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Review Article

Role of molecular testing in diagnosis of renal neoplasms

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ABSTRACT

The evolving landscape of renal neoplasms is a field that is simultaneously exciting and challenging. Next generation sequencing of renal neoplasms over the past decade has deepened our understanding of the biology underlying various entities and provided greater insight into the complexity and numerous subtypes of therein. These advancements have not only refined our comprehension but have also led to the identification of novel tumors driven by molecular alterations not previously known. This progress represents an exciting landmark moment in the field of renal oncology, but also brings its own set of challenges, such as the need to familiarize oneself with these rare subtypes and the ancillary diagnostic tools necessary for accurate diagnosis.

A comprehensive understanding of the pathogenesis and molecular biology is essential for the diagnosis of all tumors, including renal neoplasms, especially when genomic findings play a pivotal role. In this review, we explore the role of molecular testing in the diagnosis of renal tumors, addressing the scope of contemporary diagnostic approaches and their limitations, considerations for molecular testing, and provide a guide to judicious and effective molecular testing in the context of renal neoplasms. Finally, we present clinical case-based scenarios that illustrate how molecular results are incorporated into pathology reports to achieve an integrated diagnosis. Key points for each section are summarized into tables for ease of reference.

Introduction

The diagnosis of renal cell neoplasms has become increasingly complex with the recent recognition of several new subtypes and improved understanding of the clinical and morphological spectrum of established subtypes. Genomic analysis of previously unclassified renal cell carcinomas (RCC) has led to more precise classification of RCC subtypes and defining of novel subtypes, thereby reducing the size of unclassified category.[1] Also emerging from these studies are a subset of unclassified low-grade oncocytic neoplasms that lack the high-grade features previously recognized in unclassified RCCs.[2,3] In the past, these tumors may have been classified as unclassified RCCs that may be misunderstood as prototypical aggressive unclassified RCCs. In clinical practice it is important that these two entities are clearly separated.

While histomorphological features combined with immunohistochemistry (IHC) are generally sufficient for diagnosis in most instances, some cases present diagnostic challenges due to non-classical morphology, limited sampling, discordant IHC, and aberrant protein expression- some of which may be influenced by pre-analytical variables. The complexity of the morphological diagnosis is further compounded by overlapping morphological features amongst various RCC subtypes, lack of familiarity with rare and newly recognized entities, and inherent challenges of diagnosis on small biopsy and cytology samples. These challenges are particularly pronounced in high-grade neoplasms and neoplasms with papillary or oncocytic morphologies.

Moreover, many rare RCC subtypes have significant management implications, as many of these tend to be aggressive tumors. Accurate diagnosis can confer eligibility for ongoing clinical trials and highlight tumors with hereditary predisposition with implications that extend beyond the patient to screening of related family members.

Recently, the use of molecular testing for confirmatory diagnosis has been widely adopted in certain organ systems such as tumors of the nervous system, sarcomas, and hematologic malignancies.[4–6] However, its application in renal tumors remains relatively limited.[7,8] Although universal molecular testing in renal neoplasms is not recommended, findings from recent sequencing studies demonstrate its utility in the diagnosis of a significant proportion of cases that remain challenging to classify with traditional methods alone.

In this review, we provide an overview of the contemporary strategies for diagnosing RCCs, the role of molecular testing with an emphasis on when it needs to be undertaken, and finally, an approach to incorporating molecular results into pathology reports for a fully integrated

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diagnosis.

Contemporary scope of diagnosis of renal cell carcinoma largely relies on morphology and a limited panel of IHC

The contemporary diagnosis of RCCs primarily relies on careful morphologic assessment and a limited panel of IHC markers (Table 1, Fig. 1). For some of the common renal neoplasms, such as low-grade clear cell RCC (ccRCC) and papillary RCC (PRCC), classical chromophobe RCCs (ChRCC), multilocular cystic neoplasm of low malignant potential (MLCNLMP), and angiomyolipoma (AML), diagnosis can often be made solely on their distinctive morphological features. Tumors such as clear cell papillary renal cell tumor (CCPRT), tubulocystic RCC, acquired cystic disease-associated RCC, biphasic hyalinizing and psammomatous RCC/biphasic squamoid RCC, and papillary renal neoplasm with reverse polarity also exhibit unique morphological features that are typically sufficient for diagnosis.[9–12] However, anecdotal evidence suggests that a training gap may still exist among pathologists in recognizing some of these tumors, and the use of a limited IHC panel may be reasonable to exclude other diagnostic considerations.

