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BRIEF REPORT

# A novel partner of *TFE3* in the Xp11 translocation renal cell carcinoma: clinicopathological analyses and detection of *EWSR1-TFE3* fusion

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## Abstract

The renal cell carcinomas associated with Xp11 translocations (Xp11 translocation RCCs) harbor gene fusions involving *TFE3*, a member of the microphthalmia-associated transcription factor (MiTF) family. In the present study, we identified a novel partner of *TFE3*, *Ewing sarcoma breakpoint region 1* (*EWSR1*), in an Xp11 translocation RCC. A 57-year-old Japanese woman without special disease history was referred to us for treatment of an RCC. The resected tumor displayed an alveolar growth pattern with high-grade nuclei. The tumor was diffusely positive for TFE3 and cathepsin K. Anchored multiplex PCR revealed a novel fusion, *EWSR1-TFE3*. Fluorescent in situ hybridization analysis demonstrated the rearrangements of *EWSR1* and *TFE3*. RT-PCR analysis confirmed the chimeric transcript. No neoplasm with *EWSR1-TFE3* has been reported so far, in any organ. The results will expand the genomic spectrums of Xp11 translocation RCCs and contribute to better understanding of the roles of the MiTF family in the oncogenic process.

**Keywords** Chimeric transcript · EWSR1 · Microphthalmia-associated transcription factor (MiTF) · TFE3 · Xp11 translocation renal cell carcinoma

## Introduction

Microphthalmia-associated transcription factor (MiTF) family translocation renal cell carcinomas (RCCs) include Xp11 translocation RCC with transcription factors E3 (*TFE3*) rearrangement. Xp11 translocation RCCs harbor gene fusions involving *TFE3*, and it is the most common, around 40%, of RCCs in children and young adults [1]. Although adult patients have a much lower risk of developing Xp11 translocation RCC, a substantial number of RCCs histologically classified as clear cell RCC and papillary RCC in adulthood are found to harbor *TFE3* rearrangements by whole transcriptome sequencing analysis [2, 3].

Representative fusion partners of *TFE3* include *ASPSCR1* and *PRCC*. Possible differences in clinicopathological features are characterized between these two RCC subtypes. For example, *ASPSCR1-TFE3* RCCs are composed of enlarged cells that are rarely stained for cathepsin K, and sometimes show aggressive features, whereas *PRCC-TFE3* RCCs are typically composed of smaller cells that are positively stained for cathepsin K [4–6]. In addition to *ASPSCR1* and *PRCC*, whole transcriptome analyses have allowed us to detect less common subtypes including *SFPQ-TFE3*, *NONO-TFE3*, and *MED15-TFE3* [7]. Even though morphological differences between these subtypes have been characterized, it is difficult for pathologists to predict *TFE3* fusion partners from histopathological information alone. Because most subtypes of Xp11 translocation RCCs have at least partial papillary architecture and clear cell components, they may be misdiagnosed as a general type of RCC, especially in adult cases. In the present study, we present an Xp11 translocation

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RCC harboring a novel partner of *TFE3*, that is the *Ewing sarcoma breakpoint region 1* gene (*EWSR1*, previously known as *EWS*), which occurred in a middle-aged woman with no specific disease history.

## Clinical summary

A 57-year-old Japanese woman with no disease history consulted a primary care physician due to general fatigue. Liver dysfunction and a tumor of the right kidney were detected. Thorough medical examination revealed metastases to the lung and mediastinal lymph nodes. Based on the diagnosis of advanced RCC, she received Axitinib 10 mg/day for 3 months and was referred to our hospital for further treatment.

Right nephrectomy was performed. After the operation, the patient received Axitinib 10 mg/day for 32 months. Thirty-one months after nephrectomy, multifocal brain metastases

