Tissue-Based Immunohistochemical Markers for Diagnosis and Classification of Renal Cell Carcinoma

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Abstract

The development and description of renal cell carcinoma (RCC) subtypes has led to an increase in demand for tissue biomarkers. This has implications not only in informing diagnosis, but also in guiding treatment selection and in prognostication. Although historically, many immunohistochemical (IHC) stains have been widely characterized for RCC subtypes, challenges may arise in interpreting these results. These may include variations in tumor classification, specimen collection and processing, and IHC techniques. In light of the reclassification of RCC subtypes in 2016, there remains a requirement for a comprehensive outline of tissue biomarkers that may be used to differentiate between RCC subtypes and distinguish these from other non-renal neoplasms. In this review, concise summaries of the commonest RCC subtypes, including clear cell, papillary, and chromophobe RCC, have been provided. Important differences have been highlighted between chromophobe RCC and renal oncocytomas. An overview of the current landscape of tissue biomarkers in other RCC subtypes has also been explored, revealing the variable staining results reported for some markers, whilst highlighting the essential markers for diagnosis in other subtypes.

Introduction

Classifying renal cell carcinoma (RCC) into its various subtypes relies on a range of diagnostic techniques. These involve the analysis of anatomical, morphological, immunohistochemical (IHC), and molecular characteristics. IHC tissue biomarkers have maintained a useful role in aiding the diagnosis and subtyping of RCC [1]. In addition, tissue biomarkers may aid in the differentiation of non-renal neoplasms or metastatic disease. Its other uses include prognostication, as well as guidance of treatment selection [2,3].

Advances have been made in IHC staining for RCC classification; however, challenges arise in the subtyping of RCC. Substantial staining heterogeneity exists across and within tumor subtypes [4]. Variations in processing may lead to inconsistencies in reported immunoreactivity or staining patterns [4]. Ongoing revision of the classification of renal cell tumors (**Table 1**) by the World Health Organization and International Society of Urological Pathology creates difficulty in interpreting older literature [5,6]. There is increasing demand for smaller volume samples to be analyzed, as diagnostic biopsies are performed more frequently. Although some of the described markers may not yet be commonly encountered in daily clinical practice, IHC staining remains useful in indicating the presence or absence of such markers, and in relaying quantitative information such as staining extent.

Key Words

Tissue-based markers, immunohistochemistry, renal cell carcinoma, cancer subtyping.

Competing Interests

None declared.

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Abbreviations

AMACR	alpha-methylacyl coenzyme A racemase
BAP1	BRCA1-associated protein 1
CAIX	carbonic anhydrase 9
ccRCC	clear cell RCC
CD10	cluster differential marker 10
chRCC	chromophobe RCC
CK	cytokeratin
EMA	epithelial membrane antigen
hKIM-1	human kidney injury molecule-1
HMWCK	high molecular weight CK
IHC	immunohistochemical
MiT	microphthalmia-associated transcription
RCC	renal cell carcinoma
RCCM	RCC marker
RO	renal oncocytoma

This review provides an update on the current landscape of tissue biomarkers for the diagnosis and classification of common RCC subtypes.

Clear Cell RCC

Clear cell RCC (ccRCC) is responsible for approximately 75% of diagnosed RCCs [5]. Its staining profile has been widely characterized; however, markers are still being described that may aid differentiation of ccRCC from other subtypes (**Table 2**). Carbonic anhydrase 9 (CAIX), a transmembrane protein responsible for CO_2 transfer, stains along membranous non-necrotic areas [7,8]. A transmembrane mucin protein, epithelial membrane antigen (EMA), may also be present in up to 85% of specimens [9,10]. Human kidney injury molecule-1 (hKIM-1) is a type 1 transmembrane glycoprotein found in injured proximal tubules [11]. This marker can be expressed in ccRCCs but may also be found in other clear cell carcinomas of the ovaries or endometrium.

Cytokeratin (CK) staining in ccRCC may vary depending on the antibody used and the specific CK studied. Immunoreactivity in 60% of ccRCC specimens may be achieved for CK8 using CAM 5.2 antibody [12]. ccRCC does not typically stain for CK7 [8]. CK19 antibody may result in infrequent immunoreactivity in 20% of specimens [13]. Broad spectrum CK (pan-cytokeratin) may be detected using AE1/AE3 antibodies [14]. ccRCCs are not immunoreactive to 34βE12, an antibody for high molecular weight CK (HMWCK) [13]. Vimentin, an intermediate filament protein found in mesenchymal cells, may be detected in 87% of specimens [15,16]. Lectins, carbohydrate-binding proteins, may also be used as markers in RCC.

TABLE 1.

The WHO/ISUP classification of renal cell tumors, 2016

The classification of renal cell tumors as described by World Health Organization and International Society for Urological Pathology, in 2016.

