Title

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Xp11.2 translocation renal cell carcinoma with *SFPQ/PSF-TFE3* fusion gene: A case report with unusual histopathologic findings

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Abstract:

Xp11.2 translocation renal cell carcinoma (Xp11tRCC) is a subtype of renal cell carcinoma (RCC) characterized by chromosomal rearrangement of the region harboring the transcription factor for immunoglobulin heavy-chain enhancer 3 (*TFE3*). Xp11tRCCs comprises 20% to 40% of RCCs of children and adolescents and is generally associated with good prognosis. However in adult, the incidence of this tumor is relatively low (1% to 4%), suggesting a more aggressive course. *TFE3* gene is fused by translocation to numerous partner genes, and definitive molecular characteristics can be difficult to verify. In this case report, we presented a case of Xp11tRCC with the *SFPQ/PSF-TFE3* chimeric gene. The fusion gene was detected by 5'-rapid amplification of cDNA ends (5'RACE). The tumor was found to be in an advanced stage with multiple lymph node metastases. The histological characteristics of the tumor were different from those of XP11tRCC with other more frequently detected fusion genes.

Key words: Xp11.2 translocation RCC, *SFPQ/PSF-TFE3*, 5'RACE, solid variant

1. **Introduction:**

Xp11tRCC is a type of RCC characterized by the formation of a chimeric gene comprising the *TFE3* gene at Xp11.2 and its partner gene [1]. *ASPSCR1/ASPL-TFE3* and *PRCC-TFE3* are the most frequently detected chimeric genes [2], followed by *SFPQ/PSF-TFE3* [3, 6]. In addition, *Nono-TFE3*, *CLTC-TFE3* and other chimeric genes have been reported in a few cases [1, 3]. Xp11tRCC comprises 1%-4% of adult RCC cases, with an average age of onset of around 50 years [3, 4]. Other renal tumors that form the chimeric gene *SFPQ/PSF-TFE3* are Xp11 translocation perivascular epithelioid cell tumor (Xp11tPEComa) [5,6,7] and melanotic Xp11tRC (melXp11tRC) [5,8].

Histologically, Xp11tRCC is often characterized by papillary structures, which also proliferate in an alveolar or solid pattern [1]. Tumor cells are often observed to have clear cytoplasm and are sometimes observed to have eosinophilic cytoplasm [1]. The difference between RCC with *ASPSCR1/ASPL-TFE3* and *PRCC-TFE3* can be predicted based on tumor morphology. RCC with *ASPL-TFE3* is characterized by more abundant cytoplasm and more psammoma bodies, while RCC with *PRCC-TFE3* have less cytoplasm, less abundant psammoma bodies, and closely nested tumor cells [9].

In addition to the two types of RCC described above, RCC with the *SFPQ/PSF-TFE3* chimeric gene have been reported to be characterized by subnuclear vacuoles [4, 10], and

other histological images have been reported. However, in contrast to RCC with *ASPSCR 1/ASPL-TFE3* or *PRCC-TFE* [3, 4, 10, 11], only a few cases of RCC with *SFPQ/PSF-TFE3* have been reported. Definitive histological features of these tumors have not yet been determined [10].

2. Method:

2.1 Hematoxylin and Eosin (H&E) stains and Immunohistochemical (IHC) stains

Resected tissues were fixed in 10% buffered formalin and embedded in paraffin. 4μm -thick sections were cut and stained with H&E. IHC stain was performed with the primary antibodies according to the manufacturer's instructions and visualized with a Ventana BenchMark ULTRA immunostainer (Ventana Medical Systems).

2.2. Fluorescence in situ hybridization (FISH)

TFE gene break-apart FISH (Cytocell) on interphase nuclei from a paraffin-embedded 4μm-thick section was performed. The green labeled probes were situated distal to the *TFE3* gene (covering markers DXS6949 and STS-Z98763), and the red labeled probes were situated proximal to the *TFE3* gene (covering markers DXS9735 and DXS8366).