Table 1:
Ancillary tools for diagnosis of common renal neoplasms.

Group 1: Morphological diagnosis	
	• Low grade clear cell RCC • Low grade papillary RCC • Oncocytoma • Classical Chromophobe RCC • Clear cell papillary renal cell tumor • Multilocular cystic neoplasm of low malignant potential • Acquired cystic disease associated RCC • Tubulocystic RCC • Biphasic hyalinizing and psammomatous RCC • Biphasic squamoid RCC • Papillary renal cell neoplasm with reverse polarity • Angiomyolipoma
Group 2: Limited IHC panel available in most labs	
• PAX8 • CA9 • KRT7 • CD10 • p504S • High molecular weight CK (HMWCK) • CD117 • CK20 • SMARCB1 • Melanocytic markers • Smooth muscle actin (SMA) • Pan-keratin	• Clear cell RCC • Papillary RCC • Clear cell papillary renal cell tumor • Oncocytoma • Low grade oncocytic tumor • Chromophobe RCC • RCC with fibromyomatous stroma • Renal medullary carcinoma • Angiomyolipoma
Group 3: IHC stains not routinely available in all labs	
• TFE3 • Fumarate hydratase • 2 Succinocysteine (2SC) • Succinate dehydrogenase B • ALK • Cathepsin K • GPNMB • TRIM63	• *TFE3* rearranged RCC • *TFEB* altered RCC • Fumarate hydratase deficient-RCC • Succinate dehydrogenase deficient RCC • Eosinophilic solid and cystic RCC • Eosinophilic and vacuolated tumor • RCC with fibromyomatous stroma • *ALK* rearranged RCC
Group 4: Molecular assays	
• *TFE3* FISH • *TFEB* FISH • *ALK* FISH • Sequencing studies	• *TFE3* rearranged RCC • *TFEB* altered RCC • *ALK* rearranged RCC • *ELOC* mutated RCC • Eosinophilic solid and cystic RCC • Eosinophilic and vacuolated tumor • RCC with fibromyomatous stroma

Abbreviations: CK: cytokeratin, GPNMB: glycoprotein non-metastatic melanoma protein B, RCC: renal cell carcinoma.

In the next category, morphology provides significant clues for the diagnostic workup of higher-grade subtypes of ccRCC and PRCC, eosinophilic subtype of ChRCC, as well as most other subtypes. These include namely oncocytoma, CCPRT, mucinous tubular and spindle cell carcinoma, low-grade oncocytic tumor, microphthalmia-associated transcription factor family translocation-associated (MITF-RCC) RCCs, succinate dehydrogenase (SDH)-deficient RCC, fumarate hydratase (FH)-deficient RCC, renal medullary carcinoma, eosinophilic solid and cystic RCC (ESC-RCC), eosinophilic and vacuolated tumor (EVT), mixed epithelial and stromal tumors, and metanephric adenoma. For these entities, depending on the expertise of the pathologist, the diagnosis may be made on morphology alone and/or with the aid of a small panel of IHC markers available in most laboratories selected based on morphological features. Diagnostic challenges can arise due to a greater degree of heterogeneity, higher grade features, small sampling, and poor preservation of morphological features. In such instances, IHC panels can be crucial in resolving these diagnostic challenges.[13] While IHC may suffice for arriving at a pathologic diagnosis, additional molecular testing may still be warranted for definitive confirmation or to identify the causative gene and predict hereditary predisposition associated with certain entities.

In the third category, although morphology and IHC may suggest the diagnosis, molecular testing is often necessary to confirm the diagnosis, such as in *ELOC*-mutated RCC, *TFE3*-RCC, *TFEB*-altered RCCs, and *ALK*-rearranged RCC.[14–16] For some tumors such as ESC-RCC, EVT, and RCC with fibromyomatous stroma genomic findings may provide additional evidence in support of the diagnosis.

Limitations of IHC and morphology

In clinical practice, not all tumors have classical morphological features, and there is a significant overlap between different renal tumors.[17] Although IHC stains are commonly used to aid in diagnosis, they also have limitations, which are discussed in the context of specific renal tumors below (Table 2).

