

TFE3-rearranged and TFEB-altered renal cell carcinoma: from classification to real-life. Insights from a national Italian survey

Stefano Marletta^{1,2*}, Anna Calì^{3*}, Giuseppe Nicolò Fanelli⁴, Paola Bianco⁵, Angelo Giovanni Bonadio⁶, Claudia Covelli⁷, Simona Francesconi⁸, Mariia Ivanova⁹, Daniele Liscia¹⁰, Alessia Moro¹¹, Daniela Onnis¹², Maria Rosaria Raspollini¹³, Costantino Ricci¹⁴, Steno Sentinelli¹⁵, Marina Valeri¹⁶, Guido Martignoni^{3,17}

¹ Division of Pathology, Humanitas Istituto Clinico Catanese, Catania, Italy; ² Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milano; ³ Department of Diagnostics and Public Health, Section of Pathology, University of Verona, Italy; ⁴ Division of Pathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy; ⁵ Section of Pathology, Department of Surgical Sciences, University of Cagliari, Cagliari, Italy; ⁶ Pathology Unit, S. Giacomo Hospital, Novi Ligure, Alessandria, Italy; ⁷ Pathology Unit, Ospedale Desenzano del Garda, Italy; ⁸ Pathology Unit, Azienda Ospedaliera S. Maria, Terni, Italy; ⁹ Division of Pathology, IEO European Institute of Oncology IRCCS, Milan, Italy; ¹⁰ Anatomic Pathology Department, Degli Infermi Hospital, Biella, Italy; ¹¹ Pathology Unit, ASST Santi Paolo e Carlo, Milan; ¹² Pathology Department, Giuseppe Brotzu Hospital, Cagliari, Italy; ¹³ Department of Histopathology and Molecular Diagnostics, Careggi University Hospital, Florence, Italy; ¹⁴ Pathology Unit, DIAP-Dipartimento Interaziendale di Anatomia Patologica di Bologna, Maggiore Hospital-AUSL, Bologna, Bologna, Italy; ¹⁵ Department of Pathology, IRCCS "Regina Elena" National Cancer Institute, Rome, Italy; ¹⁶ Pathology Unit, Bolognini Hospital, Seriate, Bergamo, Italy; ¹⁷ Department of Pathology, Pederzoli Hospital, Peschiera del Garda, Italy

* These authors contributed equally to this work.

Summary

Objective. Ongoing discoveries in cancer research keep expanding the landscape of renal cell carcinoma classification, particularly for "molecularly-defined" tumors like TFE3-rearranged and TFEB-altered renal cell carcinoma. However, scientific updates often do not align with pathologists' daily practice and resources. Herein, we present the results from a national Italian survey assessing physicians' personal experience on TFE3-rearranged and TFEB-altered renal cell carcinomas.

Methods. An online questionnaire encompassing 26 questions was delivered to the Italian Study Group of Uropathology (GIUP) members, addressing critical concerns on their routine approach to these tumors. The answers were collected and further analyzed.

Results. Thirteen pathologists with varying uropathological experience responded to the survey. Data confirmed the rarity of these neoplasms, with 69% of participants experiencing fewer than five or none at all. Despite this, aggressive behavior was documented by half of the respondents. Unusual morphology (62%) and young age (38%) were identified as the most relevant clues for suspecting TFE3-rearranged and TFEB-altered renal cell carcinoma. However, variability was observed in the specific histological features and the age threshold. The majority of the participants (54%) agreed on the need for ancillary molecular techniques for diagnostic purposes. Regarding immunohistochemistry, all professionals relied on multiple assays, attributing a primary role to a panel including cathepsin K, melanocytic markers (HMB45 and melan-A), PAX8, cytokeratin 7, and CA9. Additionally, most (58%) reported routine TFE3 immunohistochemical staining, although generally considering it reliable as long as diffuse and intense (58%) or requiring FISH confirmation in every positive case (25%). As for this latter, variability was recorded regarding split-signals positivity cut-off.

Conclusions. The continuous evolution of renal cell carcinoma classification significantly impacts the pathologists' routine approach. Our survey underscores the importance of ongoing knowledge sharing and heightened awareness for accurately identifying TFE3-rearranged and TFEB-altered renal cell carcinoma and providing further insights on still unsolved issues.

Key words: TFE3-rearranged renal cell carcinoma, TFEB-altered renal cell carcinoma, online survey, morphology, immunohistochemistry, molecular tests

Received: July 19, 2025
Accepted: August 27, 2025

Correspondence

Guido Martignoni
E-mail: guido.martignoni@univr.it

How to cite this article: Marletta S, Calì A, Nicolò Fanelli GN, et al. TFE3-rearranged and TFEB-altered renal cell carcinoma: from classification to real-life. Insights from a national Italian survey. *Pathologica* 2025;117:384-392 <https://doi.org/10.32074/1591-951X-N1518>

© Copyright by Società Italiana di Anatomia Patologica e Citopatologia Diagnostica, Divisione Italiana della International Academy of Pathology



OPEN ACCESS

This is an open access journal distributed in accordance with the CC-BY-NC-ND (Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International) license: the work can be used by mentioning the author and the license, but only for non-commercial purposes and only in the original version. For further information: <https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>

