North Clin Istanb 2025;12(4):438–444
doi: 10.14744/nci.2024.19794

Original Article PATHOLOGY

TFE3 immunohistochemistry in renal cell carcinomas: Does the clone really matter?

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ABSTRACT

OBJECTIVE: TFE3 rearranged carcinomas constitute 5% of malignant tumours of the kidney in adults. TFE3 immunohistochemistry plays a crucial role in the diagnosis. TFE3 positivity in the appropriate histological context supports the diagnosis of Xp11 translocation renal cell carcinomas. However, there isn't any standardized approach to performing and interpreting immunohistochemical staining.

METHODS: A total of 51 renal cell carcinomas are included in the study. In this study, we compared the expression profiles of two different anti-TFE3 antibody clones (MRQ37, Cell Marque, and IHC627, GeneAbTM) on renal cell carcinoma samples that have conflicting morphologies and assessed the overall performance of these clones to identify TFE3 rearranged carcinomas.

RESULTS: There was a statistically significant difference in terms of immunohistochemical staining with TFE3-MRQ37 clone between TFE3 rearranged renal cell carcinomas and other subtypes, while no significant difference was found in staining with TFE3-IHC672. 47% of cases were stained with the TFE3-IHC672 clone and 9.8 % of cases were stained with the TFE3-MRQ37 clone at different staining intensities and proportions.

CONCLUSION: The TFE3-MRQ37 clone is easier to interpret because of the absence of background staining and is more reliable in identifying TFE3 rearranged renal cell carcinomas. However, because of various sensitivity and specificity rates, and immunoreactivity in many subtypes of renal cell carcinomas, there is a need for a standardised approach for TFE3 immunohistochemistry for diagnostic use in TFE3-tRCCs.

Keywords: Renal cell carcinoma; TFE3 rearrangement; immunohistochemistry.

Cite this article as: *Kokenek Unal TD, Kiran MM, Sungu N, Yazgan A, Gumuskaya B. TFE3 immunohistochemistry in renal cell carcinomas: Does the clone really matter? North Clin Istanb 2025;12(4):438–444.*

Renal cell carcinomas (RCC) constitute 80–85% of malignant tumours of the kidney. There are many subtypes defined so far, and new entities are being defined as pathological and molecular analyses increase [1]. MiT (microphthalmia-associated transcriptional factor) family translocation carcinomas are rare and account for 1-4% of renal tumors in adults and approximately half of the RCCs in children [2]. These tumours are characterized by fusions involving TFE3 or TFEB genes and were included as two subcategories in the 2016 WHO classification. However, with the 5th edition of the WHO Classification of Urinary and Male Genital Tumours (2022), these two entities were classified separately as TFE3-rearranged RCC (TFE3-

Received: April 03, 2024 *Revised:* July 06, 2024 *Accepted:* July 21, 2024 *Online:* August 14, 2025