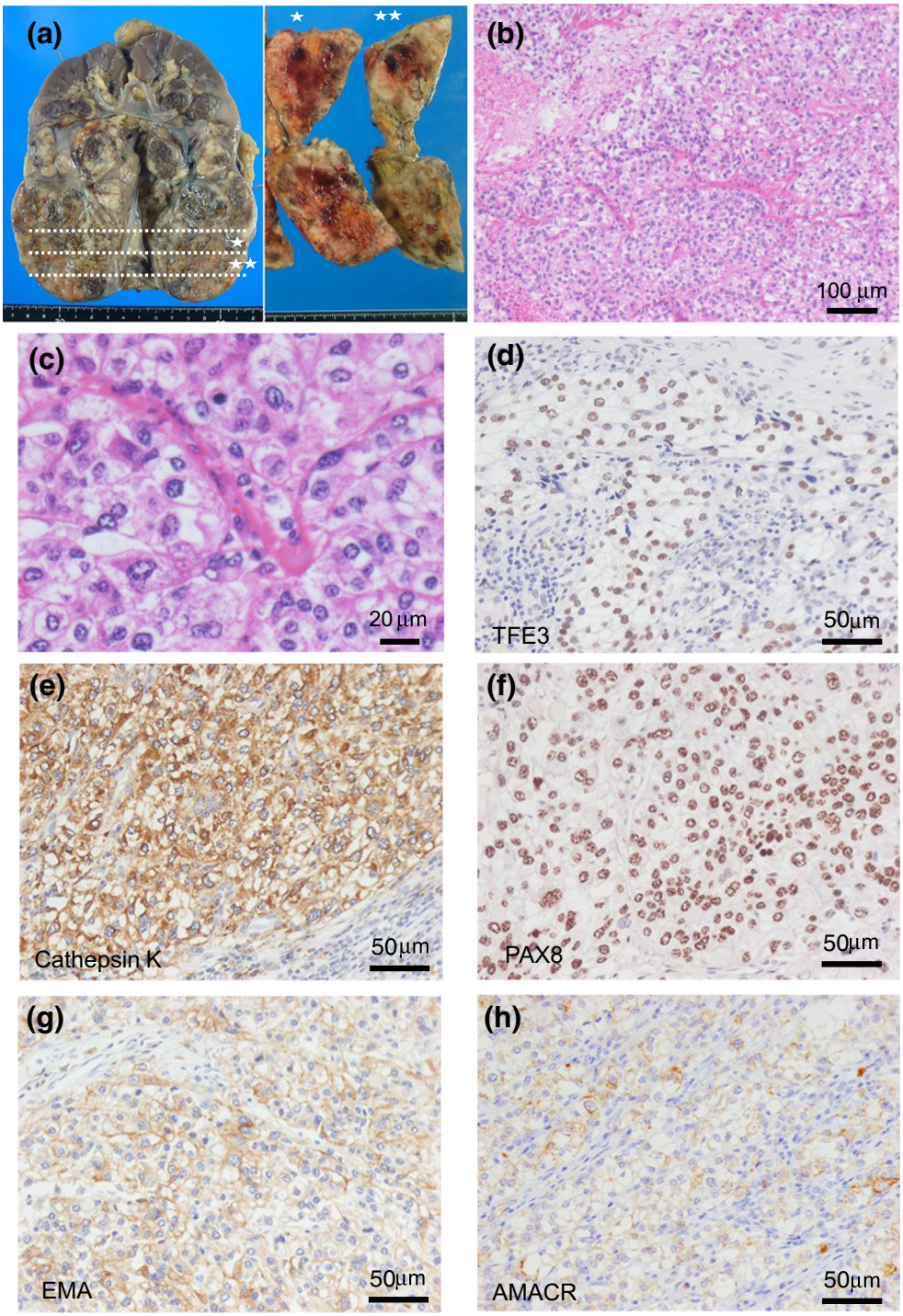


**Fig. 1** Histopathological features of the resected kidney. **a** The patient's resected right kidney (left). The dotted lines and star(s) of the frontal plane indicate axial cut sections. The cut sections of the tumor (right). Each specimen indicated by star(s) corresponds to the axial section lined in the frontal plane. **b** Hematoxylin and eosin staining of the tumor. Alveolar nested tumors have thin capillary networks. Upper left region contains necrosis. **c** Higher magnification of the tumor. Enlarged polymorphic nuclei and eosinophilic or clear cytoplasm are observed. **d–h** Representative immunostainings. Tumor cells are diffusely positive for TFE3 (**d**), cathepsin K (**e**), PAX8 (**f**), EMA (**g**), and occasionally positive for α-methylacyl-CoA racemase (AMACR) (**h**)

were detected. She received combined modality therapy including brain tumor resection, gamma rays, and whole-brain radiation. Subsequently, Nivolumab (3 mg/kg) was administrated every 2 weeks for 6 months as the second-line therapy. The response to Nivolumab was progressive disease; thus, Axitinib was administrated again, which maintained the patient in stable disease status for 2 months.