Introduction

The ongoing discovery of specific pathological signatures keeps reshaping tumor classification, modifying previously acknowledged categories, and adding novel entities. The latest 2022 WHO classification of renal cell tumors embraced such a pathogenic-centered approach by introducing the “molecularly defined renal carcinomas” category¹. This latter includes several relatively rare renal cell neoplasms characterized by peculiar driving molecular alterations carrying diagnostic, prognostic, therapeutic, and hereditary implications². Among these tumors, TFE3-rearranged and TFEB-altered renal cell carcinoma are currently recognized as separate entities according to their distinct pathological features and clinical behavior³ whereas, in past classifications, they were grouped together as “MiTF family renal cell carcinomas”⁴. Despite being uncommon, with TFE3-rearranged renal cell carcinoma accounting for about 1-4% of adult renal cell carcinomas^{5,6}, these tumors are more frequent in youngsters¹. Even pathologists who do not specialize in renal cell tumors may encounter these entities, especially given the increased number of resections for incidentally detected lesions. Due to the highly variable morphological spectrum and immunohistochemical findings along with the still ill-defined diagnostic criteria and the limited availability of gold-standard assays, pathologists often experience great difficulty in reaching an accurate diagnosis. Sharing current knowledge and maintaining a high level of awareness are crucial for properly identifying TFE3-rearranged and TFEB-altered renal cell carcinomas and avoiding misdiagnoses. In this work, we present the results of a national survey conducted by the Italian Study Group of Uropathology (“Gruppo Italiano di Studio di Uropatologia” – GIUP), which aimed to assess pathologists’ perspectives on critical issues related to the approach to TFE3-rearranged and TFEB-altered renal cell carcinomas.

Survey structure and results

SURVEY OUTLINE

Under the Italian Society of Pathology and Cytopathology (SIAPeC), the survey was led by two experienced uropathologists (A.C. and G.M.) of the GIUP and engaged pathologists from across Italy. In detail, the GIUP encompasses physicians from 15 different Italian regions, and routinely performs webinars, on-site meetings, and surveys to update its subscribers’ knowledge according to the latest evidence and to promote collaboration and scientific production.

The questionnaire was delivered online and consisted of 26 questions, listed in Table I, concerning current practice and personal experience about TFE3-rearranged and TFEB-altered renal cell carcinomas. The survey was sent to the institutional email addresses of GIUP members, enclosing a link to a Google Modules page containing the queries. In detail, the questions focused on participants’ perceived crucial clinical, morphological, immunohistochemical, and molecular findings regarding these neoplasms. Demographic information, such as years of experience and specific practice setting, was also recorded to interpret the survey results. Finally, three of the authors (S.M., A.C., and G.N.F.) collected the results via an Excel standard form (Microsoft) and then analyzed the response data.

DEMOGRAPHICS AND GENERAL PARAMETERS

Thirteen pathologists from various Italian regions, representing both public and private institutions, responded to the survey (Fig. 1). Most (54%) had a consistent background in pathology (over 20 years), while slightly more than a third had relatively shorter experience (5-10 years, 38%). The wide majority of the participants were dedicated to uropathology in their daily practice (77%). Regarding their recent personal experience,

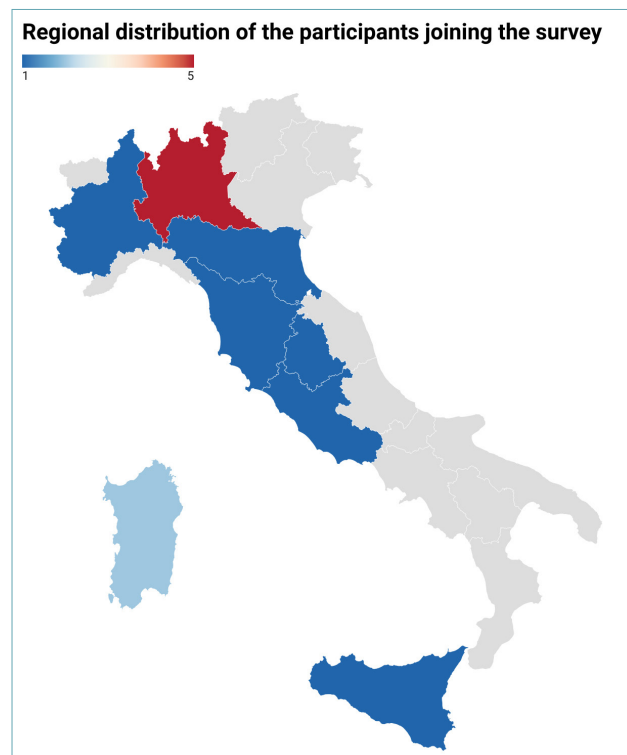


Figure 1. Schematic Italy map displaying the regional distribution of the participants joining the survey.

Table 1. Questions of the online survey regarding pathologists' perspective on key clinical and pathological issues of TFE3-rearranged and TFEB-altered renal cell carcinomas.

Query	Options					
How long have you been working in pathology?	< 5 years		5-10 years	10-15 years	> 20 years	
Are you routinely dedicated to uropathology?	Yes			No		
How many renal tumors have you been dealing with in the last five years?	< 25		15-50	50-100	> 100	
How many TFE3-translocated/TFEB-altered RCCs have you faced in the last five years?	0	< 5		5-10	10-15	> 15
How many TFEB-amplified RCCs have you diagnosed?	0			1	2	
How many metastasizing TFE3-translocated/TFEB-altered RCCs have you observed?	0		1	2		> 5
How would you rank clinical-pathological findings to suspect a TFE3-translocated/TFEB-altered RCC?	Age, gross, unusual morphology					
As for age, which threshold do you consider suspicious?	< 30 y.o.		< 40 y.o.	< 45 y.o.	< 50 y.o.	
How would you rank pathological characters to consider a diagnosis of TFE3-translocated/TFEB-altered RCC?	Papillary clear cells, calcifications, mixed architectural patterns					
Do you think nucleolar grading carries prognostic meaning in TFE3-translocated/TFEB-altered RCCs?	Yes			No		
If yes, why?						
Do you think coagulative tumoral necrosis carries prognostic meaning in TFE3-translocated/TFEB-altered RCCs?	Yes			No		
If yes, why?						
Have you ever observed sarcomatoid dedifferentiation in TFE3-translocated/TFEB-altered RCCs?	Yes			No		
If yes, in how many cases?						
Which pathological features would you rely on to diagnose a TFE3-translocated/TFEB-altered RCC?	Morphology, IHC, FISH, NGS					
Which IHC markers do you routinely test on specimens suspicious of TFE3-translocated/TFEB-altered RCC?	CD10, CA9, TFE3, HMB45, Melan-A, CK AE1/AE3, CK7, AMACR, S100A1, PAX8, Cathepsin K, vimentin					
How would you rank the following IHC markers to support a TFE3-translocated/TFEB-altered RCC diagnosis?	Cathepsin K, HMB45, Melan-A, TFE3, PAX8, CK7, CA9, CD10					
Is your laboratory furnished with a Cathepsin K IHC assay?	Yes			No		
If yes, which clone?	3F9			EPR19992		
Do you routinely employ TFE3/TFEB IHC assays?	Yes			No		
If yes, what kind of antibody do you use?						
How would you consider TFE3/TFEB IHC results?	Positive regardless of the %			Positive only if intense and diffuse	FISH confirm for every positive case	
Which FISH cut-off do you set to define a sample translocated/alters for the <i>TFE3/TFEB</i> genes?	> 10%			> 20%	> 50%	
Would you perform NGS analysis in every TFE3-translocated/TFEB-altered RCC case?	Yes			No		
If yes, for which purpose?						