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tRCC) and TFEB-rearranged RCC [3]. Although TFE3-tRCC has unique morphological features, it poses difficulties in differential diagnosis. These tumors usually have papillary morphology with eosinophilic cytoplasm and abundant psammoma bodies [4]. However, these tumours may have a morphology mimicking clear cell renal cell carcinoma, papillary renal cell carcinoma, multicystic renal cell carcinoma, oncocytoma, and even epithelioid angiomyolipoma [5]. TFE3 is a transcription factor that plays a role in cellular differentiation and is encoded by the TFE3 gene. The oncogenic activation of this gene is because of chromosomal translocation. This genetic alteration mostly involves Xp11 translocation [6]. FISH is considered to be more sensitive and specific than IHC in detecting TFE3 rearrangement, there is no clarity about when and in which cases FISH should be used and how it should be interpreted [7]. On the other hand, immunohistochemistry is more preferred because it is an easy, fast, and inexpensive method [8]. However, there is no consensus on how to perform immunohistochemistry or how to interpret it. A recent survey revealed that 16-20% of pathologists do not even know which TFE3 clone they are using [7]. According to the last edition of WHO classification, essential diagnostic criteria include presenting strong nuclear staining with TFE3 IHC in a clean background or identification of TFE3 arrangement by break-apart FISH or TFE3 gene fusion by RNA sequencing [3]. Molecular techniques are expensive and need expertise to interpret and not available for most of the pathologists [9]. In addition to that, considering the rarity of the tumour, TFE3 immunohistochemical staining is the easiest and cheapest method that can be applied in routine laboratory conditions. Because there is a lack of a standardised approach, the use of TFE3 immunohistochemistry can be confusing due to various fixation and interpretation problems. There are different brands and clones of TFE3 antibody on the market and there is no study in the literature showing the superiority of these clones over each other (if there is any). Its importance may be underestimated. In this study, we compared the expression profiles of different clones of TFE3 IHC (namely MRQ37, Cell Marque and IHC6272, GeneAbTM) on RCCs and assessed factors such as sensitivity, specificity, staining pattern, and overall performance in detecting TFE3 protein expression in tumour samples. In addition to that, we determined to reveal their differences and contributions to the definitive diagnosis of TFE3-tRCCs in challenging cases.

Highlight key points

- The 48% of renal cell carcinoma cases were weakly stained with TFE3-IHC672; and %10 of casesshowed strong nuclear positivity with TFE3-MRQ clone.
- Immunohistochemical staining with TFE3-MRQ clone was significantly different in TFE3-tRCC compared to other RCCs.
- Nosignificant difference in staining with the TFE3-IHC672 clone between TFE3-tRCCs and the others.
- Staining performed with different clones can yield highly variable results, highlighting the need for a standardized staining and evaluation algorithm in this area.

MATERIALS AND METHODS

The nephrectomy materials diagnosed between 2019 and 2023 in the Pathology Department of Ankara Bilkent City Hospital were retrospectively scanned. After ethical board approval (Ankara Bilkent City Hospital, decision ID: TABED 1-24-24 on 14.02.2024) a total of 51 patients with renal tumours that posed difficulties in differential diagnosis with their morphological features during diagnosis were included in the study. This study was conducted in accordance with 'Declaration of Helsinki'.

Demographic data on the patients was noted. Histopathological parameters such as tumour type, tumour size, tumour nuclear grade, presence of necrosis, renal sinus adipose tissue invasion, etc. were re-evaluated. For TFE3 immunohistochemical staining, 5 µ thick sections were taken from the appropriate tumour including paraffin blocks. After deparaffinization, immunohistochemistry was performed using DAKO's Autostainer Plus using TFE3 (monoclonal mouse antihuman antibody, MRQ37, Cell Marque; dilution 1/150, USA) and TFE3 (monoclonal mouse antihuman antibody, IHC672, GeneAb, dilution: 1:150, USA) according to the manufacturer's protocol. Nuclear staining for both clones as shown in Figure 1 was considered positive regardless of the intensity or the percentage of staining.

Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) Windows 22.0 (IBM Inc., Chicago, IL, USA) package program was used for statistical analysis. The distribution of the data was analysed by histogram, qq plot, and Shapiro-Wilk test. Outliers were excluded from the study. An independent t-test was used for parametric data and Mann-Whitney U test was used for nonparametric data in comparisons between two groups. For comparisons of