Renal Cell Tumors
Clear cell RCC
Multilocular cystic renal neoplasm of low malignant potential
Papillary RCC
Hereditary leiomyomatosis and renal cell carcinoma-associated RCC
Chromophobe RCC
Collecting duct carcinoma
Renal medullary carcinoma
Microphthalmia-associated transcription family translocation RCC
Succinate dehydrogenase-deficient RCC
Mucinous tubular and spindle cell carcinoma
Tubulocystic RCC
Acquired cystic disease-associated RCC
Clear cell papillary RCC
RCC, unclassified
Papillary adenoma
Renal oncocytoma

Previously, galectin-1 (51%) and galectin-3 (78%) have been detected in ccRCC [17].

It is important to note that a number of markers produce negative immunoreactivity in ccRCC. Alpha-methylacyl coenzyme A racemase (AMACR), an enzyme found in peroxisomes and mitochondria involved in fatty acid oxidation, does not typically stain in ccRCC tissue [8]. There is usually minimal immunoreactivity for parvalbumin, a calcium-binding albumin protein, and similar negative staining for claudin 7, 8, and CD117 [16,18-21]. E-cadherin is also typically not expressed, although may be detectable in tumors of higher grade [10,22,23]. Kidney-specific cadherin (Ksp-cadherin), detectable in the distal convoluted tubules, can be expressed in moderate intensity in some specimens (30%) [20]. BRCA1-associated protein 1 (BAP1), a protein with deubiquitinase properties, expresses nuclear staining in up to 81% of ccRCCs; wherein a loss of expression may be associated with higher grade of disease [24].

Sensitive but non-specific markers may prove useful in confirming RCCs, especially in determining the origin of metastatic deposits. RCC marker (RCCM), an

antibody directed at the brush border of the proximal tubule, is present in 85% of ccRCC specimens [25]. However, it may also be detected in 27% of non-renal carcinoma specimens, including Müllerian-derived tumors with clear cell morphology, rendering it a non-specific marker for RCC [26]. Similarly, cluster differential marker 10 (CD10), is expressed in 94% of ccRCC specimens but may be detectable in many non-renal tumors [27].

ccRCC may appear similar to chromophobe RCC (chRCC) because of their shared clear and eosinophilic morphology. To minimize the number of markers used to differentiate tumor subtypes, a suggested panel of IHC markers should include vimentin, RCCM, CAIX, Ksp-cadherin, CD117, and parvalbumin.

Papillary RCC

Papillary RCC (pRCC) is the second most common RCC subtype, representing 15% of diagnosed RCCs [5]. pRCCs demonstrate diffuse immunoreactivity to AE1/AE3 antibody and CAM 5.2 antibody, no reactivity to 34βE12, and strong membranous staining for CK7 [28]. Roughly 90% of pRCCs will express some CK19 on staining [13]. Other markers that are immunoreactive include AMACR, vimentin, RCCM, EMA, hKIM-1 and CD10 [11,25,27,29-31].

E-cadherin expression has been inconsistently described, likely because of variations in tumor grade or type, and in technique and processing [22]. Specimens were more likely to express E-cadherin if they were higher grade or if they were type 2 pRCC [22]. pRCCs do not express Ksp-cadherin [20]. Importantly, pRCC

TABLE 2.

Summary of immunohistochemical markers for common renal cell tumor subtypes A summary of the commonly described immunohistochemical markers and their expression is listed for clear cell RCC, papillary RCC, chromophobe RCC, and renal oncocytoma.

Immunohistochemical marker	*ccRCC*	*pRCC*	*chRCC*	*Renal oncocytoma*
Pan-cytokeratin (AE1/AE3)	+	+	+	variable
CK7	–	Type 1: + Type 2: –	+	–
CK8/CK18 (CAM 5.2)	+	+	+	+
CK19	–	+	–	–
HMWCK (34βE12)	–	–	–	–
EMA	+	+	+	+
E-cadherin	–	variable	+	+
Ksp-cadherin	–	–	+	+
CAIX	+	–	–	–
AMACR	–	+	–	–
Vimentin	+	+	–	–
Parvalbumin	–	variable	+	+
c-Kit/CD117	–	–	+	+
CD10	+	+	–	–
RCCM	+	+	–	–
hKIM-1	+	+	–	–
Caveolin-1	+	+	+	–
S100A1	+	+	–	+

+ ≥ 50% staining; – < 50% staining.

usually do not stain for CAIX, though some specimens may be weakly immunoreactive near necrotic areas [8]. There is variable staining for parvalbumin but typically negative staining for CD117 [16,19,32]. Reported data for membranous staining of claudin 7 range from 28% to 78% of pRCCs [33,34]. Claudin 8 does not stain well in pRCCs in roughly 14% of specimens [34]. Up to 83% may express galectin-1, whilst less than 6% of pRCC stain for galectin-3 [17]. BAP1 is reported to be expressed across all pRCC specimens [24].

