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BRIEF REPORT

A renal cell carcinoma with *EWSR1-TFE3* fusion gene

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Abstract

Both *EWSR1* and *TFE3* are well-known oncogenes. *EWSR1* encodes an RNA-binding protein involved in multiple soft tissue tumors, including Ewing's sarcoma/peripheral neuroectodermal tumor, desmoplastic small round cell tumor, soft tissue clear cell sarcoma (malignant melanoma of soft parts), extraskeletal myxoid chondrosarcoma, and myxoid liposarcomas. TFE3 regulates both Golgi and lysosomal homeostasis and is rearranged in renal cell carcinoma (RCC), alveolar soft part sarcoma, epithelioid hemangioendothelioma, and perivascular epithelioid cell tumors (PEComas). In this report, we found a rare case of RCC with a fusion between 5′ *EWSR1* and 3′ *TFE3*. The fusion product retained most functional motifs of TFE3. The oncogenic mechanism likely involves *TFE3* overexpression through its juxtaposition with the regulatory elements of *EWSR1* and its translocation to the nucleus, resulting in the deregulation of Golgi and lysosomal homeostasis. This is a second case of RCC containing *EWSR1-TFE3* fusion.

1 | INTRODUCTION

EWSR1 is a ubiquitously expressed RNA-binding protein, which plays roles in gene expression, RNA processing, cell differentiation, autophagy, and mitosis.[1] Due to its versatile roles, the dysregulation of EWSR1 is detrimental, causing central nervous system disorders or tumors.[2,3] *EWSR1* fusion genes are associated with various types of tumors. For example, a group of Ets-Related transcription factor genes, including *FLI1*, *FEV*, *ETV1*, *ETV4*, and *ERG*, is fused with *EWSR1* in Ewing's sarcoma/peripheral neuroectodermal tumor.[4] Other genes, including *WT1*, *CREB1/ATF1*, *NR4A3*, and *DDIT3*, when fused with *EWSR1*, cause desmoplastic small round cell tumor,[5] soft tissue clear cell sarcoma (malignant melanoma of soft parts),[6] extraskeletal myxoid chondrosarcoma,[7] or myxoid liposarcomas,[8] respectively.

TFE3, a transcription factor specifically recognizing E-box sequences,[9] is a major regulator of both Golgi and lysosomal homeostasis. In retinal pigment epithelial cells (ARPE-19), TFE3 is phosphorylated by mTORC1 and is retained in the cytoplasm by binding to 14-3-3. When these cells are starved, TFE3 rapidly dephosphorylates, translocates to the nucleus, and binds to the CLEAR (coordinated lysosomal expression and regulation) element of genes encoding autophagy and lysosomal biogenesis to promote cellular survival.[10] In cells with Golgi stress, TFE3 translocates to the nucleus and binds to the GASE (Golgi apparatus stress response element) of genes encoding glycosylation enzymes, vesicular transport components, and Golgi structural proteins to upregulate the capacity of the Golgi apparatus.[11] TFE3 oncogenic rearrangements are found in the Xp11 translocation renal cell carcinoma (RCC),[12] alveolar soft part sarcoma,[13] epithelioid hemangioendotheliomas,[14] and perivascular epitheloid cell tumors (PEComas).[15]

Here, we report a rare case of RCC harboring a fusion gene between *EWSR1* and *TFE3*, which has only reported once before.

2 | MATERIALS AND METHODS

2.1 | Targeted RNA next-generation sequencing (NGS)

Both 4 and 10 μm tissue sections were received for histologic and genetic diagnosis of the tumor. Ten-micrometer tissue sections were used for total RNA isolation using Qiagen FFPE RNeasy Kit (Qiagen, Valencia, California). One hundred nanograms total RNA was used for

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first Strand cDNA Synthesis by incubating with NEBNext First-Strand Synthesis Enzyme Mix (NEB; Ipswich, Massachusetts) for 10 minutes at 42°. The second strand synthesis was performed by incubating with NEBNext Second Strand Synthesis Enzyme Mix (NEB) for 1 hour at 16°C. cDNAs were purified with AMPure XP beads and subjected to end-polishing, phosphorylation, and dA extension by incubating with end-repair mix, Klenow exo-, and Taq polymerase (Enzymatics, Beverly, Massachusetts) for 15 minutes at 12°C, 15 minutes at 37°C, and 15 minutes at 72°C. A custom-made sequencing adaptor was added by incubating with Ligase (Enzymatics) for 20 minutes at 22°C. PCR enrichment was performed using primers specific to a group of 32 genes commonly involved in various sarcomas, and the PCR products were sequenced in an Illumina miniSeq sequencer (San Diego, California). Sequencing results were analyzed with SeqNext software (JSI, Ettenheim, Germany).