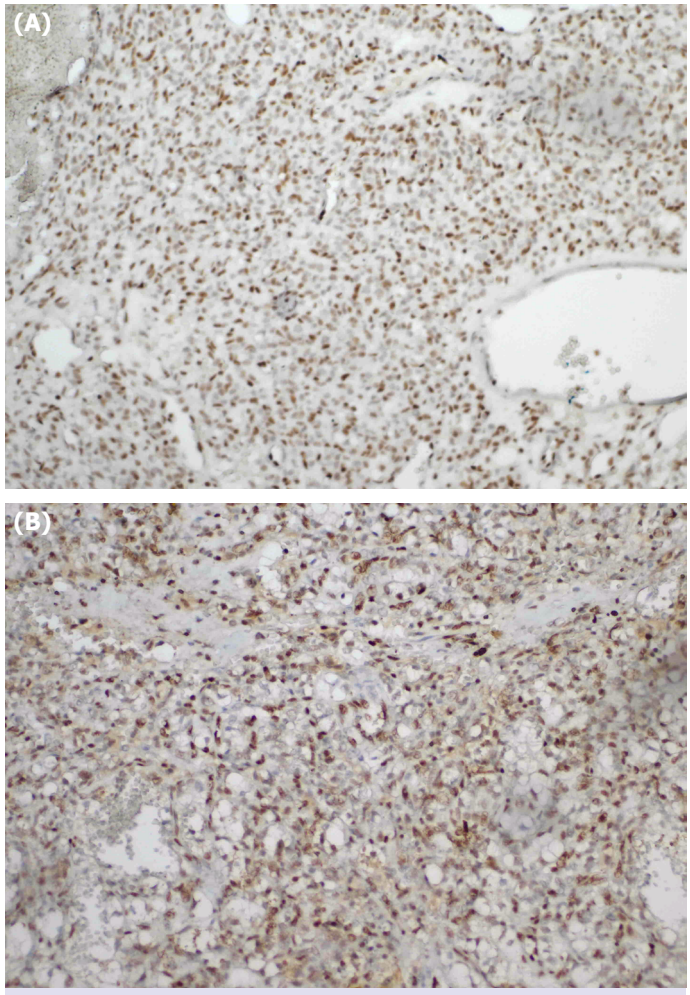

Figure 1. Strong positivity with TFE3-MRQ37 **(A)** and TFE3-IHC672 **(B)**. (x20).

three or more groups, ANOVA was used for parametric data and Kruskal-Wallis test for nonparametric data. The chi-square test was used to test for categorical variables. Sensitivity and specificity were calculated. Spearman test was used to calculate correlations between parameters. The number of units (n), median and min-max values, mean and standard deviation values were given as summary statistics. Sensitivity and specificity were calculated and the ROC curve was used to determine cutoff values. $P<0.05$ was considered statistically significant.

RESULTS

The study included 15 female (30%) and 36 male (70%) patients. The median age was 56 years (ranging from 25 to 82). 31 cases (%61) were finally diagnosed with clear cell RCC (ccRCC); 11 cases with RCC, NOS; 7 cases with chromophobe RCC (chRCC); 5 cases with papillary RCC

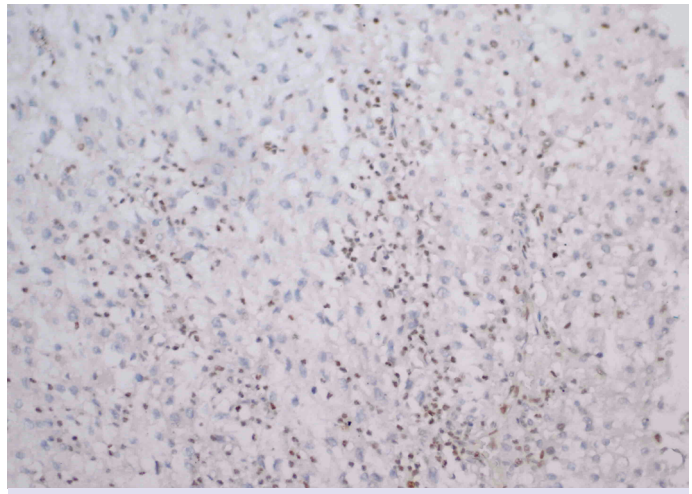

Figure 2. Tumor cells are negative but lymphocytes and stromal cells are positive with TFE3-IHC672. (x20).

