarcinoma with a Gleason score of 9 (4+5) (grade group 5). There were multiple sites of perineural and ganglional invasions implicating the aggressiveness of the tumor. In addition, multiple metastatic lesions were detected by whole body bone scan in early manifestations of the disease. Whether an aggressive phenotype of the tumor or higher clinical stage is associated with *NTRK3* gene fusion remains undetermined. *NTRK3* gene fusion was shown to be associated with more aggressive papillary thyroid carcinoma [14, 15]. The *NTRK3* via MAPK signaling pathway may play a role in neurogen-

esis and neurotropism. However, more cases of prostatic adenocarcinoma with *NTRK3* gene rearrangements are necessary for evaluating the correlation of genotype with phenotypic expression.

Patients with various histological types of *NTRK* fusion-positive cancers could be treated by the US Food and Drug Administration granted approval of the TRK inhibitors such as larotrectinib or entrectinib. High response rates of greater than 75% have been reported [13]. In our present case, multiple metastatic lesions were minimally reduced despite two years of leuprolide therapy. To reach the goal of clinical remission, larotrectinib or entrectinib may be an alternative treatment option [14, 15].

Mutations of phosphatidylinositol 3-kinase (*PI3K*) gene and dysregulation of PI3K signaling pathways were described in many types of human cancers such as non-small cell lung carcinoma, colorectal adenocarcinoma, breast carcinoma, lymphoma, melanoma, and prostatic adenocarcinoma [16]. PI3K/AKT/mTOR pathway inhibitors buparlisib (BKM120) or dactolisib (BEZ235) monotherapy or combination therapy with other therapeutic agents were under clinical studies for treating castration-resistant prostate cancer [17]. In our present case, point mutations of the *PI3KCA* were detected and this altered gene may serve as a biomarker for precision targeted therapy.

Conclusion

NTRK gene fusions are reported in many different histological types of cancers. We report a prostatic adenocarcinoma with a Gleason score of 9 (4+5), grade group 5, multiple perineural and ganglional invasions, *ASXL1*, *KIF5B*, *MED12*, *PIK3CA gene mutations*, and a novel *PRPSAP1-NTRK3* translocation. *NTRK3* and *PIK3CA* may serve as biomarkers for oncogenic-targeted therapy.

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Disclosure of conflict of interest

None.

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