Clear cell tumors

Prototypical among tumors with clear cell features is ccRCC, characterized by nests of clear cells separated by a rich, arborizing, delicate vasculature. This preserved architecture in regions of higher-grade tumors or those with sarcomatoid and rhabdoid morphology can suggest the diagnosis.[18] The diagnosis is further supported by a diffuse membranous staining pattern of carbonic anhydrase 9 (CA9) in non-necrotic areas, typically accompanied by a lack of KRT7 staining. Distinguishing ccRCC from its mimics is clinically important because of the different treatment strategies utilized and the metastatic potential of ccRCC that may warrant prolonged follow-up.[19]

CA9, KRT7, and CD10 are the most frequently used stains for making the distinction in this category of tumors. Diffuse KRT7 staining is unexpected in ccRCC and is widely used to differentiate it from low-grade mimics such as CCPRCT, RCC with fibromyomatous tumors and *ELOC*-mutated RCC.[14,20,21] Reliance on KRT7 for this distinction could be a potential diagnostic pitfall as a significant number of ccRCC may demonstrate variable extent of KRT7 staining, especially in low-grade cystic and tubulopapillary areas.[7,13] Therefore, KRT7 should be interpreted in the context of morphological and other IHC features.

The intricate micro and macrocystic branching tubulopapillary pattern with linear abluminal arrangement of nuclei, positivity for HMWCK, and lack of CD10 staining help in the diagnosis of CCPRCT.[12] Furthermore, the cup-shaped pattern of CA9, often described in literature, in our experience, is not always reproducible.

RCC with fibromyomatous stroma may exhibit *ELOC* or *MTOR* pathway alterations, sharing overlapping histological and IHC features