(pRCC), 3 cases with TFE3-tRCC; and 2 cases with oncocytic tumour. The median tumour size was 65 mm (range: 20 to 180 mm). Radical and partial nephrectomy were performed in 39 cases (77%) and 12 cases (23%), respectively. Patient and tumour characteristics are described in Table 1. In the TNM staging, 20 (39%) cases were categorised as pT1a-b, 7 cases (13%) as pT2a-b, and 24 cases (47%) as pT3a, but none were categorised as pT4. Regional lymph nodes were neither dissected nor metastasis was present in our cases, but distant metastases were present in 12% of cases (3 ccRCC; 1 pRCC; 1 chRCC; and 1 TFE3-tRCC) at the time of diagnosis. Four cases with distant metastasis showed weak nuclear positivity with the TFE3-IHC672 clone. The median follow-up period was 33 months (min. 21 to max. 49 months. All patients were alive at the end of the follow-up period.

A total of 24 cases including 13/31 ccRCCs, 3/7 chRCCs, 2/5 pRCCs, 2/2 oncocytic tumours, and 3/3 TFE3-tRCCs showed weak nuclear staining with TFE3-IHC672. On the other hand, nuclear staining was observed with TFE3-MRQ37 in 2/31 ccRCCs, 1/5 pRCC and 2/3 TFE3-tRCC (Table 2). All TFE3-tRCCs showed strong nuclear positivity with the TFE3-IHC672 clone, while two of them showed strong nuclear staining with TFE3-MRQ37 clone. This case had been diagnosed based on the histomorphological findings and positive staining of the TFE3-IHC627 clone in addition to ancillary immunohistochemistry. Stromal cells and lymphocytes were also positively stained, regardless of tumour cells in 45/51 cases with the TFE3-IHC672 clone. Positively stained stromal cells and lymphocytes can be seen in Figure 2. In only 2 ccRCC cases, stromal cells and

Table 1. Characteristics of 51 renal cell carcinoma cases

	Mean (Min–Max) or percentage		Mean (Min–Max) or percentage		Mean (Min–Max) or percentage
Age	56 (25–82)	Nuclear grade		Renal capsule invasion	
Gender		NA	8	Present	16
Female	29	Grade 1	4	Absent	84
Male	71	Grade 2	33	Lymphovascular invasion	
Site		Grade 3	33	Present	86
Right	59	Grade 4	12	Absent	14
Left	41	pT		Renal vein invasion	
Localisation		T1a	25	Present	8
Lower pole	33	T1b	14	Absent	92
Middle pole	32	T2a	8	Perinephric tissue invasion	
Upper pole	35	T2b	6	Present	22
Operation		T3a	47	Absent	78
Parsiyel nephrectomy	24	Necrosis		Metastasis	
Radical nephrectomy	76	Present	53	Present	12
Histological subtype		Absent	47	Absent	88
RCC, NOS	6	Differentiation			
ccRCC	60	Present	4		
chRCC	14	Absent	96		
TFE3-tRCC	6	Renal sinus invasion			
pRCC	10	Present	39		
Oncocytic tumour	4	Absent	61		

Min: Minimum; Max: Maximum; RCC, NOS: Renal cell carcinoma, not otherwise specified; ccRCC: Clear cell renal cell carcinoma; chRCC: Chromophobe renal cell carcinoma; TFE3-tRCC: TFE3 rearranged renal cell carcinoma.