2.3. mRNA extraction, 5'RACE, and sequencing

Total mRNA was extracted using RecoverAll TM total nucleic Acid Isolation Kit (Life Technologies). For 5' RACE, we used kit of 5'-Full RACE Core Set (Takara). 5 'phosphorylated primer 5'-(P)-CTCGGTCTCAGAGA-3', degraded hybrid RNA and, cyclization or concatenated of single-stranded cDNA by ligation reaction. For the first round of PCR, we used primer 5'-GGAACTGGGCACTCTCAT-3' and 5'-CGTTTGATGTTGGGCAGCTC-3': 1 cycle of 94°C for 3min, 25 cycles of 94°C for 30sec, 55°C for 30sec, and 72°C for 30sec. The second round of PCR was performed using primer 5'-TCAAGCAGATTCCCTGACAC and 5'-GAACAAGGGCACCATCCTGA-3': 27cycles of 94°C for 30sec, 55°C for 30sec, and 72°C for 30sec. After purification of PCR product, two bands were obtained by electrophoresis. They were cut separately and sequenced.

3. Case presentation

A 57-year-old female underwent a CT examination in a local hospital because of macroscopic hematuria. The patient was referred to the Shimane University hospital for further analysis. A mass of renal hilus with regional lymph node swellings in the middle of the left kidney was detected in the CT scan. A contrast-enhanced CT was performed,

which additionally revealed a multinodular invasive tumor with a size of approximately 5 cm in the left kidney. Invasion of the tumor into the peripelvic fat and multiple lymph node metastases with a maximum diameter of 8 cm in the para-aortic region at the left renal hilar level were observed (Figure 1a). The preoperative clinical stage was cT3aN2M0. Transabdominal left nephrectomy and lymph node dissection were performed (Figure 1b).

3.1. Pathological findings

Macroscopically, the cut surface of the tumor appeared grayish brown and solid with focal hemorrhage. Numerous intravenous tumor emboli were observed around the tumor. Necrotic areas were distributed throughout the mass (Figure 1c). Microscopic findings demonstrated solid sheets of neoplastic cells (Figure 2a) with comedo-like necrosis (Figure 2b) in a wide area within the tumor. Pseudorosette formation could not identified in these area, but trabecular pattern was identified focally (Figure 2c). Pseudopapillary pattern was observed in the small range with in the tumor (Figure 2d), but true papillary structure could only be identified in a small part of the tumor (Figure 2e). Melanin deposition and psammoma bodies were not observed. The neoplastic cells were weakly eosinophilic and appeared clearly around the nucleus (Figure 2f); however, subnuclear

vacuoles in tumor cells were not observed. The tumor cells had a relatively high N/C ratio with irregularly shaped nuclei. Immunohistochemical analysis revealed that the tumor cells were diffusely and strongly positive for CD10 (Figure 3a), but negative for vimentin (Figure 3b), CK7 (Figure 3c), Melan A, and HMB45. The tumor cells stained very weakly positive for PAX8. TFE3 overexpression was observed (figure 3d), the tumor was highly suspected to be Xp11tRCC.

3.2. FISH analysis

Results of the *TFE3* gene break-apart assay showed a high percentage of split signals (Figure 4a, b).

3.3. Molecular analysis

Adequate total mRNA was extracted from the frozen specimens and then subjected to 5'RACE analysis. Expression levels of the chimeric *SFPQ-TFE3* transcripts were measured. Exon 7 of the *SFPQ* gene was found to be fused with exon 9 of *TFE3* gene (Figure 4c). Thus, the diagnosis was confirmed to be Xp11tRCC with *SFPQ-TFE3* fusion gene.

4. Discussion:

Xp11tRCC was first established by the World Health Organization (WHO) as an independent subtype in 2004 [11]. The Xp11tRCC tumor is defined by a harbor gene fusion involving members of the MiT family of transcription factors, including *TFE3* gene [11]. The tumor is defined by both papillary and clear cell morphology with a nested/alveolar architecture, and the type of gene translocation can be determined based on the tumor morphology [11].

Considering the possibility of Xp11tRCC, we employed IHC and FISH, which confirmed the overexpression of TFE3 protein levels (IHC of TFE3 protein is highly sensitive and specific assay for neoplasms bearing *TFE3* gene fusions [12]) and the presence of a break in the *TFE3* gene, respectively. Then, RT-PCR was performed to identify the partner gene, focusing on *ASPSCR1/ASPL* and *PRCC*. However, considering that the gene fusions were not identified, we screened for other partner genes by conducting 5'RACE. The analysis identified the *SFPQ/PSF-TFE3* chimeric gene as a partner gene. RCC with *SFPQ/PSF-TFE3* is the third most frequent gene fusion detected in Xp11tRCC [3].