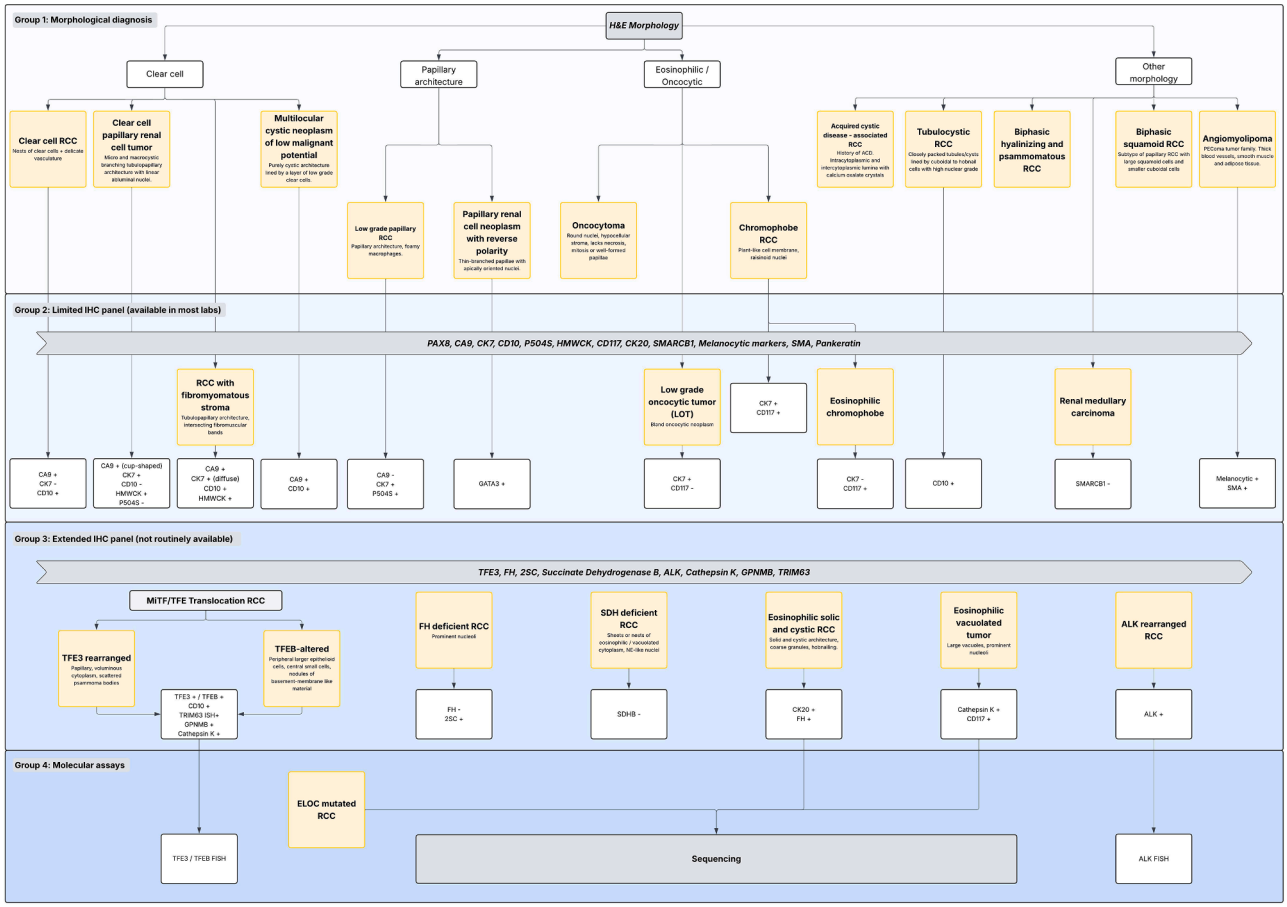

Fig. 1. An algorithmic diagnostic approach to renal neoplasms.

Table 2:
Salient limitations of Immunohistochemical stains used for diagnostic work up of renal neoplasms.

- KRT7 staining may be seen in a subset of clear cell RCCs
- KRT7 staining may be variable in chromophobe RCC, especially in eosinophilic subtype
- TFE3 IHC should be interpreted as positive with diffuse strong nuclear positivity
- TFE3, Cathepsin K, Melanocytic markers and GPNMB staining may be seen in other tumors with alterations in TSC/MTOR/FLCN genes.
- FH deficient tumors may exhibit heterogeneous staining pattern
- SDHB staining may be fraught with interpretation challenges, weak diffuse cytoplasmic blush is considered negative

with ccRCC.[14,22] *ELOC*-mutated RCCs were identified through sequencing efforts of ccRCC revealing tumors that lacked *VHL* and chromosome 3p alterations.[23] Instead, these tumors are characterized by hotspot mutations in *ELOC* (formerly called *TCEB1*), a component of the *VHL* E3 ligase complex, accompanied by monosomy of chromosome 8, acting as an alternate mechanism of HIF pathway activation.[18] These tumors are immunoreactive for CA9 and KRT7[24] and display a nodular appearance with intersecting fibromuscular bands and a tubulopapillary architecture with clear cells.[25] Given these overlapping features and lack of a distinctive IHC profile, molecular testing remains the cornerstone for detecting *ELOC* mutations.[26] IHC for glycoprotein nonmetastatic B (GPNMB), a marker that is expressed in tumors with constitutive activation of mTORC1 activation may be helpful in identifying tumors with *MTOR* pathway alterations,[27] although this is not yet routinely available.

Oncocytic renal neoplasms

KRT7 is an important stain in distinguishing between oncocytoma, which characteristically shows negative or patchy KRT7 staining, and ChRCC, which typically exhibits diffuse KRT7 positivity. However, this distinction is not always straightforward as there is a spectrum of overlapping morphological features and KRT7 positivity within this category of tumors.[28,29] Additionally, eosinophilic ChRCCs may exhibit subtle perinuclear clearing and lack of KRT7 staining, making the differentiation even more challenging and a controversial area in urologic pathology.[29,30] When the morphological and IHC features are inconclusive, it may be reasonable to classify them descriptively as renal oncocytic neoplasm. The term "Oncocytic renal neoplasm of low malignant potential, not further classified" has been proposed to describe solitary sporadic cases with overlapping features of oncocytoma and eosinophilic ChRCC but lacking unequivocal diagnostic criteria.[29] Importantly, the diagnosis of oncocytoma, a benign entity, should be based on a strict criteria including absence of coagulative necrosis, absence of well-formed papillary formations, at most rare mitotic figure and absence of atypical mitotic activity.[29]

Recently described oncocytic tumors have emerged from a spectrum of oncocytic neoplasms that do not fit the description of oncocytoma or ChRCCs and include SDH-deficient RCC, low-grade FH-deficient RCC, LOT, ESC-RCC, and EVT.[31–35] These tumors exhibit classical morphology and characteristic IHC profiles but can be challenging to diagnose when there is a limited sample, a lack of familiarity with these

entities, morphological variability, or discordant IHC results. Molecular testing may be helpful in identifying underlying molecular alterations, enabling a diagnosis in such cases.