Abbreviations: RCC: renal cell carcinoma, y.o.: years old, IHC: immunohistochemistry, FISH: fluorescence in situ hybridization, NGS: next generation sequencing, CK: cytokeratin, AMACR: alpha-methylacyl-CoA racemase.

nearly half of the involved physicians (46%) dealt with more than 100 renal tumors in the past 5 years. Only two responders, both not routinely dedicated to uropathology, had observed fewer than 25 (16%). During the same period, about two-thirds of the professionals reported encountering fewer than 5 (46%) TFE3-rearranged or TFEB-altered renal cell carcinomas, or even none at all (23%). Similarly, a noteworthy percentage of the participants (83%) reported having faced TFEB-amplified renal cell carcinomas just once (42%) or never (41%). In terms of clinical outcome, half of the responders (50%) documented aggressive behavior in TFE3-rearranged and TFEB-altered renal cell carcinomas they had encountered, confirmed by the development of distant metastases.

CLINICAL AND MORPHOLOGICAL FEATURES

An unusual morphological appearance compared to other more common renal cell tumors was identified as the clue that most frequently (62%) triggered the suspicion of TFE3-rearranged and TFEB-altered renal cell carcinomas, followed by patients' age (38%) (Fig. 2A). As for this latter, most pathologists set an age threshold of less than 40 (41%) or 30 years old (17%) to raise the suspicion of TFE3-rearranged/TFEB-altered renal cell carcinomas, with the remaining moving the cut-off to patients younger than 45 (17%) or even 50 years old (25%). On characterizing morphological features, variability was observed, with a relevant role mainly attributed to a clear cell-made papillary pattern (62%), followed by the presence of mixed architectural patterns (31%) (Fig. 2B). Although less useful, calcifications were identified as secondary significant findings by roughly two-thirds of the physicians (62%). As far as further morphological features were concerned, just 30% of the respondents considered nucleolar grading prognostically important in TFE3-rearranged/TFEB-altered renal cell carcinomas, primarily because of examples of its correlation with clinical outcome. Likewise, for the same reasons, coagulative tumor necrosis was claimed to carry prognostic meaning by only slightly more than a third (36%) of the participants. Finally, just one physician reported having observed sarcomatoid dedifferentiation in a TFE3-rearranged/TFEB-altered renal cell carcinoma case.

IMMUNOHISTOCHEMICAL AND MOLECULAR FINDINGS

Nearly half (46%) of the participants considered morphology and immunohistochemistry reliable enough for a definitive diagnosis of TFE3-rearranged/TFEB-altered renal cell carcinoma. Conversely, the remaining (54%) retained the integration of additional tests like fluorescence in situ hybridization (FISH) mandatory

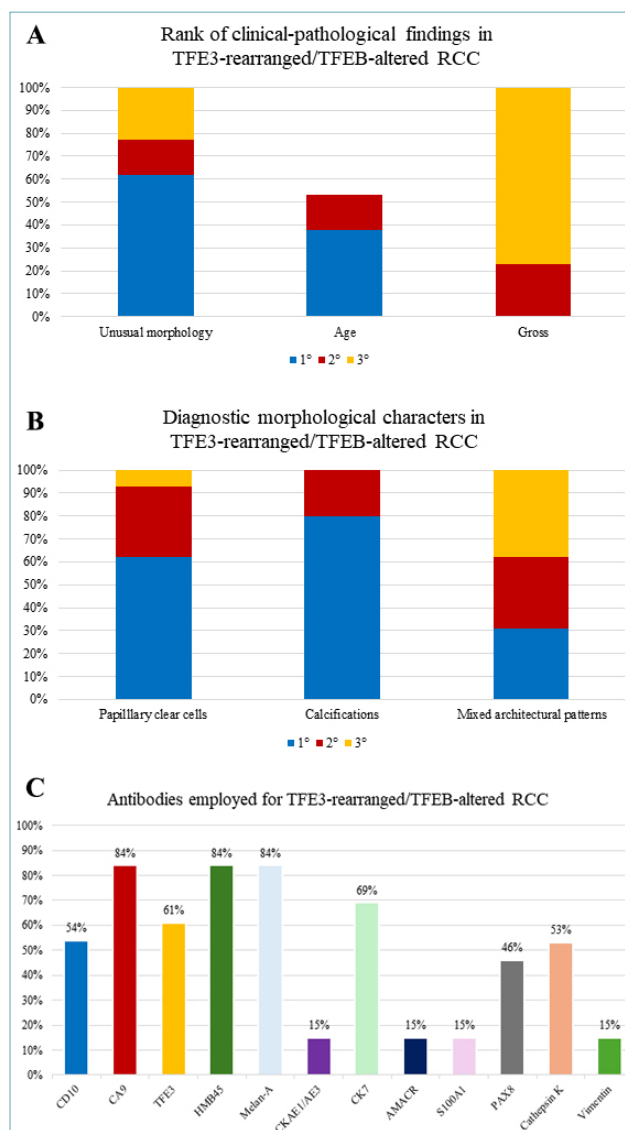


Figure 2. Charts showing the participants' rank of different clinical-pathological findings (A), morphological features (B), and routinely employed immunohistochemical antibodies (C) for the diagnosis of TFE3-rearranged and TFEB-altered renal cell carcinoma.