Table 2. The histological subtypes of renal cell carcinomas and immunohistochemical expressions of tumor cells with different TFE3 antibody clones

	RCC, NOS	ccRCC	chRCC	pRCC	TFE3-tRCC	Oncocytic tumour
TFE3-MRQ37						
Positive	0/3	2/31	0/7	1/5	2/3	0/2
Negative	3/3	29/31	7/7	4/5	1/3	2/2
TFE3-IHC627						
Positive	1/3	13/31	3/7	2/5	3/3	2/2
Negative	2/3	18/31	4/7	3/5	3/3	0/2

RCC, NOS: Renal cell carcinoma, not otherwise specified; ccRCC: Clear cell renal cell carcinoma; chRCC: Chromophobe renal cell carcinoma; TFE3-tRCC: TFE3 rearranged renal cell carcinoma.

lymphocytes were stained with the TFE3-MRQ37 clone as well as tumour cells. A background staining which is seen in Figure 3 was observed in some slides. There was a statistically significant difference in staining with the TFE3-MRQ37 clone between TFE3-tRCCs and the others (p=0.001). However, we couldn't find any significant difference in staining with the TFE3-IHC672 clone between TFE3-tRCCs and the others. Focal staining and staining at different intensities were two important and challenging situations for all RCCs in our group.

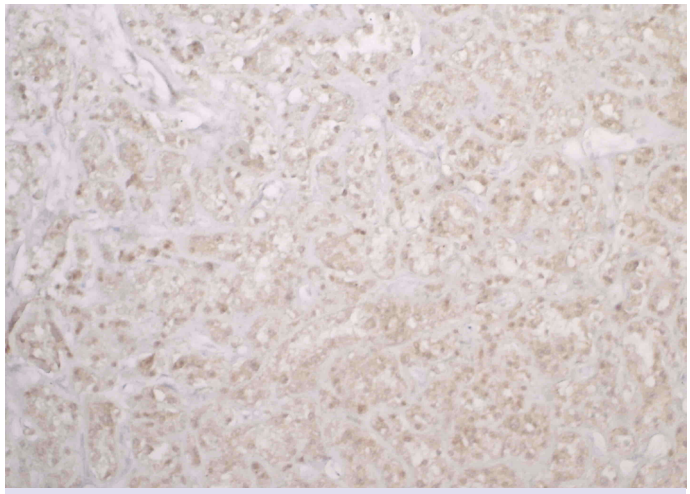

Figure 3. Background staining with TFE3-IHC372. (x20).

In this study, the specificity of the TFE3-MRQ37 clone was 94%, and the sensitivity was 67% for detecting TFE-tRCC cases. On the other hand, for TFE3-IHC672, the specificity was 56% and the sensitivity was 100%. No significant correlation was found between immunohistochemical staining with either TFE3-IHC672 or TFE3-MRQ37 clones and the other histopathological parameters examined.

DISCUSSION

In our study, we found that only staining with the TFE3-MRQ37 clone was statistically significant in TFE3-tRCCs compared to the other histological subtypes of RCCs.

TFE3-tRCCs are a group of tumour that can be easily underestimated because they can morphologically resemble many other RCC subtypes. TFE3 immunohistochemistry IHC can be used to show translocation because it detects the abnormal expression of TFE3 protein, indicating the presence of the gene rearrangement. In our study, we investigated the performance of commercially available and most commonly used antı-TFE3 clones (TFE3-MRQ37 and TFE3-IHC672).

Our results demonstrated that the TFE3-MRQ37 clone showed statistically different staining in TFE3-tRCCs compared to other RCCs. With the TFE3-IHC627 clone, there is no significant difference between TFE3-tRCCs and the other RCCs. In our study, the TFE3-IHC672 clone showed positive staining in all TFE3-tRCCs and 44% (21/48) of nonTFE3-tRCCs and therefore its specificity was low similar to the literature. An important problem with TFE3 immunohistochemistry emerges as having low specificity rates despite high sensitivity rates in the literature [10]. On the other hand, the TFE3-MRQ37 clone stained positively in only 6% (3/48) of cases of nonTFE3-tRCCs and had a high specificity rate. In the literature, different sensitivity and specificity rates were reported for TFE3 immunohistochemistry IHC [7]. Our results also reveal the different rates between antibody clones and all these findings decrease the reliability of immunohistochemistry.

