A Simplified Diagnostic Approach on *TFE3* Gene Fusion–Associated Renal Cell Carcinoma

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The transcription factor E3 (*TFE3*) gene is located at the Xp11.2 locus,[1] and its rearrangement accounts for the majority of microphthalmia-associated transcription factor (MiT) family translocation renal cell carcinomas (RCCs),[1] about 40% of pediatric RCCs, and up to 5% of adult RCCs.[2] Young adults seem to be particularly affected, although TFE3 RCC can be seen in any age group, statistically more in adults than in the pediatric age group.[1,2] TFE3 RCC may present with various macroscopic features, such as solid mass with a flesh-colored cut surface (Figure 1, A) or multicystic appearance.[3] Large tumor cells with abundant clear or eosinophilic cytoplasm with at least focal true papillary formations (Figure 1, B), with occasional psammoma bodies and/or intracytoplasmic hyaline globules, have been underscored as classical histomorphologic features. However, microscopic features also vary significantly (Figure 1, C), partly because of the *TFE3* gene's promiscuous fusion arrangements by multiple gene partners.[4] Herein we share our thought process on TFE3 RCC in light of the current literature, including our recently published single-institutional experience on 85 fluorescence in situ hybridization (FISH)–confirmed TFE3 RCCs,[5] intending to facilitate the diagnostic approach on the subject.

At our institution, we perform TFE3 IHC assay using mrq-37 clone (Cell Marque, Rocklin, California) ready-to-use antibody with antigen retrieval at low PH on an automated Ultra Vision detection system (30/10/30/10; Thermo Fisher Scientific, Waltham, Massachusetts) on the Dako Omnis platform (Agilent Technologies, Santa Clara, California). TFE3 RCC tumor is used as a positive control, as it is known to increase the specificity of TFE3 IHC.[6] We semiquantitatively grade TFE3 protein expression as weak (+1), moderate (+2), and strong (+3) based on the staining intensity, and also estimate the ratio of TFE3-overexpressing tumor cells to all tumor cells as negative (0%), focal (<25%), regional (≥25%–74%), or diffuse (>75%). A FISH assay with break-apart probes detects *TFE3* gene rearrangement (Figure 1, D) and is considered the gold standard for the TFE3 RCC diagnosis, with very rare false negativity due to intrachromosomal translocations.[7] These translocations include, but are not limited to, RBM10 and NONO.[8] In these circumstances, molecular testing may help establish the diagnosis.

In our report on TFE3 RCC, classical morphology was documented in only about half of the TFE3 RCC (42 of 85; 49.4%).[5] This result may seem in contrast with the findings of recently published TFE3 RCC cohorts,[3,4,9,10] although 65 of 121 TFE3 RCC cases (53.7%) in these studies were identified in patients younger than 40 years, whereas in our cohort, a much smaller proportion of patients (21 of 85; 24.7%) were younger than 40 years. Among these studies, Argani et al[4] described that classical microscopic features were present in the majority of TFE3 RCC cases regardless of the *TFE3* gene fusion partner. Nevertheless, younger age and classical morphology should be used as important indicators to initiate workup for TFE3 RCC.

Initial reports have shown that immunohistochemical (IHC) documentation of nuclear TFE3 protein overexpression is sensitive and specific (Figure 1, B and C insets) in the detection of *TFE3* gene rearrangements, with 97.5% sensitivity and 99.6% specificity.[11] Also, negative or decreased expression of cytokeratins (eg, AE1/AE3, cytokeratin 7) and cathepsin K expression (in a subset of TFE3 RCC) may aid the diagnostic approach.[12] Recent studies have questioned the overall reliability of the TFE3 IHC. First of all, TFE3 IHC was found to have lower sensitivity than previously reported,[11] with substantial weak or focal expression in cases without *TFE3* gene rearrangement.[13] Technical difficulties in the TFE3 IHC assay may have a negative impact on the assay's specificity by the type of positive control used for initial antibody titration and variable fixation quality (particularly in automated stainers).[6,14] Moreover, the presence of TFE3 nuclear overexpression has been reported in other RCCs[9] as well as in tumors that do not have *TFE3* gene rearrangement.[15] In Akgul et al,[5] most FISH-confirmed TFE3 RCC cases (76 of 85; 89.4%) were positive for TFE3 IHC, with diffuse and strong/moderate expression in 45 of 85 (52.9%). Compared with 20 *TFE3* FISH-negative cases, diffuse and strong TFE3 IHC had specificity to 91.7% with 64% specificity, with exceptional positive predictive value of 95.5% and a modest negative predictive value of 47.8%.[5] In multivariate analysis, TFE3 IHC was the only statistically significant parameter to detect a positive *TFE3* FISH test.[5]