to confidently reach such a diagnosis, whether (15%) or not (39%) integrated with molecular sequencing. With regard to immunohistochemical markers, most pathologists reported employing carbonic anhydrase 9 (CA 9, 84%), HMB45 (84%), melan-A (84%), cytokeratin 7 (CK7, 69%), TFE3 (61%), CD10 (54%), and cathepsin K (53%). Fewer relied on PAX8 (46%), while just a minority routinely adopted CKAE1/AE3 (15%), alpha-methylacyl-CoA racemase (AMACR, 15%), S100A1 (15%), or vimentin (15%) (Fig. 2C). When asked to rank the former assays, half of pathologists considered the combination of cathepsin K

and melanocytic markers as the most diagnostically significant (50%). On the other hand, a minority attributed a primary role to PAX8, TFE3, and CK7 (33%) or CD10 combined with melanocytic markers (17%). As for cathepsin K, roughly half of the participants' laboratories were effectively equipped with such a marker (45%), all employing the 3F9 (Abcam) clone. Similarly, more than half (58%) of the participants reported routinely using TFE3/TFEB immunohistochemical assays, all employing a laboratory self-made staining protocol. Most physicians (58%) employing these latter claimed to consider a positive immunohistochemical TFE3/TFEB staining as long as intense and diffuse, while a minority (17%) accomplished as diagnostically relevant every positive case, regardless of the intensity and percentage. To note, a significant proportion (25%) of the respondents would rather obtain a FISH confirmation for each positive case. As for the FISH technique, there was considerable variability in the positivity cut-off, with an equal proportion (40%) of physicians using either a 20% or 50% split-signal threshold. Finally, in case of resource availability, most responders (73%) would perform molecular sequencing in TFE3-rearranged/TFEB-altered renal cell carcinoma, mainly to either confirm a doubtful immunohistochemical result or to further characterize the underlying molecular alterations.

The responses of the participants to each question of the survey, along with their corresponding percentages, are summarized in Supplementary Table I.

Discussion

Advances in cancer research keep on changing the current classification of renal tumors, defining specific biologically distinct categories, such as molecularly defined renal cell carcinoma. However, due to their rarity and complexity, the recognition of the peculiar pathological features as well as the application of strict diagnostic criteria often lags behind in routine practice. Thus, in the present study, we aimed to investigate in a real-world setting pathologists' personal experience and perspective on TFE3-rearranged and TFEB-altered renal cell carcinoma.

Our survey embraced a heterogeneous population of physicians from various institutions, with different levels of uropathological experience in both their backgrounds and daily activities. Firstly, our data confirm the rarity of these tumors, with most participants observing fewer than 5 cases in the last 5 years. This aligns with the reported incidence of about 1-4% for TFE3-rearranged renal cell carcinoma^{5,6} and 0.02% for TFEB-altered renal cell carcinoma³ among adult

renal cell carcinomas. According to recent data⁷, renal cell carcinoma should account for roughly 11,000 novel cancer cases in Italy in 2024, once upper urinary tract carcinomas are ruled out. Hence, within the same period, about 100-400 TFE3-rearranged renal cell carcinomas and 2-3 TFEB-altered renal cell carcinomas should be diagnosed. Since the first report in 2001⁸, more than 1200 TFE3-rearranged renal cell carcinomas with documented *TFE3* gene rearrangement have been described⁹. It is also likely that the incidence of such neoplasms would further increase thanks to the wider availability of novel diagnostic assays. Thus, pathologists should be aware that such tumors may also be faced by professionals who are not routinely dedicated to uropathology and should not be misdiagnosed as unclassified renal cell carcinoma. This consideration is of particular prognostic concern, because TFE3-rearranged and TFEB-altered renal cell carcinoma may display a severe outcome, as underlined by our data of half participants (50%) documenting at least one metastasizing tumor in their personal experience. Specifically, published evidence claims about half of TFE3-rearranged renal cell carcinomas to develop either local recurrence, distant metastases, or both⁹. Conversely, TFEB-rearranged renal cell carcinomas appear to behave more indolently, with local or distant recurrences observed in just 17% of the cases³. However, it is worth mentioning that neoplasms carrying amplifications, rather than translocations, of the *TFEB* gene have been found to have a more severe prognosis^{10,11}.

Secondly, the herein questionnaire strengthens the importance of specific clinical and pathological features in suspecting TFE3-rearranged and TFEB-altered renal cell carcinoma. Namely, while minor significance was attributed to gross appearance, an unusual morphology (62%) and patient's age (38%) were indicated as the most relevant clues. As for this latter, the majority set a threshold of less than 40 years old, while a minority used a 50-year-old cutoff. Notably, both these tumors had been initially described in childhood, with TFE3-rearranged renal cell carcinoma globally accounting for 40% of pediatric renal cell carcinomas¹. Nevertheless, neoplasms in older adults have since been documented, so that nowadays the average age is approximately 35-40 years old for TFE3-rearranged renal cell carcinoma⁹ and 30-35 years old for TFEB-altered renal cell carcinoma^{3,12}. With regard to morphology, architectural growth patterns and microscopic findings unusual for other conventional renal cell carcinoma histotypes are the most typical hint that triggers the suspicion of TFE3-rearranged and TFEB-altered renal cell carcinoma. Among these, the evidence of papillary structures made up of clear cells was ranked