Renal carcinomas with papillary features

Renal carcinomas with papillary features represent the most heterogeneous category of renal tumors.[36] Mimics of low-grade papillary tumors include CCPRCT, metanephric adenoma, and mucinous tubular and spindle cell carcinoma. On the other hand, high-grade papillary features are often seen in FH-deficient RCC, MIT-family RCC, renal medullary carcinoma, and collecting duct carcinoma.[29]

Papillary renal cell carcinoma

The diagnosis of papillary renal cell carcinoma can in many instances be based on a combination of characteristic morphology of papillary architecture, with entrapped aggregates of histiocytes in papillary cores, and typically diffuse KRT7 and AMACR positivity. These stains are not specific and reduced KRT7 expression may be seen in eosinophilic papillary tumors. Given the significant morphologic heterogeneity and overlap with other RCC subtypes, a low threshold for ancillary IHC and molecular testing may be reasonable.[17] Characteristic molecular alterations include gain of chromosomes 7 and 17 and loss of Y or *MET* gene mutations.[36]

Microphthalmia-associated transcription factor family translocation-associated RCCs (MITF-RCC)

Microphthalmia-associated transcription factor family translocation-associated RCCs include tumors with *TFE3* rearrangement (TFE3-RCC), *TFEB* rearrangement, *TFEB* amplification, and *MITF* rearrangement.[37]

TFE3-RCC

Distinctive features of *TFE3*-RCCs include papillary architecture with eosinophilic and clear cells that have voluminous cytoplasm and scattered psammoma bodies.[38,39] As more *TFE3* rearranged RCCs were identified through sequencing efforts, the morphological spectrum expanded to include features that overlap with several known RCC subtypes including ccRCC, PRCC, CCPRCT, *TCEB1/ELOC* mutated RCC, MCRNLMP, oncocytoma, and epithelioid angiomyolipoma.[29,37,40] The phenotypic heterogeneity was, in many instances, reflective of the *TFE3* fusion partner tumor, which was also shown to be associated with differences in biological behavior and prognosis.[40,41]

Melanocytic markers and cathepsin K have routinely been used for the diagnosis of MITF-RCCs, and PEComas/AMLs, and are frequently considered in this diagnostic consideration. Emerging data suggests that there may be more biological overlaps between *TSC/MTOR* pathway and tumors with *TFE3, TFEB,* and *FLCN* alterations than previously understood.[42] Consequently, the expression of melanocytic markers and other transcriptional targets of *TFE3* and *TFEB,* such as GPNMB, can be seen across these diverse tumor types and are not tumor specific.[43]

TFE3 gene rearrangement results in overexpression of the fusion oncoprotein, which can be detected by a C-terminus binding site TFE3 antibody.[41] However, the TFE3 IHC is technically challenging to adapt to most automated platforms and does not reliably detect all possible translocations.[44] The sc-5958 goat polyclonal TFE3 antibody binds to the C-terminal downstream of exon 4. *TFE3* fusion transcripts that do not retain the downstream exons 5, 6, and 7 could potentially explain the reduced affinity of TFE3 in a subset of tumors.[41]

Importantly, the interpretation of the TFE3 stain is also fraught with challenges, as a weak nuclear blush is not uncommon. Comparing the stain to adjacent benign renal parenchyma and limiting positive interpretation to diffuse strong nuclear positivity in the tumor can aid in accurate interpretation of this stain.

TFE3 positivity is not specific to *TFE3*-RCCs and can be seen in other renal and extra-renal tumors, even in the absence of underlying *TFE3* rearrangements.[41] Therefore, confirmatory testing by FISH or sequencing is ideal, if available.