2.2 | Fluorescence in situ hybridization (FISH)

FISH was performed on 5 μm tissue sections with two colored split apart probes for *TFE3* and *EWSR1*. Briefly, the tumor area on the slides was marked with a diamond-tipped pen. The slides were deparaffinized in xylene, rehydrated, treated in 750 U/mL pepsin

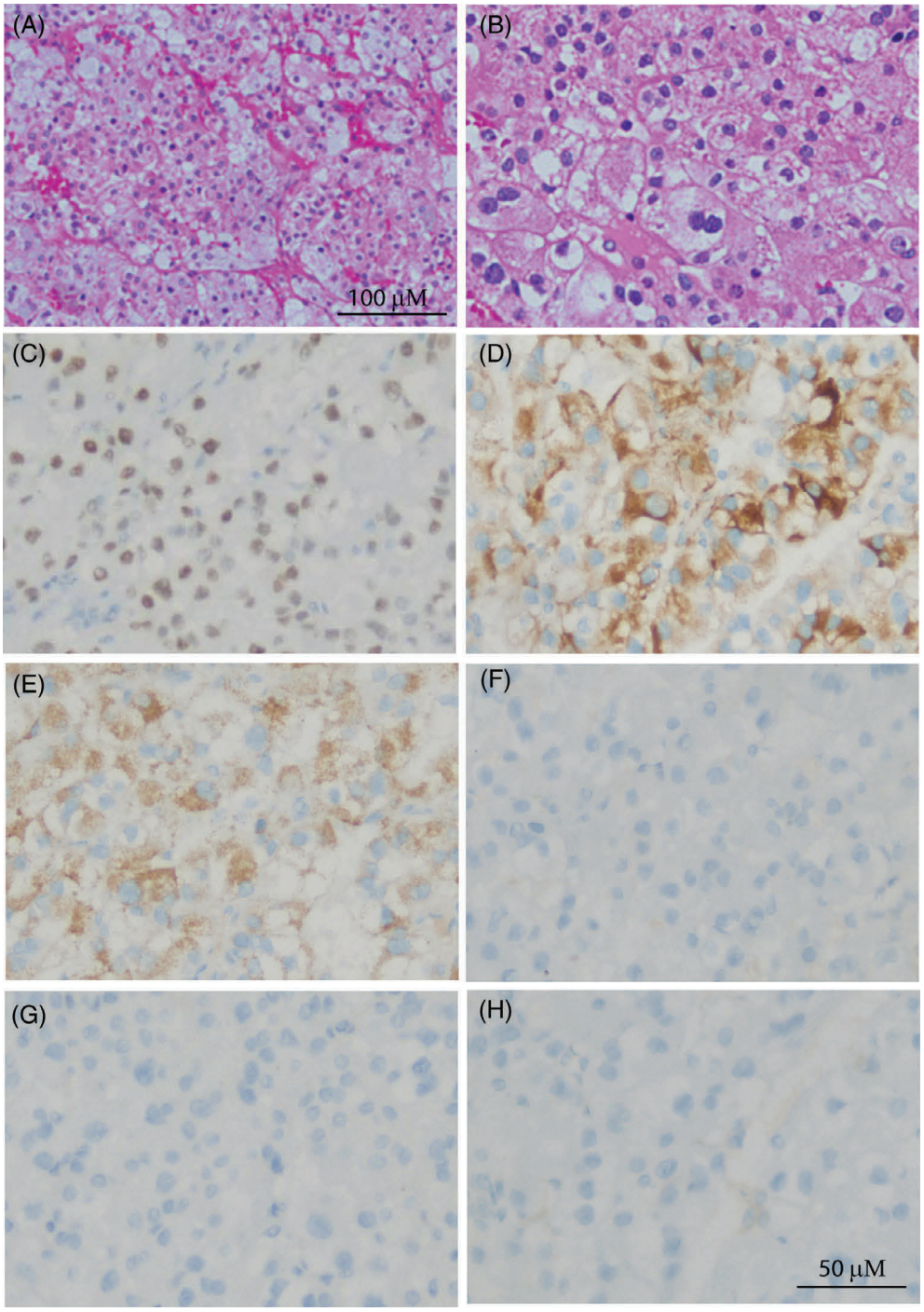

FIGURE 1 H&E and immunohistochemistry (IHC) staining of the tumor tissue sections. A, H&E staining showed alveolar nested cells with a rich capillary network. B, Tumor cells had eosinophilic or clear cytoplasm with polymorphic nuclei. C-H, IHC staining was positive for TFE3 (nuclear staining) (C), Vimentin (cytoplasmic staining) (D), and P504S (cytoplasmic staining) (E), and negative for CD10 (F), CK7 (G), and CD117 (H) [Color figure can be viewed at wileyonlinelibrary.com]

digest solution (Sigma-Aldrich, Natick, Massachusetts) for 10 minutes, and incubated in 10% buffered formalin for 10 minutes. The slides and probes were separately denatured, and hybridization performed at 37°C overnight. Post-hybridization wash was done in 0.4×SSC/ 0.3% NP-40 at 73°C for 3 minutes and slides counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

2.3 | Reverse transcriptase PCR (RT-PCR) and Sanger sequencing

Total RNA from 10 μm FFPE slides were isolated, and cDNA was synthesized with random priming and SuperScript IV reverse transcriptase (ThermoFisher, Waltham, Massachusetts). PCR was performed with primers specific for *EWSR1* and *TFE3* (F_EWSR1: 5′-CAGGAGAGAACCGGAGCATGAGTG and R_TFE3: 5′-CTCACGCCTCTCCTGCTCCTG; or F_TFE3: 5′-GAGCCGTGTTCGTGCTGTTGGAG and R_EWSR1: 5′-CATCTAGAGTCACACTGTCATTTAATCCTTGTAC). The first PCR condition was 94°C 3 minutes for 1 cycle followed by 30 cycles of 94°C 30 seconds, 62°C 30 seconds, and 72°C 60 seconds. 