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A novel ETV6-NTRK3 gene fusion in primary renal fibrosarcoma

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Abstract. – INTRODUCTION: Primary renal fibrosarcoma is a relatively uncommon tumor in the urinary system of adults, in fact only 6 cases have been reported in the English literature so far worldwide. The etiology of renal fibrosarcoma is incompletely understood. It is still lacking in simple and specific tissue-based biomarkers to assist the diagnosis of renal fibrosarcoma. Among the previously reported cases in the literature, the *ETV6-NTRK3* gene fusion could be detected in the congenital (or infantile) fibrosarcoma, and this rearrangement may play a vital role in initiation of congenital fibrosarcoma. However, the *ETV6-NTRK3* expression has not been reported in adult-type fibrosarcoma in the literature so far.

CASE PRESENTATION: A 66-year-old male patient admitted to our hospital because of chills, fever, and a right indwelling percutaneous nephrostomy catheter. Compared to normal kidney, the right renal had a thinner cortex and no function. After a week of anti-infective treatment, the patient underwent retroperitoneal laparoscopic right nephrectomy. The postoperative pathological result was fibrosarcoma of the right kidney.

CONCLUSIONS: Aberrant expression of EVT6-NTRK3 may contribute to the development of renal fibrosarcoma.

Key Words:
Primary renal fibrosarcoma, Gene fusion, ETV6-NTRK3.

Introduction

Compared to epithelial neoplasms, primary malignant renal mesenchymal neoplasms are relatively rare, and while leiomyosarcoma is the most common type, fibrosarcoma is seldom seen. Specific immunologic marker doesn't exist in primary renal fibrosarcoma. Routine methods, such as hematoxylin-eosin (H&E) staining and immunohistochemistry staining, have little significance in the diagnosis of renal fibrosarcoma, which mainly happens through exclusion of other diseases. With the rapid development of molecular pathology in recent years, specific fusion genes have been discovered in a variety of soft tissue tumors. This discovery of fusion gene undoubtedly adds an effective means for pathological diagnosis. In view of this, research on the fusion gene was carried out and explained in this paper.

Case Presentation

Case Selection

A 66-year-old man presented to the urology clinic with severe hydronephrosis of the right renal. Imaging examination showed that the renal cortex was thin, and the patient underwent retroperitoneoscopic nephrectomy for the severe hydronephrosis complicated with infection. After excluding other soft tissue sarcoma, results showed fibrosarcoma of right kidney, and remained a part of fresh samples[1] stored at -70°C, which were diagnosed as primary renal fibrosarcoma by the Department of Pathology. H&E stained sections and immunohistochemical staining of this case were reviewed.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and DNA Sequencing

Total RNA was extracted from frozen tissues of primary tumor using TRIzol reagent (Invitrogen, CA, USA), following the manufacturer's instructions, and converted into complementary deoxyribonucleic acid (cDNA) as described. The presence of the tumor in the tissue was confirmed on by microscopic analysis of a parallel frozen section stained with H&E. The expression of *ETV6-NTRK3* gene was detected by RT-PCR assay as previously reported[2], using oligonucleotide *ETV6* primers 114 (5′-GACGCCACTTCATGTTCCAGTG-3′) and *NTRK3* primer *TRKC-2* (NTRK3 nt 1816-1838: 5′-CCGCACACTCCATAGAACTTGAC-3′). PCR reagents and cycling conditions were as previously reported[3]. The ABI Prism 377 DNA sequencer detection system was used to analyze the sequences.

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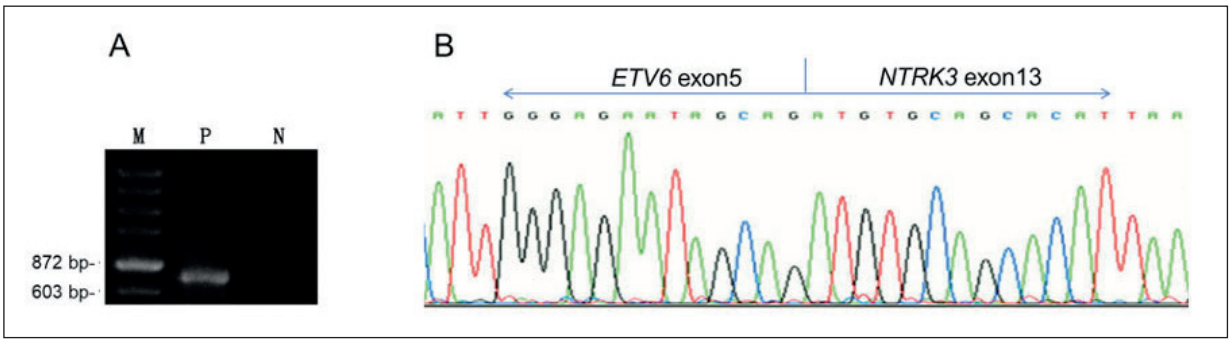

Figure 1. A novel ETV6-NTRK3 gene fusion was identified in our case. **A**, RT-PCR detection of a ETV6-NTRK3 gene fusion transcripts are seen in this tissue. M=Marker, P=Patient, Negative control; **B**, Subsequent Sanger sequencing of the RT-PCR fragment of renal fibrosarcoma shows an in-frame fusion of ETV6 exon 5 and NTRK3 exon 13.