TFEB altered RCCs

TFEB alterations in RCC can be seen either as rearrangements or amplification of the gene. *TFEB*-rearranged RCC, also known as t(6;11) RCC,[29] is less common than *TFE3*-RCC.[26,37] *TFEB* rearranged RCCs often have a biphasic morphology, with peripheral larger epithelioid cells, and central smaller cells arranged around nodules of pink basement membrane-like material resembling the Call-Exner bodies of adult granulosa cell tumor.[29] *TFEB* amplified RCCs are less distinctive, with high-grade oncocytic cells arranged in a papillary configuration. The preferred diagnostic test for *TFEB*-rearranged RCC is *TFEB* break-apart FISH that can also be used for identifying amplifications.[45]

Fumarate hydratase-deficient RCCs

FH-deficient RCC exhibits a wide spectrum of architectural patterns, including papillary, tubulocystic, cystic, cribriform, sieve-like, and solid patterns.[46] These tumors have high-grade cytology with eosinophilic cytoplasm and prominent inclusion-like nucleoli. The vast majority of the FH-deficient RCCs are associated with either germline mutations or deletions of the *FH* gene,[29] with somatic alterations seen in a subset.

The diagnosis of FH-deficient RCC is often made using IHC, which shows a loss of staining in the presence of retained staining of background non-neoplastic cells. However, while FH IHC is highly specific, the sensitivity is not optimal and may not identify a small subset of tumors. FH IHC may yield false negative results due to a non-functional protein that retains protein epitopes and expression. Additionally, FH staining can be heterogeneous, and in such cases,[47,48] 2-Succinocysteine (2SC) staining or sequencing should be undertaken for confirmation.

2SC is a highly sensitive IHC stain, albeit less specific than FH, and still not widely available. Positive staining is indicated by strong nuclear and cytoplasmic staining.[49]

Succinate dehydrogenase deficient RCCs

SDH-RCC results from germline mutation in any of the four *SDH* genes, *SDHA, SDHB, SDHC*, and *SDHD*.[29] SDH-deficient RCCs have distinctive features of sheet-like or compact nests of cells with eosinophilic to vacuolated cytoplasm and cytoplasmic inclusions or flocculation. Diagnosis requires confirmation of loss of SDHB staining resulting from biallelic inactivation of any of the genes of the *SDH* complex.[29] The interpretation of SDHB stain requires loss of staining in the tumor cells, with retained staining in non-neoplastic internal positive control, which exhibit dark and granular mitochondrial staining. Interpretation can be challenging when a weak diffuse cytoplasmic blush is present and is considered negative.[50]

As the diagnosis of renal neoplasms continues to evolve, additional IHC stains are becoming available. Chief among these are Cathepsin K, GPNMB, TRIM63 and Merlin. Cathepsin K and GPNMB are markers of dysregulated MTOR pathway and may be positive in a diverse range of tumors.[27] TRIM63 is a highly specific marker for *TFE3*-RCC and *TFEB* altered RCC.[51] Merlin is a protein product of *NF2*[52] that may help identify RCCs with *NF2* gene alterations. However, these IHC stains are not widely available, and there is a general unfamiliarity with their interpretation among pathologists.

Role of molecular testing in evaluating renal neoplasms

Many tumors, especially high-grade tumors, exhibit a varied range of morphologies, and aberrant IHC patterns are not uncommon. These patterns can still be very useful for narrowing down the differential diagnosis and guiding subsequent testing.[13,53] In cases that defy morphological and IHC classification, molecular testing can facilitate a

specific diagnosis.

The underlying molecular alterations in RCCs include mutations, structural variations, and copy number changes. Therefore, the selection of the assay should be directed by diagnostic considerations. When the diagnostic considerations are limited, a targeted molecular assay may suffice. In contrast, for unclassified tumors, with broader diagnostic considerations, a comprehensive sequencing panel that encompasses the range of alterations seen in RCCs and includes the gene(s) of interest should be considered.

While IHCs can frequently suggest a diagnosis, molecular testing is often required for confirmation and to guide subsequent genetic counseling and evaluation of hereditary germline predisposition. Molecular confirmation is particularly important for tumors such as *MITF*-RCC, FH-RCC, SDH-RCC, *ALK*-RCC, *ELOC*-RCC, or when clinical findings suggest hereditary associations. If highly specific antibody clones such as D5F3 are used, these can be sufficient to establish the diagnosis of *ALK*-RCC.