as the most common finding in TFE3-rearranged and TFEB-altered renal cell carcinoma (62%), followed by a mixture of distinct architectural patterns (31%). In this view, it should be underlined that a papillary-arranged clear cell proliferation is certainly one of the most frequent patterns in TFE3-rearranged renal cell carcinoma, especially if psammoma-like calcifications are also noted^{8,13} (Fig. 3), but many other patterns can also occur. TFE3-rearranged tumors mimicking clear cell or papillary renal cell carcinoma¹⁴, clear cell papillary renal cell tumor¹⁵, and multilocular cystic renal neoplasm of low malignant potential¹⁶ are on record, as well as TFEB-altered neoplasms resembling oncocytoma¹⁷, chromophobe renal cell carcinoma, and epithelioid angiomyolipoma/pure epithelioid PEComa^{18,19}. Hence, when dealing with unusual renal tumors, a high level of awareness is the key to addressing appropriate further analyses and getting the proper diagnosis. Moreover, similar to other molecularly defined renal cell carcinomas such as fumarate hydratase-deficient renal cell carcinoma²⁰, intratumoral architectural and cellular heterogeneity has been observed in TFE3-rearranged and TFEB-altered renal cell carcinoma^{21–24}. Speaking of this, just a minority of the participants considered nucleolar grading (30%) and the presence of coagulative tumor necrosis (36%) as prognostically useful in TFE3-rearranged and TFEB-altered renal cell carcinoma. The clinical meaning of such histological findings is still partially unsolved in these neoplasms. While nucleolar grading failed to reach statistical meaning²⁵, in some cohorts, tumor necrosis correlated to patients' prognosis both in TFE3-rearranged²⁵ and TFEB-altered renal cell carcinoma²¹. However, as most studies rely on limited case series, further wider studies are indeed warranted to further

address these topics. Finally, all but one participant reported never noticing sarcomatoid dedifferentiation in TFE3-rearranged and TFEB-altered renal cell carcinoma, indicating that this feature is extremely rare in such tumors, even in the most pathologically aggressive cases.

Although it is indeed critical to initially suspect TFE3-rearranged and TFEB-altered renal cell carcinoma, morphology alone is not sufficient for a definitive diagnosis. According to all survey participants, integration with ancillary techniques such as immunohistochemistry, FISH, and molecular sequencing is mandatory. However, the respondents were divided, with 46% relying solely on immunohistochemistry and 54% using immunohistochemistry along with other molecular tests. Regarding immunohistochemistry, the majority of the answers routinely employed CA9, HMB45, Melan-A, CK7, TFE3, CD10, and cathepsin K, while only a minority of laboratories used PAX8, CKAE1/AE3, AMACR, S100A1, and vimentin. The adoption of a reasoned panel of antibodies may be significantly helpful in addressing the diagnosis of TFE3-rearranged and TFEB-altered renal cell carcinoma, especially when more expansive molecular assays are not available²⁶. In this context, the majority of physicians attributed the greatest importance to cathepsin K, melanocytic markers, TFE3, PAX8, CK7, CA9, and CD10 assays, which aligns with the published literature. Namely, both types of tumors are generally negative for keratins¹, like CK7, and CA9, while a noteworthy proportion of TFE3-rearranged (35%)⁹ and TFEB-altered (90%)²⁵ renal cell carcinomas stain for melanocytic markers HMB45 and melan-A. Similarly, growing published evidence underscores the diagnostic role of cathepsin K in these tumors, be-

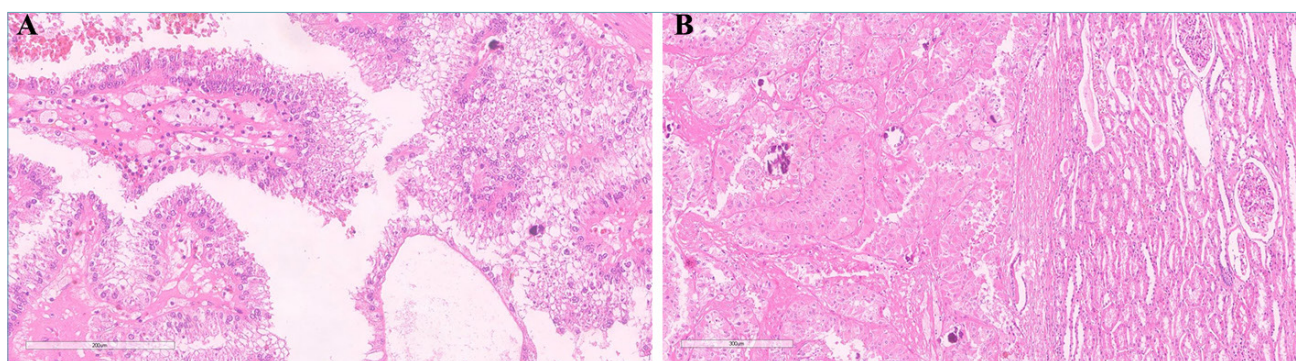


Figure 3. Representative hematoxylin and eosin images revealing diagnostic clues in TFE3-rearranged and TFEB-altered renal cell carcinoma: papillary-arranged structures (A) associated with deposits of psammoma-like calcifications (B). Notably, the neoplasms from both pictures are made up of a variable proportion of clear and eosinophilic cells, underscoring the architectural and cytological heterogeneity typical of these tumor entities.