0.1 μL of the first PCR product was subjected to nested PCR with corresponding nested primers (F_EWSR1 nest: 5′-GATTTGATCGTGGAGGCATGAGCAG and R_TFE3 nest: 5′-CGCAGCAAGACCCTCGATGAAGAAG; or F_TFE3 nest: 5′-CTTGATCCTGACAGCTTCTACGAGCTC andR_EWSR1 nest: 5′-GTCAGAGTCTTCATCTGGATCTACAGGTG). The nested PCR condition was 94°C 3 minutes for 1 cycle followed by 30 cycles of 94°C 30 seconds, 65°C 30 seconds, and 72°C 60 seconds. 5% of dimethyl sulfoxide (DMSO) was added to PCR reactions for *EWSR1-TFE3* due to high GC content in the region.

Quantitative RT-PCR was performed using iTaq Universal SYBR Green One-Step Kit (Bio-Rad, Hercules, California). Two hundred nanograms of total RNA was added to PCR reactions containing iScript reverse transcriptase, SYBR Green, iTaq DNA polymerase, and *EWSR1*- and *TFE3*-specific primers (F_EWSR1 nest and R_TFE3 nest; F_TFE3 nest andR_EWSR1 nest) or ACTB-specific primers (F_ACTB: CAATGTGGCCGAGGACTTTG; R_ACTB: CATTCTCCTTAGAGAGAAGTGG). The reactions were incubated at 50°C for 10 minutes for reverse transcription and subjected to 40 cycles of 95°C for 10 seconds and 65°C for 60 seconds. The expression level of the *EWSR1-TFE3* and *the TFE3-EWSR1* was normalized to *ACTB*.

This study is approved by the institutional review board at respective institutions.

3 | RESULTS

3.1 | Case report

Abdominal and pelvic CT scan of a 33-year-old male incidentally identified a 2.8 cm left kidney mass with high signal intensity. A thorough