In clinical practice, for a small subset of unclassified and high-grade RCCs, the morphological features may provide limited clues, necessitating an extensive workup that may require several IHC stains. In such cases, an upfront comprehensive sequencing panel may be more economically judicious in overall cost versus multiple series of IHC stains when used in the correct context.

Some may question the need for a confirmatory diagnosis in low-grade tumors. However, it is clinically relevant to establish a definitive diagnosis (as best as possible with contemporary technology and medical literature) for tailored surveillance and management of patients in the current era of precision medicine. The diagnostic considerations for some low-grade tumors may include those with metastatic potential. Without a definite diagnosis, many patients with indolent and low-grade tumors may undergo unnecessary prolonged surveillance, while a watchful approach to tumors with metastatic potential could result in preventable metastasis. Additionally, a definite diagnosis can direct therapeutic strategies and enable enrollment in clinical trials for some rare subtypes.

Guide to molecular testing

The selection of molecular tests should always be guided by diagnostic considerations to determine whether a single assay or a more comprehensive panel is optimal for a given instance. When selecting a panel, it is important to review the technical information available to confirm the following: (1) the relevant gene(s) of interest are included in the panel, (2) the assay can detect the relevant alteration type(s) and (3) the available lesional tissue meets the input specifications of the assay with regards to quantity and type, such as formalin fixed tissue, cell blocks, or smears (Table 3).

The adequacy of the lesional content is an important consideration when selecting an assay. Scant lesional material within a background of benign inflammatory or stromal cells (resulting in low tumor cellularity) may not be optimal for sequencing studies. However, such specimens can be suitable for IHC and FISH assays, which allow direct visualization of lesional cells.

Before requesting molecular tests, it is also important to remember that decalcified specimens are not suitable for molecular assays including FISH, unless a modified decalcification protocol is employed to preserve DNA integrity.

In some situations, such as *TFE3*-RCC, a single assay may be appropriate. A FISH assay can often detect these rearrangements and establish the diagnosis, except for some cryptic *TFE3* rearrangements due to paracentric inversions such as *RBM10::TFE3, GRIPAP1::TFE3, RBMX::TFE3*, and *NONO::TFE3*.[41] *TFE3* break-apart FISH assays have a higher specificity than automated TFE3 IHC, providing more consistent results in formalin-fixed tissue.[29,44]

When considering a broader differential, wherein the molecular alterations may include multiple alteration classes, such as mutations (for example FH or SDH-deficient RCCs), rearrangements (for example *TFE3*-RCC or *ALK*-RCC), or copy number changes (for example chromophobe RCC, papillary RCC, or *TFEB* amplified RCC), a comprehensive panel that can detect these alteration types should be selected. A comprehensive panel can also provide important information related to prognostic biomarkers such as *BAP1, PBRM1,* and *SETD2* in ccRCC[54,55] and *PTEN* and*TP53* in ChRCCs.[56]

Incorporating molecular results in pathology reports

Access to molecular testing remains limited for many pathologists, often requiring send-out tests to external laboratories. It is essential to collaborate closely with the laboratory performing the tests to understand the scope and limitations of the assay and establish a communication channel. This is particularly important as the external laboratory pathologists may lack some key clinical information relevant to the case, and the pathologist responsible for the case may not fully understand the assay and interpretation of its findings.

Once the report is available, incorporating it into the final pathology is crucial for making an integrated final diagnosis. This can be facilitated by good communication with the laboratory performing the test and/or discussions within multidisciplinary teams. The understanding of the molecular biology of renal tumors becomes important in this context, as shown by some examples encountered in our clinical practice (Table 4).

The first scenario involves a small renal mass biopsy from a patient with von-Hippel-Lindau syndrome, with scant PAX8-positive atypical cells submitted for sequencing for diagnostic confirmation. The results revealed a *VHL* loss-of-function mutation at 47% mutation allele frequency with no other alterations identified, albeit with a disclaimer regarding the low lesional content of the sample. Without complete information, one might be tempted to over-interpret these findings as indicative of ccRCC. However, the *VHL* mutation identified is the patient's germline mutation; the mutation allele frequency supports this and does not correlate with the tumor content of the sample. The diagnosis of ccRCC requires two hits, one often due to a mutation in *VHL* and the second through loss of chromosome 3p on which the gene resides or promoter methylation of the *VHL* gene. In the absence of two hits, caution is warranted in making a diagnosis of ccRCC, and these results should be interpreted in the clinical context.