cause, in contrast to other epithelial renal neoplasms, it is expressed in about 60% TFE3-rearranged renal cell carcinomas and nearly all TFEB-altered renal cell carcinomas²⁷. However, it is advisable to also perform PAX8 staining and, if possible, CD68 (clone PG-M1) when dealing with these tumors for the differential diagnosis of renal mesenchymal neoplasms, such as pure epithelioid PEComa (so-called epithelioid angiomyolipoma) or TFE3-rearranged PEComas, which may share almost overlapping morphology and immunophenotype^{9,18,19,28}. About cathepsin K, it is also worth noting that all the laboratories furnished with it (55%) employed the 3F9 (Abcam) clone. This latter has been associated with higher sensitivity and specificity rates^{29,31} than a recently developed assay (clone EPR19992) which has instead proven to stain a significant proportion of other renal tumors, like oncocytomas and chromophobe renal cell carcinoma³². Of note, investigating cathepsin K expression may also carry therapeutic implications as it could partially explain response to cabozantinib, one of the most commonly employed systemic options for patients diagnosed with metastatic TFE3-rearranged and TFEB-altered renal cell carcinoma. In detail, this drug's activity in these patients has been correlated to modulation of osteoclast differentiation, causing down-regulating cathepsin K³³, and blockage of various kinases including MET, a known oncogenic driver in such tumors^{25,34}. Thus, in the current hardly desert landscape, apart from direct TFEB inhibitors like eltrombopag³⁵ still in early experimental phases, demonstrating positive cathepsin K staining may strengthen the adoption of specific therapeutic options for TFE3-rearranged and TFEB-altered renal cell carcinoma patients.

Most pathologists joining the survey (58%) routinely performed TFE3/TFEB immunohistochemical assessment when suspecting TFE3-rearranged and TFEB-altered renal cell carcinoma. Among them, a minority (25%) would require further FISH confirmation, while the majority would consider reliable TFE3/TFEB staining as long as intense and diffuse (58%) or even regardless of the pattern of expression (17%). It should be noted that the role of such immunohistochemical assays is still controversial. Despite being potentially helpful in specific settings (i.e. subtle paracentric inversions easily missed by FISH), they have been associated with potential false-negative and false-positive rates^{24,28,36,37}. Thus, to date, documenting genetic alterations by FISH or molecular sequencing remains the gold standard to definitely prove a diagnosis of TFE3-rearranged and TFEB-altered renal cell carcinoma¹. As far as FISH is concerned, it has been historically considered the ground truth test for identifying TFE3-rearranged and TFEB-altered renal

cell carcinomas, thanks to its accuracy and lesser influence by fixation issues²³. However, no universally acknowledged positivity cut-off has been established, as evidenced by the variable thresholds set by participants, ranging from 10% to 50% split signals. In addition, false-negative FISH results may be caused by slight paracentric inversions involving the Xp11 chromosome, like *TFE3::RBM10* and *TFE3::NONO*, among others^{38,39}. In such instances, sequencing techniques like RNA-sequencing or RT-PCR are the keys in identifying the underlying molecular alterations and then confirming the diagnosis in suspicious cases (i.e. morphological features, positive cathepsin K and TFE3 immunohistochemistry)⁴⁰. Although not generally required to sort the diagnostic quandary out, most participants (73%) agreed they would routinely perform molecular sequencing if their laboratories were equipped with it. Beyond diagnostic purposes, the rationale of this trend lies in the pathological and prognostic correlations of these techniques. Specific alterations often reflect peculiar morphological patterns, such as a papillary architecture in *TFE3::PRCC* carcinomas⁴¹, a biphasic TFEB-like or pseudorosettes pattern in *TFE3::SFPQ* neoplasms⁹, and a cystic growth in *TFE3::MED15* tumors¹⁶. Furthermore, differences in clinical behavior have been associated with distinct molecular alterations: for instance, *TFE3::MED15* neoplasms usually have an indolent behavior⁴², while an increased gene copy number may be associated with poorer outcome in cases with *TFEB* amplification^{11,18,43,44}.

Conclusions

The adoption of novel diagnostic techniques and the modification of renal cell carcinoma classification may significantly impact physicians' daily practice and pathology reports. Herein, we have reported the results of a national Italian survey on TFE3-rearranged and TFEB-altered renal cell carcinoma. Our data further demonstrates that constant updating and sharing of knowledge, along with a high level of awareness, are the keys to the correct recognition of such tumors and to avoiding misdiagnoses, in order to ultimately provide patients with the best clinical management.

CONFLICTS OF INTEREST STATEMENT

The authors have nothing to disclose.

FUNDING

This study was funded by: "Bando di ricerca finalizzata 2021 (PC: GR-2021-12374462)".

AUTHORS' CONTRIBUTION

Conceptualization: AC and GM. Methodology: SM, AC, and GN. Formal analysis and investigation: AC and GN. Writing — original draft preparation: SM and AC. Writing — review and editing: PB, AGB, CC, SF, MI, DL, AM, DO, MRR, CR, SS, and MV. Supervision: AC and GM.