Fluorescence In Situ Hybridization (FISH) Analysis

ETV6-NTRK3 Dual Color Break Apart Probe (ZytoVision, Bremerhaven, Germany) was designed for the detection of this variation. The images were taken with a Leica DMRB (Leica, Wetzlar, Germany) fluorescence microscope. 100 cells were analyzed and criteria from Hopman et al[4] to evaluate the fluorescence signals were adapted.

Results

RT-PCR and Sequencing

Nested RT-PCR analysis was performed to identify the *ETV6-NTRK3* fusion. The results revealed~400-bp fragments in the tissue (Figure 1A). The results showed that exon 5 of ETV6 was fused in frame to exon 13 of NTRK3 (Figure 1B). No sequence variant was detected in fusion transcripts.

FISH

Dual-color FISH probes revealed fusion of *ETV6* and *NTRK3*. The results exhibited three fusion signals, polyploidy and negative detection of the *ETV6/NTRK3* dual fusion probe, in which separate green and red signals are shown (Figure 2).

Discussion

Primary renal fibrosarcoma is so rare that only 6 cases have been reported so far[1,5-9]. The etiology of renal fibrosarcoma is still largely unknown, and the difficulty to postoperatively diagnose the renal fibrosarcoma with accuracy still exist. Renal fibrosarcoma remains exclusive in a diagnosis due to inadequacy of pathology and diagnostic criteria.

In the infantile fibrosarcoma, reviews[10,11] from the literature have indicated that the t (12; 15) (p13; q25) could produce *ETV6-NTRK3* fusion gene,

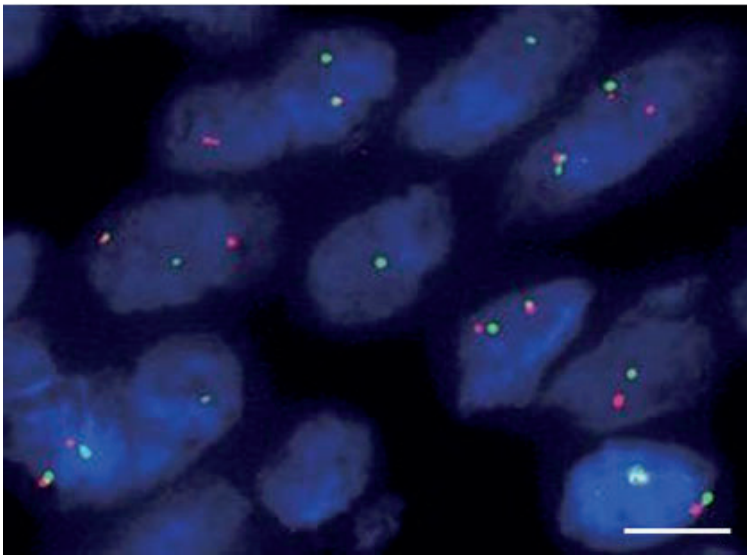

Figure 2. FISH image was acquired at ×100 magnification. Positive result is detected in renal fibrosarcoma with one fused signal pair and one split green and red signal. Scale bar: 25 μm.

which was a unique chromosomal rearrangement associated with various cancers. At present, there are two main methods for detecting fusion genes in tissues: FISH and RT-PCR. In this case, *ETV6-NTRK3* gene fusion was detected using both RT-PCR and FISH, and results showed that the expression of *ETV6-NTRK3* was confirmed. However, it should be noted that *ETV6-NTRK3* fusion gene is not a completely specific marker of renal fibrosarcoma. This fusion gene had also been detected in rare tumors, such as secretory carcinoma of the breast and salivary gland. This is just a case report, but the *ETV6-NTRK3* gene fusion expression needs more clinical data and basic research to support. *NTRK3* is a receptor belongs to neurotrophic tyrosine receptor kinase family, which can control cell survival and the vertebrate nervous system's normal function, and it may play a significant guiding role in chemotherapy[12]. Few cases of infantile fibrosarcoma with *ETV6–NTRK3* gene fusion have been reported, making tumors more sensitive to TRK inhibitors, such as entrectinib and crizotinib, with an objective response rate (ORR) of 93%[10,13]. Nagasubramanian et al[14] reported a successful targeted therapy experience of infantile fibrosarcoma with *ETV6-NTRK3* fusion-positive with *LOXO-101*, suggesting that treatment may bring new options for people with chemotherapy resistance.

Conclusions

In summary, our study found that expression of *ETV6-NTRK3* gene fusion was detected, resulting in *ETV6-NTRK3* rearrangements which may be unique to treat primary renal fibrosarcoma among soft-tissue spindle cell sarcomas. Aberrant *EVT6-NTRK3* gene expression may be a critical oncogenic driving force in primary renal fibrosarcoma. The high specificity and sensitivity of the RT-PCR assay may be helpful in distinguishing among morphologically similar fibroblastic sarcoma.

Acknowledgments

Not applicable.

Ethics Statement

The trial was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was given by the Fifth Affiliated Hospital of Zunyi Medical University (Grant No.07ZMU012).

Conflicts of Interest

The author declares no conflict of interest.

Informed Consent

Written informed consent for the publication of this report was obtained from the patient.

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