The second scenario involves interpreting low-level rearrangements. Most FISH break apart assays have a limit of detection set at 10–30% break-apart signals. It is important to remember that the rearrangements in RCCs are driver alterations and are expected at high levels. Interpretation of FISH on formalin-fixed tissue can be challenging, and low-level signals should be carefully evaluated for false-positive calls that may occur due to technical artifacts, crushed, transected, or spindled cells. If low-level fusions are identified, correlation with histomorphological and IHC findings becomes particularly relevant.

In the third scenario, alterations may not be specific to one RCC subtype and will require correlation with histomorphological features. A good example is of alterations in the *TSC/MTOR* pathway that may define LOT, ESC-RCC, EVT, AML/PEComa and RCC with fibromyomatous stroma, but can also be seen as secondary alterations in other RCC

Table 3:
Checklist for selecting molecular test(s).

- Select test(s) based on the differential diagnosis considered
- Confirm alteration type and gene (s) are included in the panel
- Available tissue is not decalcified and meets the tests specification in terms of adequacy of lesional content and processing

Table 4:
Considerations for interpreting molecular test results.

- Not all *TFE3* rearrangements are detected by *TFE3*-FISH assay
- Low level FISH rearrangements could be false positive
- Alterations in some genes may be passenger alterations
- A single hit to a tumor suppressor gene may occur if a patient is a carrier and not necessarily be a driver alteration
- Germline alterations are also identified on sequencing of the tumor sample
- Interpretation of molecular results may require additional investigations, discussion in multidisciplinary teams and importantly correlation with clinical and histopathological findings

subtypes.[57] For example, in a clinical case of a CK20 negative renal oncocytic neoplasm on a biopsy, detection of *MTOR* mutation upon sequencing and re-review of the morphology was helpful in establishing a diagnosis of a CK20 negative ESC-RCC. Similarly, alterations in *SMARCB1* and *NF2* may occur as driver alterations in renal medullary carcinoma and *NF2*-mutated RCC, respectively, or as passenger alterations in several RCC subtypes, requiring clinical and histopathological correlation for an integrated diagnosis.[29,58]

In the fourth scenario, it is important to remember that many of the genes implicated in RCCs are tumor suppressor genes that require inactivation of both alleles to have a functional impact. Alterations in these genes can also occur in patients who are carriers, and such alterations may not necessarily be causative in tumorigenesis. An example would be a characteristic ccRCC with an incidental *SDHA* mutation identified in a patient who is a carrier for *SDHA* mutation. The patient's germline alterations will also be detected in the tumor sample, and distinction between somatic and germline alterations requires dedicated testing of a normal sample, such as non-neoplastic tissue, peripheral blood, or buccal swab.

Lastly, comprehensive sequencing, which increasingly includes transcriptomic studies, may reveal lesser-known or novel findings requiring further investigation to understand their significance in a case or discussion in multidisciplinary forums. For example, a renal biopsy received for review reported amplification of the *CCND3* gene. Further investigation revealed that *CCND3* is co-located with *TFEB* gene suggesting a diagnosis of *TFEB*-amplified RCC. Reflex testing for *TFEB* by FISH and additional melanocytic markers confirmed the diagnosis in a case, which might otherwise have been diagnosed as an unclassified RCC.[59]

Conclusion

In summary, while RCC subtypes can in most instances be diagnosed based on morphological features and a limited IHC panel, molecular testing is critical in establishing a definitive diagnosis for a small subset of cases. An accurate diagnosis is important in guiding management strategies including therapeutic decisions, clinical trials eligibility, and further evaluation for hereditary predisposition. Molecular testing can also provide additional prognostic information. In the era of precision medicine, molecular testing should be judiciously incorporated into diagnostic algorithms.

CRediT authorship contribution statement

Timothy Gilpatrick: Writing – review & editing, Writing – original draft, Conceptualization. **Natalia G. Sanchez:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Nancy Y. Greenland:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Deepika Sirohi:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

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