References

- WHO Classification of Tumours Editorial Board, ed. WHO Classification of Tumours, Urinary and Male Genital Tumours. 5th ed. Lyon: IARC press; 2022.
- Caliò A, Marletta S, Brunelli M, et al. WHO 2022 Classification of Kidney Tumors: what is relevant? An update and future novelties for the pathologist. *Pathologica* 2022;115:23–31. <https://doi.org/10.32074/1591-951X-814>.
- Caliò A, Segala D, Munari E, et al. MiT Family Translocation Renal Cell Carcinoma: from the Early Descriptions to the Current Knowledge. *Cancers (Basel)*. 2019;11. <https://doi.org/10.3390/cancers11081110>.
- Moch H, Humphrey PA, Ulbright TM, et al. WHO Classification of Tumours of the Urinary System and Male Genital Organs. 4th ed. Lyon: IARC press; 2016.
- Komai Y, Fujiwara M, Fujii Y, et al. Adult Xp11 translocation renal cell carcinoma diagnosed by cytogenetics and immunohistochemistry. *Clin. Cancer Res.* 2009;15:1170–1176. <https://doi.org/10.1158/1078-0432.CCR-08-1183>.
- Sukov WR, Hodge JC, Lohse CM, et al. TFE3 rearrangements in adult renal cell carcinoma: clinical and pathologic features with outcome in a large series of consecutively treated patients. *Am. J. Surg. Pathol.* 2012;36:663–670. <https://doi.org/10.1097/PAS.0b013e31824dd972>.
- Associazione Italiana Oncologia Medica. I numeri del cancro in Italia.; 2024.
- Argani P, Antonescu CR, Illei PB, et al. Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: a distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. *Am. J. Pathol.* 2001;159:179–192. [https://doi.org/10.1016/S0002-9440\(10\)61684-7](https://doi.org/10.1016/S0002-9440(10)61684-7).
- Marletta S, Caliò A, Pierconti F, et al. SFPQ::TFE3-rearranged PEComa: Differences and analogies with renal cell carcinoma carrying the same translocation. *Pathol. Res. Pract.* 2025;270:155963. <https://doi.org/10.1016/j.prp.2025.155963>.
- Kammerer-Jacquet S-F, Gandon C, Dugay F, et al. Comprehensive study of nine novel cases of TFEB-amplified renal cell carcinoma: an aggressive tumour with frequent PDL1 expression. *Histopathology* 2022. <https://doi.org/10.1111/his.14683>.
- Peckova K, Vanecek T, Martinek P, et al. Aggressive and non-aggressive translocation t(6;11) renal cell carcinoma: comparative study of 6 cases and review of the literature. *Ann. Diagn. Pathol.* 2014;18:351–357. <https://doi.org/10.1016/j.anndiagpath.2014.10.002>.
- Smith NE, Illei PB, Allaf M, et al. t(6;11) renal cell carcinoma (RCC): expanded immunohistochemical profile emphasizing novel RCC markers and report of 10 new genetically confirmed cases. *Am. J. Surg. Pathol.* 2014;38:604–614. <https://doi.org/10.1097/PAS.000000000000203>.
- Argani P, Antonescu CR, Couturier J, et al. PRCC-TFE3 renal carcinomas: morphologic, immunohistochemical, ultrastructural, and molecular analysis of an entity associated with the t(X;1) (p11.2;q21). *Am. J. Surg. Pathol.* 2002;26:1553–1566. <https://doi.org/10.1097/00000478-200212000-00003>.
- Hayes M, Peckova K, Martinek P, et al. Molecular-genetic analysis is essential for accurate classification of renal carcinoma resembling Xp11.2 translocation carcinoma. *Virchows Arch.* 2015;466:313–322. <https://doi.org/10.1007/s00428-014-1702-7>.
- Wang X-T, Xia Q-Y, Ye S-B, et al. RNA sequencing of Xp11 translocation-associated cancers reveals novel gene fusions and distinctive clinicopathologic correlations. *Mod. Pathol.* 2018;31:1346–1360. <https://doi.org/10.1038/s41379-018-0051-5>.
- Song Y, Yin X, Xia Q, et al. Xp11 translocation renal cell carcinoma with morphological features mimicking multilocular cystic renal neoplasm of low malignant potential: a series of six cases with molecular analysis. *J. Clin. Pathol.* 2021;74:171–176. <https://doi.org/10.1136/jclinpath-2020-206681>.
- Argani P. MiT family translocation renal cell carcinoma. *Semin. Diagn. Pathol.* 2015;32:103–113. <https://doi.org/10.1053/j.semdp.2015.02.003>.
- Caliò A, Brunelli M, Segala D, et al. T(6;11) renal cell carcinoma: A study of seven cases including two with aggressive behavior, and utility of CD68 (PG-M1) in the differential diagnosis with pure epithelioid PEComa/epithelioid angiomyolipoma. *Mod. Pathol.* 2018;31:474–487. <https://doi.org/10.1038/modpathol.2017.144>.
- Caliò A, Harada S, Brunelli M, et al. TFEB rearranged renal cell carcinoma. A clinicopathologic and molecular study of 13 cases. Tumors harboring MALAT1-TFEB, ACTB-TFEB, and the novel NEAT1-TFEB translocations constantly express PDL1. *Mod. Pathol.* 2021;34:842–850. <https://doi.org/10.1038/s41379-020-00713-6>.
- Caliò A, Marletta S, Stefanizzi L, et al. Comparison of Primary and Metastatic Fumarate Hydratase-Deficient Renal Cell Carcinomas Documents Morphologic Divergence and Potential Diagnostic Pitfall With Peritoneal Mesothelioma. *Mod. Pathol.* 2024;37:100561. <https://doi.org/10.1016/j.modpat.2024.100561>.
- Xia Q-Y, Wang X-T, Fang R, et al. Clinicopathologic and Molecular Analysis of the TFEB Fusion Variant Reveals New Members of TFEB Translocation Renal Cell Carcinomas (RCCs): Expanding the Genomic Spectrum. *Am. J. Surg. Pathol.* 2020;44:477–489. <https://doi.org/10.1097/PAS.0000000000001408>.
- Argani P, Olgac S, Tickoo SK, et al. Xp11 translocation renal cell carcinoma in adults: expanded clinical, pathologic, and genetic spectrum. *Am. J. Surg. Pathol.* 2007;31:1149–1160. <https://doi.org/10.1097/PAS.0b013e318031ffff>.
- Skala SL, Xiao H, Udager AM, et al. Detection of 6 TFEB-amplified renal cell carcinomas and 25 renal cell carcinomas with MITF translocations: systematic morphologic analysis of 85 cases evaluated by clinical TFE3 and TFEB FISH assays. *Mod. Pathol.* 2018;31:179–197. <https://doi.org/10.1038/modpathol.2017.99>.
- Akgul M, Williamson SR, Ertay D, et al. Diagnostic approach in TFE3-rearranged renal cell carcinoma: a multi-institutional international survey. *J. Clin. Pathol.* 2021;74:291–299. <https://doi.org/10.1136/jclinpath-2020-207372>.
- Caliò A, Brunelli M, Segala D, et al. Comprehensive analysis of 34 MiT family translocation renal cell carcinomas and review of the literature: investigating prognostic markers and therapy targets. *Pathology* 2020;52:297–309. <https://doi.org/10.1016/j.pathol.2019.11.006>.
- Caliò A, Marletta S, Brunelli M, et al. TFE3 and TFEB-rearranged renal cell carcinomas: an immunohistochemical panel to differentiate from common renal cell neoplasms. *Virchows Arch.* 2022. <https://doi.org/10.1007/s00428-022-03380-x>.
- Caliò A, Brunelli M, Gobbo S, et al. Cathepsin K: A Novel Diagnostic and Predictive Biomarker for Renal Tumors. *Cancers (Basel)*. 2021;13. <https://doi.org/10.3390/cancers13102441>.
- Caliò A, Marletta S, Brunelli M, et al. TFE3-Rearranged Tumors of the Kidney: An Emerging Conundrum. *Cancers (Basel)*. 2024;16. <https://doi.org/10.3390/cancers16193396>.