Moreover, background staining and positivity in lymphocytes and stromal cells were observed in 88% of the cases when staining with the TFE3-IHC672 clone. Similarly, background staining was mentioned as an important problem in the evaluation of immunohistochemistry [7]. Our study showed that TFE3-MRQ37 is superior to TFE3-IHC627 clone in terms of ease of interpretation because of the clarity of staining and consistency of results.

In our study, we observed nuclear positivity with both antibody clones in all subtypes of RCCs at varying proportions and intensities. Similarly, it is reported that the positive staining pattern varied from weakly focal to diffusely strong staining in 111 of the 114 RCCs [7]. Moreover, Sharain et al. [10] found different staining patterns and proportions among other TFE3 rearranged tumours even with the same clone between two different laboratories. These data show us that immunohistochemical results are changeable depending on many different factors such as antibody clone and dilution, laboratory conditions, etc. Moreover, recent studies indicate that the presence of TFE3 protein overexpression in RCCs has prognostic implications regardless of the presence of TFE3 rearrangement. Tumours showing TFE3 expression have a poor prognosis [8, 11, 12]. In contrast to these studies, we couldn't find any statistically significant correlation between immunohistochemical expressions of both clones and histopathological parameters.

This study has potential limitations. As proposed in the WHO Blue Book, diagnosis of TFE3-tRCC is only possible by the demonstration of TFE3 arrangement by immunohistochemistry, FISH, or molecular testing. Firstly, we used only immunohistochemistry to diagnose cases and couldn't validate our positive cases with break-apart FISH or RNA sequencing. These tests are not available in many laboratories including ours. Although the FISH break apart probe has been validated in many studies, immunohistochemically positive but FISH-neg-

ative cases and vice versa were also reported in the literature [13]. Because available FISH probes do not cover all translocations regarding TFE3 gene, the false negative results with FISH may be explained by intrachromosomal translocations with rare partners other than XP11. FISH analysis is quite expensive and needs to Secondly, we had a small number of TFE3-tRCC cases in the study. However, TFE3-tRCC is a rare tumour and every study is important to expand our understanding of the histological and immunohistochemical properties and the behaviour of tumour. Lastly, the follow-up period was short in our study, so we didn't obtain reliable results regarding prognosis and survival.

Conclusion

In conclusion, TFE3 immunohistochemistry plays a crucial role in the diagnosis, subtyping, and prognostication of renal cell carcinoma, particularly in identifying cases with TFE3 gene rearrangement and TFE3 protein overexpression. It enhances the accuracy of diagnosis and provides valuable information for patient management. By comparing different aspects of different clones of TFE3 immunohistochemistry, we demonstrated insights into their relative performance and suitability for detecting TFE3 protein expression in RCC samples. In our study, the TFE3-MRQ137 clone gave more accurate results for the diagnosis of TFE3-tRCC and therefore may be preferred for detecting TFE3 rearrangement in routine practice. Different subtypes of RCC can be positively stained by TFE3 regardless of TFE3 rearrangement. In addition to that, various sensitivity and specificity rates of clones and different staining results depending on antibody and/or laboratory conditions are potential problems in application and interpretation of immunohistochemical staining for TFE3-tRCCs. Our results demonstrated that different TFE3 antibody clones have different staining properties. For these reasons, we hope that our results can increase the awareness of this indisputable need for developing a standardised approach for TFE3 immunohistochemistry IHC such as clones, staining procedure, interpretation of staining, and its clinical implications etc. A standardised evaluation helps guide the selection of the most appropriate clone for diagnostic or research purposes, prevents false positive diagnoses, and eliminates possible financial losses. Additionally, validation studies should be performed to ensure that the selected clone performs optimally in the intended application.