Xp11 translocation cancers are characterized by a variety of gene fusions involving *TFE3* gene [1]. The common fusion partners of *TFE3* gene include *ASPSCR1/ASPL*,

PRCC, and *SFPQ/PSF*, and its rare fusion partners include *CLTC, NONO, RBM10, PARP14, LUC7L3, KHSRP, DVL2, MED15,* and *GRIPAP1* [3]. Next-generation sequencing is the ideal method for identifying these partner genes but has the disadvantage of very high cost. If there are many reciprocal candidate genes as chimeric genes like Xp11tRCC, screening for a partner gene by performing RT-PCR [13, 14] or detecting fusion signals by FISH [14, 15] can be inefficient; therefore, RACE could serve as the most suitable method [3].

TFE3 gene translocation and TFE3 protein expression in RCC are correlated with poor prognosis [16] and exhibits worse prognosis than papillary RCC, which is similar to that of clear cell RCC [3, 15]. Xp11tRCC with *ASPSCR1/ASPL-TFE3* fusion has been reported to have worse prognosis than Xp11tRCC with other fusion partners [3]. The findings of Ellis CA et al. indicated that RCC with *ASPACR1/ASPL-TFE3* is more likely to be detected during the advanced stage than RCC with *PRCC-TFE3*; however, the latter tended to recur during the late stage and thus warrants long-term follow-up [2]. The incidence of *SFPQ-TFE3* RCC is lower compared to those of the above two tumors; however, precise prognosis of the *SFPQ-TFE3* RCC tumor remains a challenge.

Typical histological features of Xp11tRCC include mixed papillary and nested/alveolar architectures, comprising cells with clear and/or eosinophilic cytoplasm, which is

granular and voluminous with discrete borders. The nuclei have vesicular chromatin and prominent nucleoli [1]. Extensive psammoma bodies is frequently observed [1]. *ASPSCR1/ASPL-TFE3* RCC is comprised of epithelioid cells with more abundant clear to eosinophilic cytoplasm with papillary or pseudopapillary and nested growth [1]. Psammoma bodies can be frequently observed. On the other hand, *PRCC-TFE3* RCC is characterized by less cytoplasm and less abundant psammoma bodies with smaller papillary and nested pattern.

RCC with *SFPQ-TFE3* is slightly different in appearance compared to the former two, which are frequently associated with subnuclear vacuoles, leading to palisading of nuclei, similar to the appearance of clear cell papillary RCC [4, 8]. Psammoma bodies can be observed [4]. However, many uncommon findings have been reported in RCC *SFPQ-TFE3*, such as trabecular [17], epithelioid [17], sarcomatous morphology, pseudorosette formation, hyalinized stroma, marked foam cells [4,10]. In our case, most of the tumor cells proliferated in solid sheets with comedo-necrosis (solid-variant). Thick trabecular patterns were also identified continuously from the solid area. Although pseudopapillary pattern (resulting from the degenerative process of the solid area which can be seen at solid-pseudopapillary tumor of the pancreas) were observed in a small area, but true papillary structure was less identified only in the limited area. There were no subnuclear

vacuoles, same as the paper reported by Wang XT, et al [17].

Currently, there is no difference in treatment between Xp11.2tRCC and clear cell RCC. However, regardless of the type of chimeric gene, Xp11tRCC is known to progress to advanced clinical stages, including lymph node metastasis at the time of detection [2]. In such cases, adjuvant chemotherapy will indicated after nephrectomy. Molecular targeted drugs such as tyrosine kinase inhibitors (ex. Sunitinib, Sorafenib) and mTOR inhibitors are used. The latter has reported that effective in cases of Xp11.2tRCC [18]. Recently, using of immune checkpoint inhibitors has also increased [19].

However, the prognostic details of RCC with *SFPQ-TFE3* alone remain unclear. The number of accumulated cases is limited, and accurate data on disease prognosis are lacking. In our case, nuclear atypia of the tumor cells was prominent, and numerous intravenous tumor emboli and many lymph node metastases were observed around the aorta at the time of surgery. New metastatic lesions were identified at left pubic bone after total nephrectomy and lymph node dissection, indicating that RCC with *SFPQ-TFE3* is a highly malignant tumor.