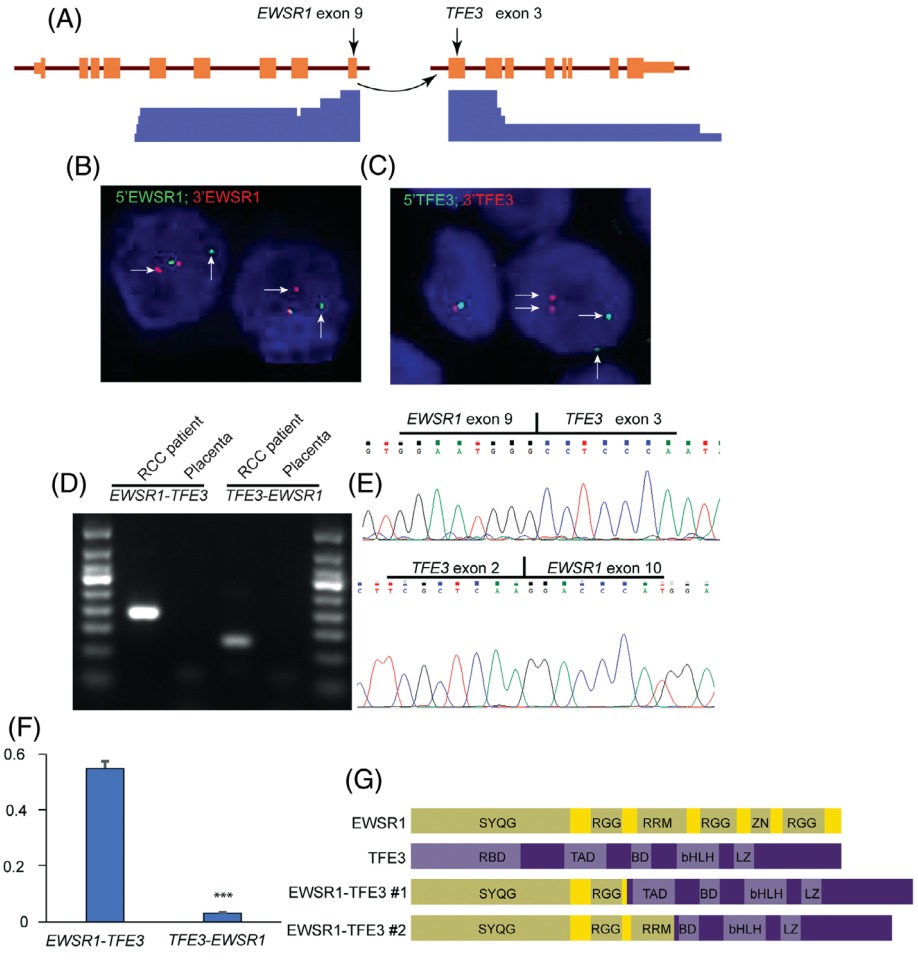

FIGURE 2 Characterization of the *EWSR1-TFE3* fusion. A, Targeted RNA NGS showed reads span exon 9 of *EWSR1* and exon 3 of *TFE3*, consistent with *EWSR1-TFE3* fusion. B, FISH with an *EWSR1* split-apart probe showed the separation of the 5′*EWSR1* (green) from the 3′*EWSR1* (red), consistent with *EWSR1* rearrangement. C, FISH with a *TFE3* split-apart probe showed the separation of the 5′*TFE3* (green) from the 3′*TFE3* (red), consistent with *TFE3* rearrangement. D, RT-PCR assays showed the expression of both *EWSR1-TFE3* and *TFE3-EWSR1* transcripts. E, Sanger sequencing confirmed the same breakpoints on both *EWSR1* and *TFE3* as seen in RNA NGS. F, Real-time quantitative RT-PCR showed a dominant *EWSR1-TFE3* expression. ***$P<0.001$. G, Predicted functional motifs of two cases of EWSR1-TFE3 fusions in renal cell carcinoma (RCC). EWSR1-TFE3 #1 is our case; EWSR1-TFE3 #2 is the previously published case[16] [Color figure can be viewed at wileyonlinelibrary.com]