## Pathological findings

A 12 × 6.5 × 7.8 cm-sized nodular infiltrative mass replaced the lower pole of the right kidney (Fig. 1a). The resected tumor grew in a solid manner and exhibited whitish, bloody, and necrotic features. Microscopically, the solid lesions were composed of nested alveolar architectures, compartmentalized by thin-walled capillary vasculature (Fig. 1b). At a higher magnification, the tumor cells had large nuclei with nucleoli. Nuclear pleomorphism was noted with occasional bizarre nuclei. The tumor was classified as grade 4 according to the WHO/International Society of Urologic Pathologists (Fuhrman grade 4) (Fig. 1c). The cytoplasm was voluminous, eosinophilic and fluffed, and was also vacuolated. Psammoma body and melanin pigments were undetectable. Immunohistochemically, the tumor cells were positively stained for TFE3, cathepsin K, PAX8, EMA, and α-methylacyl-CoA racemase, as well as focally positive for melanosome-associated antigen (clone HMB 45), Melan-A and E-cadherin. The tumor was negative for CD10, cytokeratin (clone AE1/AE3, CAM 5.2, CK5/6, CK7), high-molecular weight cytokeratin (clone 34βE12), S-100 protein, smooth muscle actin, and neuroendocrine markers CD56, synaptophysin, and chromogranin A (Fig. 1d–h and data not shown). The histological features, especially alveolar architecture with high grade nuclei and diffuse TFE3 immunoreactivity, were indicative of Xp11 translocation RCC. Dual-color fluorescent in situ hybridization (FISH) using break-apart *TFE3* probe showed a rearranged pattern in 69 of 97 counted nuclei (71%) of tumor cells (Fig. 2a). The tumor was diagnosed as an Xp11 translocation RCC. Lymphovascular invasion and infiltration to the renal pelvis were detected, thus the TNM stage was pT3aN0Mx.

After obtaining written informed consent and institutional review boards approvals, the patient's tumor was analyzed for gene fusion. The RT-PCR for common partner genes (*ASPSCR1*, *PRCC*, *NONO*, and *SFPQ*) did not make fusion products; thus, anchored multiplex PCR was done. The tumor was found to have an *EWSR1-TFE3* fusion. To confirm the result, FISH and RT-PCR were done. Dual-color FISH using a break-apart *EWSR1* probe displayed split signals in 70 of 138 counted nuclei (51%) of the tumor area (Fig. 2b). RT-PCR and sequencing analysis allowed us to define the chimeric *EWSR1-TFE3* transcript, which joined *EWSR1* exon 12 to *TFE3* exon 6 (Fig. 2c).

## Discussion

We diagnosed the present tumor with "Xp11 translocation RCC" based on the current WHO classification; however, the diagnostic rationale warrants further consideration. The

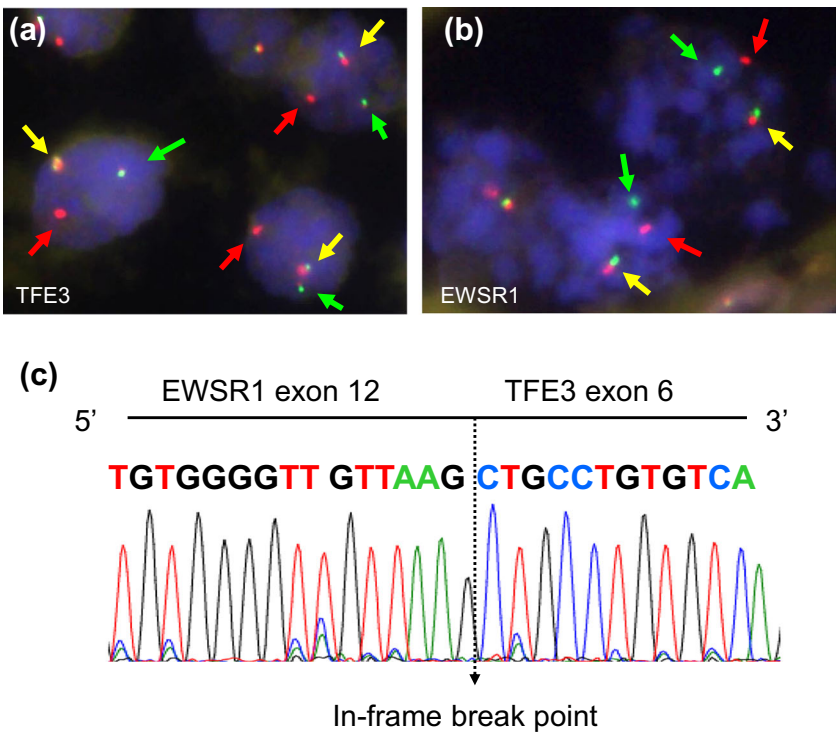


**Fig. 2** Analysis of *EWSR1-TFE3* fusion. **a** Dual-color break-apart FISH assays using the probes for *TFE3* (**a**) and *EWSR1* (**b**). The 5′ and 3′ ends are labeled with FITC and Texas Red, respectively. A yellow arrow (fused color) indicates a full-length transcript in one allele. A single colored arrow (green and red) indicates that the gene rearrangement occurs in the other allele harboring *TFE3* (**a**) and *EWSR1* (**b**). **c** mRNA was extracted from the renal tumor, and reverse transcription-PCR was performed. Sequencing analysis demonstrates the chimeric transcript composed of *EWSR1* and *TFE3*. The in-frame break point is indicated by a dotted arrow