In summary, it is difficult to differentiate Xp11tRCC from other RCC by radiological examination but it is known that the findings on MRI are similar to papillary RCC [20]. Staining of the TFE3 protein is recommended to estimate disease prognosis [16], even if

the possibility classical papillary RCC is considered. Moreover FISH analysis for *TFE3* break-apart and is generally preferable to TFE3 immunohistochemistry with few exceptions.

Conflict of interest

None of the authors have any conflict of interest to disclose.

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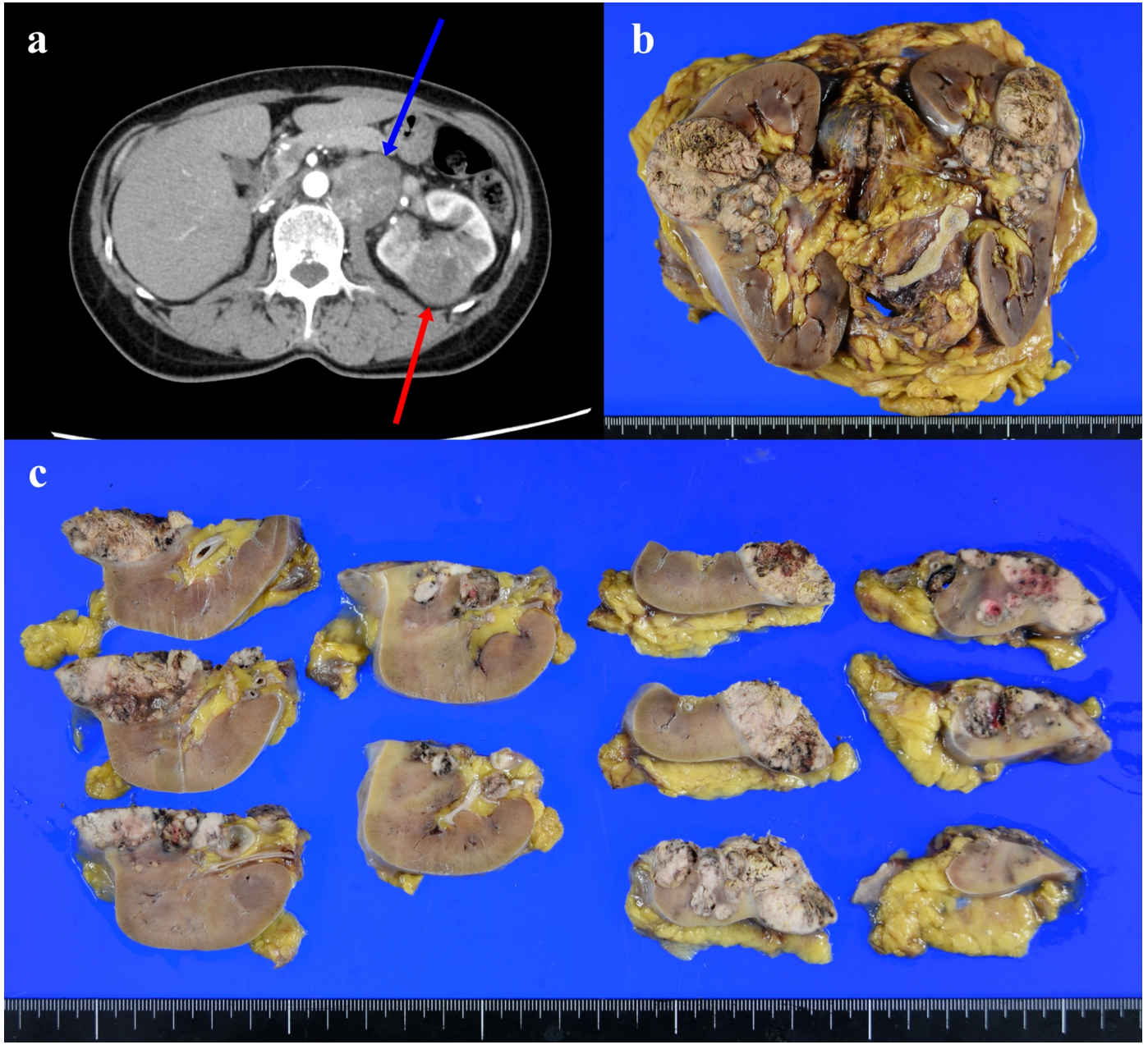
Figure legends:

Figure 1: a. Enhanced CT showing large mass originating from the lt kidney. Red arrow: Invasion into the peripelvic fat. Blue arrow: Paraaortic lymph node. b. Sagittal cut view of formaline-fixed resected kidney. c. Horizontal cut surfaces of the kidney.