medical examination found no metastasis. Partial nephrectomy removed a piece of 2.5 × 2.3 × 2.5 cm kidney containing a well-defined tumor measured 1.8 × 1.5 × 1.3 cm. Hematoxylin and eosin (H&E) staining of the tumor tissue sections showed an alveolar nested tumor with rich capillary networks. Tumor cells had clear or eosinophilic cytoplasm and polymorphic nuclei with 1-2 nucleoli (Figure 1A, B). Occasional Psammoma bodies and melanin pigments were noted. Immunohistochemistry showed that the tumor cells were positive for TFE3, Vimentin, and P504S and negative for CD10, CK7, and CD117. While both Vimentin and P504S were cytoplasmic, TFE3 showed nuclear staining (Figure 1C-H). An RNA NGS targeted fusion panel for sarcoma, which includes 32 genes commonly involved in sarcomas as "bait" genes, detected an *EWSR1-TFE3* fusion transcript (Figure 2A). Subsequent FISH with *EWSR1*- and *TFE3*-specific probes confirmed the rearrangements of both genes (Figure 2B, C). We next performed RT-PCR and Sanger sequencing, which showed that both *EWSR1-TFE3* and its reciprocal product *TFE3-EWSR1* were expressed (Figure 2D, E). The reading frames were maintained for both fusion transcripts. A real-time quantitative RT-PCR showed that the expression of *EWSR1-TFE3* was >10 times greater than that of *TFE3-EWSR1* (Figure 2F). *EWSR1-TFE3* contained the first 9 exons of *EWSR1* and the last 8 exons of *TFE3*. The predicted functional motifs of the EWSR1-TFE3 fusion are shown in Figure 2G, and include the SYQG-rich transcriptional activation domain, the RGG domain from EWSR1, the transcriptional activation domain (TAD), the basic helix-loop-helix domain, and the leucine zipper from TFE3. The breakpoints on both *EWSR1* and *TFE3* in our case were different from those of the published RCC, which had the first 12 exons of *EWSR1* and the last 5 exons of *TFE3*.[16] The fusion protein in our case retained all-important functional motifs; however, the previous case contained only the basic helix-loop-helix domain and the leucine zipper with no TAD, suggesting that TAD is likely unnecessary for RCC oncogenesis (Figure 2G).

Clinical follow-up 6-month after surgery (without adjuvant therapy) showed unremarkable physical examination, normal blood test including normal serum creatinine, and no sign of tumor recurrence by chest, abdominal, and pelvic CT.

4 | DISCUSSION

TFE3 belongs to microphthalmia transcription factor (MiT) gene family that includes the three other members TFEB, TFEC, and MiTF. The founding member MiTF is associated with small eye phenotype in mice.[17] Three of the four MiT members play roles in RCC tumorigenesis; while *MiTF* germline mutation is associated with inherited RCC,[18] somatic *TFE3* and *TFEB* rearrangements leading to oncogenic fusion proteins are found in spontaneous RCC. A total of 14 fusion partners of *TFE3* have been found so far in RCC, including *PRCC*, *ASPSCR1*, *SFPQ*, *NONO*, *CLTC*, *MED15*, *MATR3*, *FUBP1*, *RBM10*, *KHSRP*, *PARP14*, *LUC7L3*, *DVL2*, and the recently reported *EWSR1*.[19,20] Among those, only seven were recurrent fusions: *PRCC-TFE3*, *ASPSCR1-TFE3*, *SFPQ-TFE3*, *NONO-TFE3*, *RBM10-TFE3*, *CLTC-TFE3*, and *MED15-TFE3*. Our report confirms the eighth recurrent fusion of *TFE3*, that is, *EWSR1-TFE3*.

The oncogenic mechanisms of these TFE3 rearranged RCC are not fully understood. The MiTF germline mutation E318K impairs SUMOylation of MITF and enhances its binding capability to a targeting promoter.[18] *TFEB* fused with a non-coding RNA gene *MALAT1*, and the fusion transcript retaining the entire coding region of *TEFB*, suggest a gain of function or dysregulation of *TFEB* by promoter swap. Along these lines, the various fusion partner genes of *TFE3* likely contribute new sets of gene regulatory elements for *TEF3*, leading to its dysregulation or overexpression. Consistently, all *TFE3* fusion partner genes including *EWSR1* are ubiquitously expressed with constitutive active promoters. In addition, wild type TFE3 is mostly cytoplasmic by its interaction with 14-3-3; however, as seen in this case, rearranged TFE3 is translocated to the nucleus (Figure 1C).[21] The 14-3-3 binding site of TFE3 includes Ser321, which can be phosphorylated by mTORC1. Both RCC cases of *EWSR1-TFE3* fusion retain the TFE3 Ser321. It is possible that the fusion of TFE3 with EWSR1 affects its interaction with mTORC1 and therefore blocks its phosphorylation and interrupts its binding with 14-3-3, leading to nuclear translocation of the EWSR1-TFE3.

In summary, we described a rare fusion involving two important oncogenes, that is, *EWSR1* and *TFE3*. The oncogenic mechanism likely involves *TFE3* overexpression through its juxtaposition with the regulatory elements of *EWSR1* and its translocation to the nucleus, resulting in deregulation of Golgi and lysosomal homeostasis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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