- ²⁹ Martignoni G, Bonetti F, Chilosi M, et al. Cathepsin K expression in the spectrum of perivascular epithelioid cell (PEC) lesions of the kidney. *Mod. Pathol.* 2012;25:100–111. <https://doi.org/10.1038/modpathol.2011.136>.
- ³⁰ Martignoni G, Pea M, Gobbo S, et al. Cathepsin-K immunoreactivity distinguishes MiTF/TFE family renal translocation carcinomas from other renal carcinomas. *Mod. Pathol.* 2009;22:1016–1022. <https://doi.org/10.1038/modpathol.2009.58>.
- ³¹ Martignoni G, Gobbo S, Camparo P, et al. Differential expression of cathepsin K in neoplasms harboring TFE3 gene fusions. *Mod. Pathol.* 2011;24:1313–1319. <https://doi.org/10.1038/modpathol.2011.93>.
- ³² Iakymenko OA, Delma KS, Jorda M, et al. Cathepsin K (Clone EPR19992) Demonstrates Uniformly Positive Immunoreactivity in Renal Oncocytoma, Chromophobe Renal Cell Carcinoma, and Distal Tubules. *Int. J. Surg. Pathol.* 2021;29:600–605. <https://doi.org/10.1177/1066896921991588>.
- ³³ Fioramonti M, Santini D, Iuliani M, et al. Cabozantinib targets bone microenvironment modulating human osteoclast and osteoblast functions. *Oncotarget* 2017;8:20113–20121. <https://doi.org/10.18632/oncotarget.15390>.
- ³⁴ Rizzo M, Pezzicoli G, Santoni M, et al. MiT translocation renal cell carcinoma: A review of the literature from molecular characterization to clinical management. *Biochim. Biophys. Acta. Rev. Cancer* 2022;1877:188823. <https://doi.org/10.1016/j.bbcan.2022.188823>.
- ³⁵ Lin Y, Shi Q, Yang G, et al. A small-molecule drug inhibits autophagy gene expression through the central regulator TFEB. *Proc. Natl. Acad. Sci. U. S. A.* 2023;120:e2213670120. <https://doi.org/10.1073/pnas.2213670120>.
- ³⁶ Argani P, Lal P, Hutchinson B, et al. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am. J. Surg. Pathol.* 2003;27:750–761. <https://doi.org/10.1097/00000478-200306000-00005>.
- ³⁷ Sharain RF, Gown AM, Greipp PT, et 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. <https://doi.org/10.1016/j.humpath.2019.02.008>.
- ³⁸ Argani P, Zhang L, Reuter VE, et al. RBM10-TFE3 Renal Cell Carcinoma: A Potential Diagnostic Pitfall Due to Cryptic Intrachromosomal Xp11.2 Inversion Resulting in False-negative TFE3 FISH. *Am. J. Surg. Pathol.* 2017;41:655–662. <https://doi.org/10.1097/PAS.0000000000000835>.
- ³⁹ Xia Q-Y, Wang Z, Chen N, et al. Xp11.2 translocation renal cell carcinoma with NONO-TFE3 gene fusion: morphology, prognosis, and potential pitfall in detecting TFE3 gene rearrangement. *Mod. Pathol.* 2017;30:416–426. <https://doi.org/10.1038/modpathol.2016.204>.
- ⁴⁰ Harada S, Calì A, Janowski KM, et al. Diagnostic utility of one-stop fusion gene panel to detect TFE3/TFEB gene rearrangement and amplification in renal cell carcinomas. *Mod. Pathol.* 2021;34:2055–2063. <https://doi.org/10.1038/s41379-021-00858-y>.
- ⁴¹ Tretiakova MS, Wang W, Wu Y, et al. Gene fusion analysis in renal cell carcinoma by FusionPlex RNA-sequencing and correlations of molecular findings with clinicopathological features. *Genes. Chromosomes Cancer* 2020;59:40–49. <https://doi.org/10.1002/gcc.22798>.
- ⁴² Tretiakova MS. Chameleon TFE3-translocation RCC and How Gene Partners Can Change Morphology: Accurate Diagnosis Using Contemporary Modalities. *Adv. Anat. Pathol.* 2022;29:131–140. <https://doi.org/10.1097/PAP.0000000000000332>.
- ⁴³ Yan M, Wang R, Guan W, et al. A clinicopathological and molecular series of five TFEB-altered renal cell carcinoma (RCC) cases: highlighting an aggressive subset of TFEB-rearranged RCC concomitant with TFEB amplification/gene copy number gains. *Virchows Arch.* 2024;485:1041–1051. <https://doi.org/10.1007/s00428-024-03968-5>.
- ⁴⁴ Zhang M, Xian J, Tang J, et al. Clinicopathologic and Molecular Study of TFEB-altered Renal Cell Carcinomas: Tumors With Frequent PDL1 Expression. *Am. J. Surg. Pathol.* 2025. <https://doi.org/10.1097/PAS.0000000000002458>.