Figure 2: a. Solid sheets of neoplastic cells. b. Comedo-like necrosis. c.Trabecular pattern. d. Focal area of pseudopapillary patterm. e. Focal area of true papillary pattern with fibrovascular cores. f. Clearly to lightly eosinophilic cytoplasm. Mitosis (red arrow) and nuclear inclusion (blue arrow) of neoplastic cell.

Figure 3: a. Tumor cells are diffusely positive for CD10 b. Negative for vimentin c. Negative for CK7 d: Overexpression of TFE3

Figure 4: a, b. *TFE* break-apart FISH. c. Sequence after 5' RACE. Fusion gene of *SFPQ* and *TFE3*.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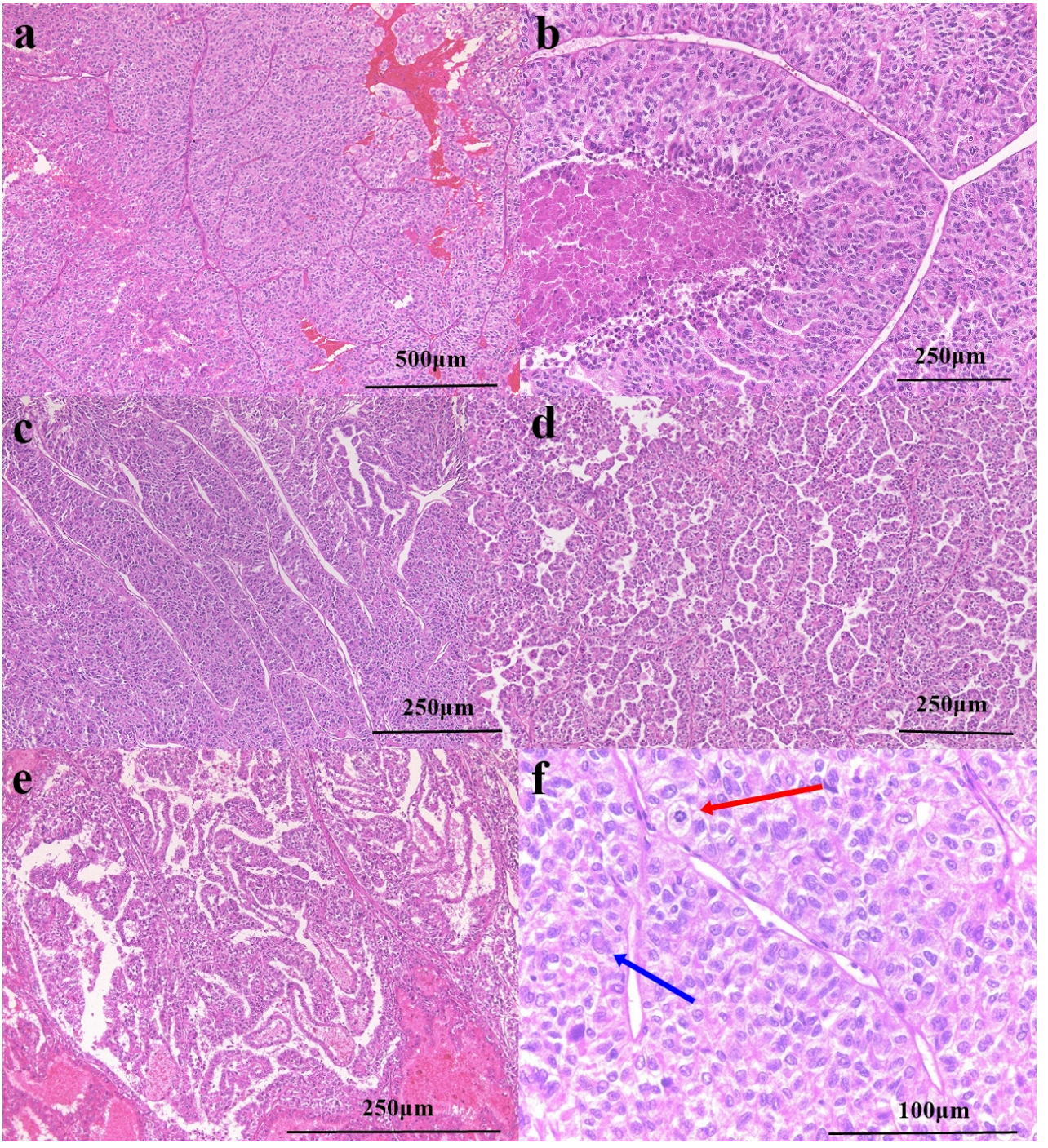
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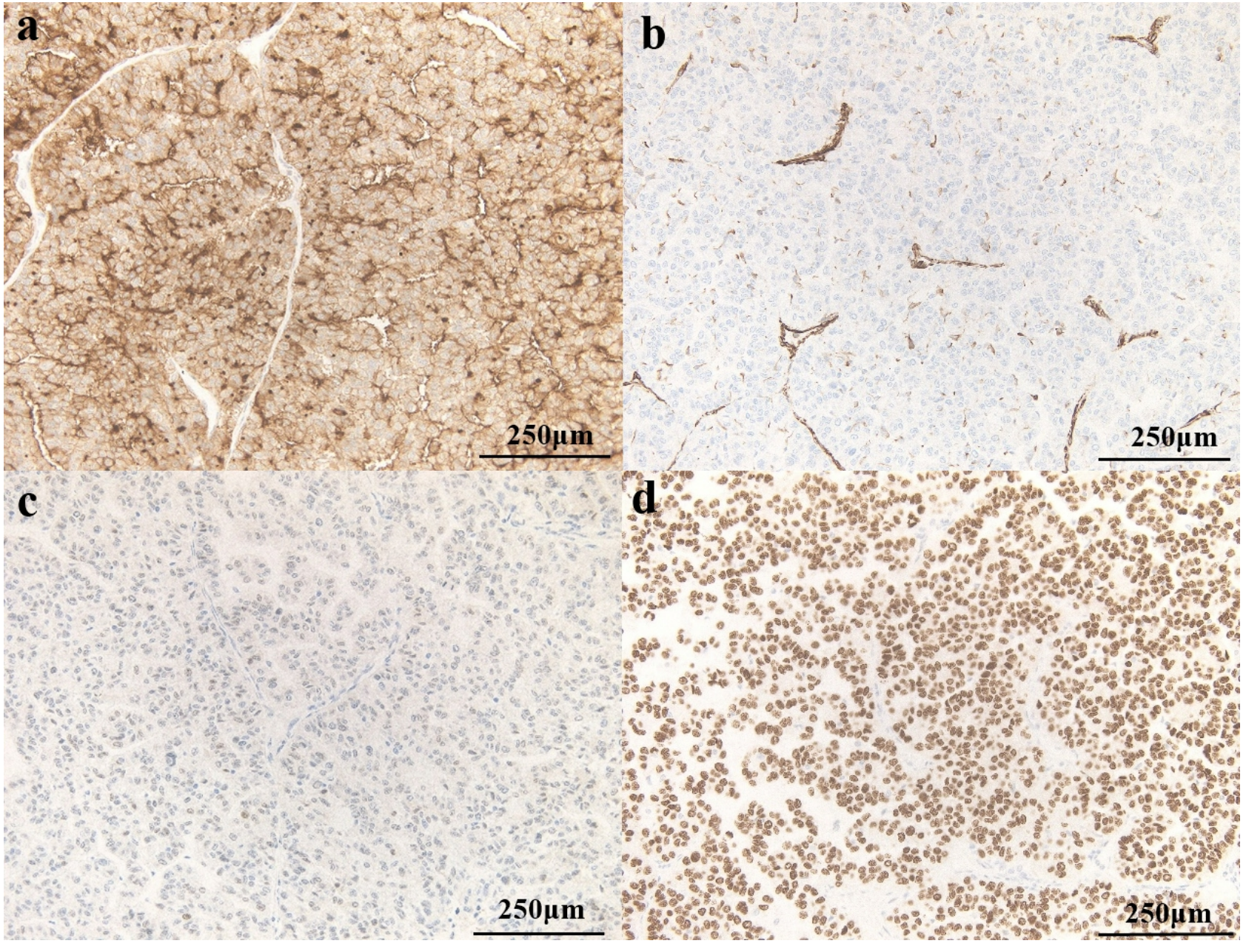
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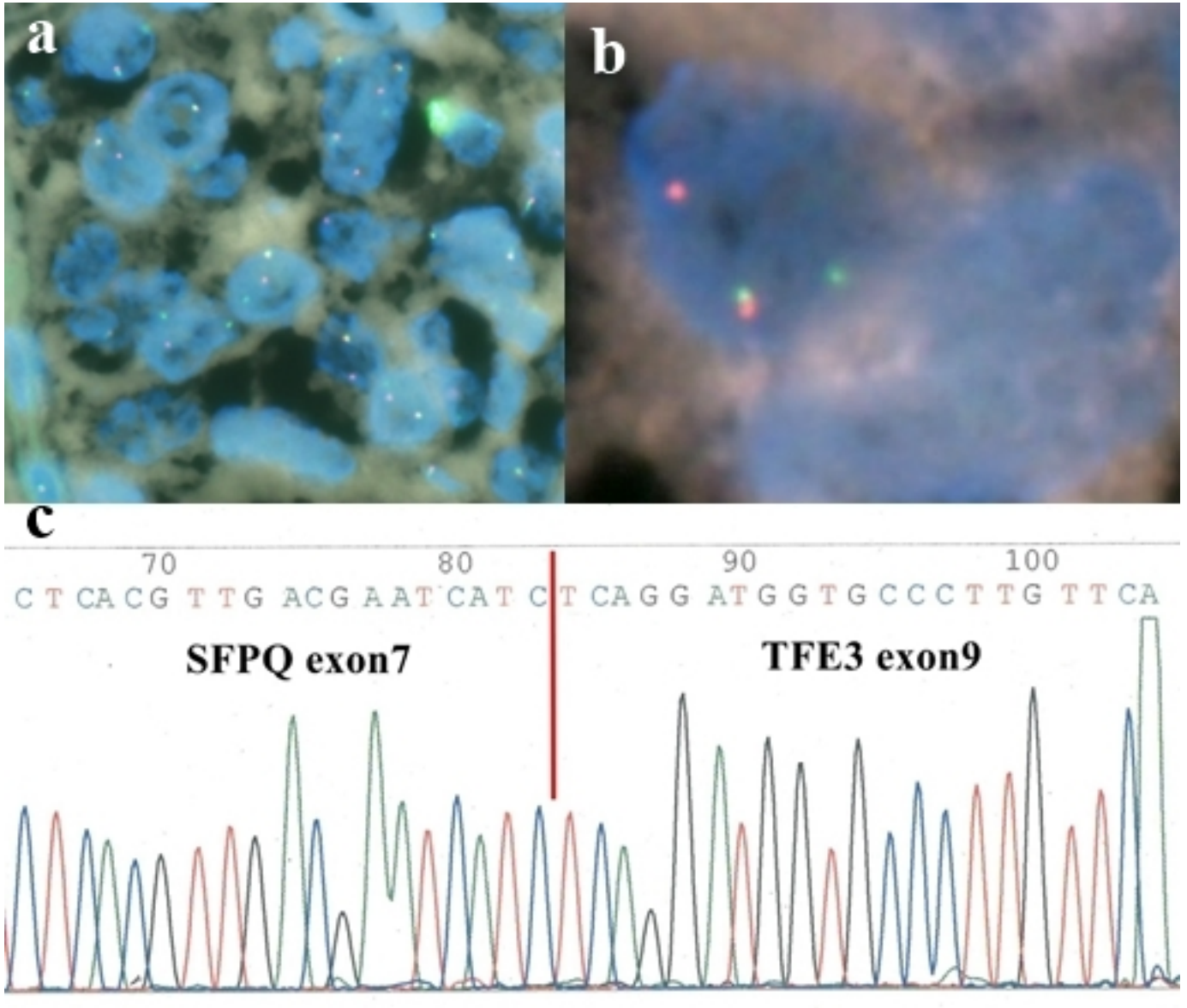
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TFE3 exon9