Ethics Committee Approval: The Ankara Bilkent City Hospital Ethics Committee granted approval for this study (date: 14.02.2024, number: TABED-1-24-24).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: We didn't use artificial intelligence(AI)- assisted technologies to produce the submitted paper.

Authorship Contributions: Concept – BG, TDKU; Design – TDKU, NS; Supervision – BG, AY; Fundings – TDKU; Materials – MMK, TDKU; Data collection and/or processing – MMK, TDKU; Analysis and/or interpretation – BG, TDKU, NS; Literature review – AY, MMK; Writing – TDKU, NS; Critical review – BG, AY, TDKU.

Peer-review: Externally peer-reviewed.

REFERENCES

1. Trpkov K, Williamson SR, Gill AJ, Adeniran AJ, Agaimy A, Alaghehbandan R, et al. Novel, emerging and provisional renal entities: The Genitourinary Pathology Society (GUPS) update on renal neoplasia. Mod Pathol 2021;34:1167-84. [Crossref]
2. Ellati RT, Abukhiran I, Alqasem K, Jasser J, Khzouz J, Bisharat T, et al. Clinicopathologic Features of Translocation Renal Cell Carcinoma. Clin Genitourin Cancer 2017;15:112-6. [Crossref]
3. Alaghehbandan R, Siadat F, Trpkov K. What's new in the WHO 2022 classification of kidney tumours? Pathologica 2023;115:8-22. [Crossref]
4. Argani P. MiT family translocation renal cell carcinoma. Semin Diagn Pathol 2015;32:103-13. [Crossref]
5. Trpkov K, Hes O, Williamson SR, Adeniran AJ, Agaimy A, Alaghehbandan R, et al. New developments in existing WHO entities and evolving molecular concepts: The Genitourinary Pathology Society (GUPS) update on renal neoplasia. Mod Pathol 2021;34:1392-424. [Crossref]
6. Cimadamore A, Cheng L, Scarpelli M, Massari F, Mollica V, Santoni M, et al. Towards a new WHO classification of renal cell tumor: What the clinician needs to know-a narrative review. Transl Androl Urol 2021;10:1506-20. [Crossref]
7. Akgul M, Williamson SR, Ertoy Di, Argani P, Gupta S, Caliò A, et al. Diagnostic approach in TFE3-rearranged renal cell carcinoma: A multi-institutional international survey. J Clin Pathol 2021;74:291-9. [Crossref]
8. Lee HJ, Shin DH, Kim SY, Hwang CS, Lee JH, Park WY, et al. TFE3 translocation and protein expression in renal cell carcinoma are correlated with poor prognosis. Histopathology 2018;73:758-66. [Crossref]
9. Tang J, Baba M. MiT/TFE Family Renal Cell Carcinoma. Genes (Basel). 2023;14:151. [Crossref]
10. Sharain RF, Gown AM, Greipp PT, Folpe AL. Immunohistochemistry for TFE3 lacks specificity and sensitivity in the diagnosis of TFE3-rearranged neoplasms: a comparative, 2-laboratory study. Hum Pathol 2019;87:65-74. [Crossref]
11. Lin J, Tang Z, Zhang C, Dong W, Liu Y, Huang H, et al. TFE3 gene rearrangement and protein expression contribute to a poor prognosis of renal cell carcinoma. Heliyon 2023;9: e16076. [Crossref]

12. Takamatsu D, Kohashi K, Kiyozawa D, Kinoshita F, Ieiri K, Baba M, et al. TFE3-immunopositive papillary renal cell carcinoma: A clinicopathological, immunohistochemical, and genetic study. Pathol Res Pract 2023;242. [Crossref]

13. Akgul M, Saeed O, Levy D, Mann SA, Cheng L, Grignon DJ, et al. Morphologic and immunohistochemical characteristics of fluorescent in situ hybridization confirmed TFE3 -Gene fusion associated renal cell carcinoma: A single institutional cohort. Am J Surg Pathol 2020;44:1450-8. [Crossref]