Accepted for publication August 14, 2020.
Published online November 4, 2020.
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The authors have no relevant financial interest in the products or companies described in this article.
doi: 10.5858/arpa.2020-0360-ED
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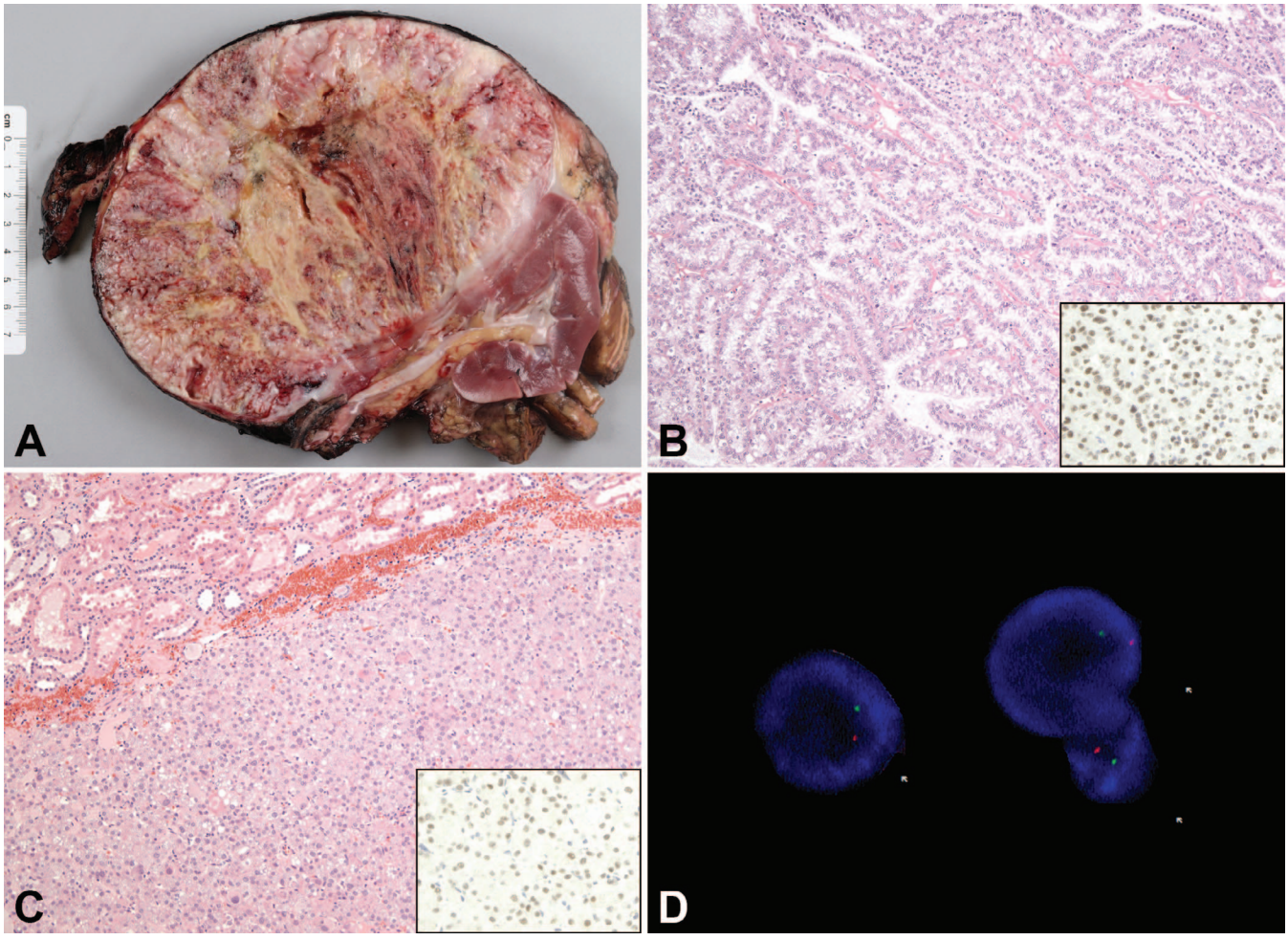