fusions *ASPSCR1-TFE3* and *SFPQ-TFE3* are not limited to Xp11 translocation RCCs but have also been detected in mesenchymal neoplasms such as alveolar soft part sarcoma and perivascular epithelioid cell tumor (PEComa) [8]. Wang et al. proposed that the Xp11 translocation RCC and melanotic Xp11 neoplasm sharing a *SFPQ-TFE3* gene fusion need to be differentiated [9]. Similarly, the diagnosis of "RCC" in the present case may not be conclusive. The patient was an adult without special medical history. The tumor developed in the renal parenchyma, and tumor cells displayed epithelioid morphology with immunoreactivities for PAX8 and EMA. These findings support RCC. The possibility of melanomas is unlikely, given lack of melanin pigment and negative staining for S-100 protein. On the other hand, PEComa and some other mesenchyme neoplasms carrying *TFE3* rearrangements can show solid and alveolar patterns with immunoreactivity for cathepsin K, and often lack spindle cell differentiation [10]. Although we cannot exclude the possibility of a mesenchymal tumor showing epithelioid morphology, no information is currently available about soft tissue tumors harboring an *EWSR1-TFE3* fusion. Whether tumors with *EWSR1-TFE3* fusion are detectable in any organs is a subject for further investigation. If such tumors appear and possess a sarcoma- or PEComa-like nature, then the diagnosis of the current case should be re-considered.

*EWSR1* gene is located on 22q12.2 chromosome and spans about 40 kb of DNA with 17 exons. *EWSR1* gene rearrangements are frequently detected in Ewing family tumors and some other mesenchymal tumors. In addition, tumors of epithelial origin, such as papillary thyroid carcinoma and hyalinizing clear cell carcinoma of the salivary gland also have *EWSR1* translocations [11, 12]. The chimeric proteins produced by *EWSR1* usually consist of the N-terminal transcriptional activation domain of EWSR1 fused to the C-terminal DNA-binding domain of the transcription factor. Representative translocation partners are E 26 family and homeobox gene superfamily. Leucine-zipper transcription factors family such as ATF1 and CREB1 can also form a fusion transcript with TFE3. The structure of *EWSR1-TFE3* conforms to the prototype mentioned above. Most tumors with *EWSR1* chimeric transcripts have break points between exons 7–10 [13]. The present case had a break point at exon 12. Although the inclusion of the additional exons would predict transcription of an arginine-glycine-glycine (RGG) box and a part of RNA recognition motif of the C-terminal RNA-binding domain, subcellular localization of this transcript may not be different from those of common *EWSR1* chimera with break points at exons 7–10 [14]. The unique combination will be added as a target for developing new therapeutic approaches to block aberrant molecular pathways induced by fusion transcripts in cancers.

A series of molecular studies of RCC using whole transcriptome analyses has revealed that chimeric transcripts are not limited to MiT family-associated types. Substantial numbers of histologically diagnosed clear cell- and papillary RCCs, and even Xp11 translocation RCCs, possess various chimeric transcripts other than TFE3 [15]. Currently, it is largely unknown whether and how miscellaneous chimeric transcripts contribute to the biology of each RCC. Not all chimeric transcripts were increased at the mRNA level, indicating that some chimeric transcripts may participate in renal carcinogenesis through dysfunction of tumor-related genes [15]. In the present case, TFE3 and its downstream molecule, cathepsin K, were overexpressed as detected by immunohistochemistry. Similar to the other subtypes such as *ASPSCR1-TFE3* and *SFPQ-TFE3* with *TFE3* break point at exon 6, the present chimeric *TFE3* transcript also encodes exons 6–8, which includes the basic region and helix-loop-helix/leucine-zipper domains that mediate dimerization and DNA binding. Although TFE3 overexpression predicts constitutive activation of target promoters, detailed effects of the novel chimeric transcript in oncogenic signaling will require further study.

The present case added a new *TFE3* partner to Xp11 translocation RCCs. Further studies of TFE3-mediated signaling in the oncogenic process will help us manage the affected patients with this type of RCC and also with the mesenchymal neoplasms involving TFE3 such as alveolar soft part sarcoma and PEComa.

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**Authors' contributions** HF, IK, MF, RT, and YN analyzed and interpreted the data. TT and TK contributed to clinical management. IK, MF, and YN contributed to writing the manuscript. All authors read and approved the final manuscript.

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## Compliance with ethical standards

**Ethical approval** This study was approved by the institutional review boards of Tokyo Women's Medical University (No.330) and Yokohama City University (A170928009).

**Informed consent** Written informed consent for the study was obtained from the patient.

**Conflict of interest** The authors declare that they have no conflict of interest.

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