Figure 1. TFE3 *gene fusion–associated renal cell carcinoma (TFE3 RCC) may have a gross appearance of flesh-colored soft mass with necrosis (A). True papillary formation with epithelioid cells with abundant clear or eosinophilic cytoplasm (B) is a common microscopic feature, although unusual microscopic presentation such as exclusively eosinophilic tumor cells in the well-delineated tumor (C) can be seen. Diffuse and strong nuclear overexpression of TFE3 protein is helpful in diagnosing TFE3 RCC (B and C insets), and* TFE3 *gene rearrangement documented by break-apart signals by fluorescence in situ hybridization (D) is more sensitive and specific (hematoxylin-eosin, original magnification ×100 [B and C], ×200 [B and C insets], and ×1000 [D]).*

We have formulated a clinically and histomorphologically driven diagnostic approach for the accurate and cost-effective diagnosis of TFE3 RCC with the aid of both IHC and FISH testing. This approach is formulated with the use of ancillary studies with regard to the patient's age and morphologic features of the tumor (Figure 2). Renal cell carcinoma in younger patients, particularly with tumors showing classical features, should be handled as TFE3 RCC unless proven otherwise; therefore, *TFE3* FISH can be prioritized as the initial ancillary assay (Figure 2). Further studies, such as next-generation sequencing, should be considered in the presence of negative *TFE3* FISH in younger patients.

In advanced age, TFE3 RCC is essentially a diagnosis of exclusion, and morphologic features impact the further interpretation of ancillary studies. In our institution, "classical" TFE3 RCC morphology coupled with diffuse and strong TFE3 IHC has an excellent correlation with *TFE3* FISH and is considered sufficient for the TFE3 RCC diagnosis (Figure 2). In the case of weak and/or focal TFE3 IHC expression extent and intensity, *TFE3* FISH is performed.

It is not infrequent to encounter TFE3 RCC in tumors with nonclassical or morphologic features overlapping with other recognized RCC; therefore, TFE3 IHC should be used as a screening tool (Figure 2), along with a panel of biomarkers. Weak or negative TFE3 IHC is considered negative, and *TFE3* FISH performed if moderate/strong expression is evident, even focally.

Because of the lack of a consolidated approach for diagnosing these challenging cases, enormous inconsistencies and differences in the use of ancillary studies exist. We suggest that this simplified and easy-to-implement algorithm based on clinical and morphologic characteristics, along with TFE3 IHC and FISH, will help guide the pathologist in making an appropriate and timely decision, regardless of the practice setting.

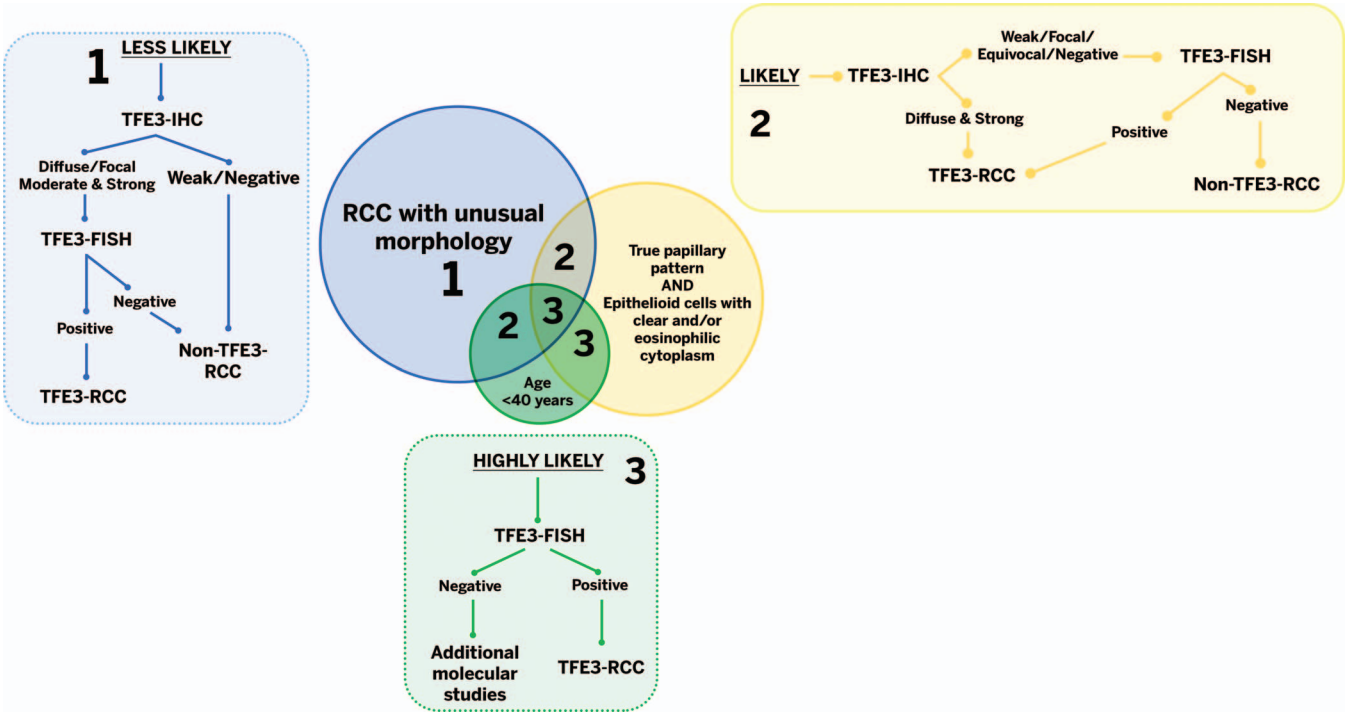

Figure 2. *Inclusion of* TFE3 *gene fusion–associated renal cell carcinoma (TFE3 RCC) in renal cell carcinoma differential should be based on morphologic features as well as the patient's age. TFE3 RCC with unusual morphologic presentation is not infrequent, and TFE3 RCC along with a comprehensive morphology-oriented biomarker panel should be used (scenario 1). Moderate and strong TFE3 RCC expression, particularly diffuse, is predictive of positive TFE3 fluorescence in situ hybridization (FISH) assay as a screening method in these cases. Cases with weak/equivocal TFE3 immunohistochemical (IHC) expression may no further be pursued for TFE3 RCC. Renal cell carcinoma with classical TFE3 RCC morphology (as detailed in yellow circle, scenario 2), particularly in a young patient (scenario 3), should be thoroughly evaluated for TFE3 RCC. Strong/moderate and diffuse TFE3 IHC can substitute for TFE3 FISH in cases where there is morphologically strong suspicion for TFE3 RCC and where TFE3 FISH is not available. In young patients with classical morphology, TFE3 RCC should first be ruled out and TFE3 FISH can be first used instead of initial TFE3 IHC screening.*

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