To assist with classifying a RCC with papillary features, a marker panel should consist of CK7, AMACR, CD10, RCCM, TFE3, and CD57 [30,35].

Chromophobe RCC

Chromophobe RCC (chRCC) represents up to 11% of diagnosed RCCs [7]. chRCC specimens are typically immunoreactive to CAM 5.2 antibody, AE1/AE3 antibody, as well as CK7 [7]. chRCC do not usually express CK19 or HMWCK [13].

Typically, chRCCs stain positive for EMA, E-cadherin, and Ksp-cadherin [10,20,22,36]. chRCCs are immunoreactive for parvalbumin and CD117 in most specimens [16,37]. chRCCs may also demonstrate immunoreactivity for tight junction proteins, claudin 7 (91%), and less frequently, claudin 8 (27%) [21,34]. These tumor subtypes highly express galectin-1 (100%), as well as galectin-3 (63%) [17]. BAP1 may be expressed in up to 77% of specimens [24]. Rh family C glycoprotein (RHCG) as a membranous stain, is homogeneously expressed across chRCC specimens [38]. It is also expressed in renal oncocytomas, although staining patterns may differ in comparison [38]. Long non-coding RNA LINC01187 has also been studied. chRCC specimens demonstrate widespread expression of LINC01187 using RNA in situ hybridization, although this may also be identified in renal oncocytomas [38].

chRCCs do not express vimentin, although specimens may stain positive in sarcomatoid areas [7,15,39]. CAIX, hKIM-1, CD10, RCCM and AMACR are also not typically expressed in chRCCs [7,11,27,29].

Renal Oncocytoma

Renal oncocytomas (ROs) remain challenging to diagnose because they share morphological and IHC features with chRCC. The IHC profile of ROs consist of variable immunoreactivity to AE1/AE3 antibody (49%) and CK19 (40%), while no expression is demonstrated for HMWCK [13]. ROs may stain for CAM 5.2 antibody [40].

ROs can also be immunoreactive for parvalbumin, CD117, E-cadherin, and sometimes EMA (52%) [10,16,36,37]. They may express Ksp-cadherin in up to 76% of specimens [20]. There can be membranous staining for claudin 7 in 55% and mixed pattern expression of claudin 8 in 92% of specimens [21,33,34]. It is important to note that ROs stain negative for vimentin, AMACR, CAIX, CD10, and RCCM [27,29,30,39,41]. Limited staining has been reported for hKIM-111.

Several markers to distinguish between ROs and chRCCs have been investigated. ROs typically stain negative for Hale's colloidal iron stain; however, variability in processing and technique has affected the interpretability and reproducibility of this staining technique [10]. CK7 staining may demonstrate focal positivity in ROs, which is in contrast to the diffuse staining observed in chRCCs [39]. S100A1, a calcium-binding protein, demonstrates consistent and diffuse cytoplasmic staining in ROs compared with chRCC, in which the positive staining rate for S100A1 is considerably lower [42]. Caveolin-1, a scaffolding protein, which was originally described as demonstrating expression in chRCC and not in ROs, has since been reported with variable expression and staining pattern, likely due to inconsistencies among subtypes [39,43-45].

Additional markers are currently being explored. Amylase α1A, a salivary-type digestive enzyme, produces 100% staining in RO specimens, compared with only 13% of chRCCs [46]. Wnt-5a, involved in tumor development, similarly produces 100% staining in RO specimens while only 16% of chRCCs may stain positive [47]. FXYD2, a marker coding for a subunit of a distal tubule Na/K ATPase, stains in 17% of ROs, compared with 96% in chRCCs [48]. Ankyrin-repeated protein with a proline-rich region, a muscle protein, was present in 86% of ROs, compared with 0% in chRCC specimens [49]. CD63, a glycoprotein investigated for differential staining patterns, produces apical/polar staining in 94% of ROs, which is in contrast to the diffuse staining pattern observed in 96% of chRCCs [50]. Transforming growth factor β1, a cytokine, demonstrates predominantly cytoplasmic staining in ROs, while producing membranous staining in chRCC specimens [51].

Other novel markers include FOXI1, a transcription factor identified in intercalated cells (positive in ROs) [52], ELA, a ligand of apelin receptor (positive in ROs) [52], caspase 3, a protease involved in apoptosis (positive in chRCCs) [53], nuclear expression of leptin (in ROs) [54], loss of RB1 (in chRCCs) [55], and nuclear staining of tyrosine kinase ERBB4 [55].

Other RCC Subtypes

In clear cell papillary RCC (ccpRCC), there is diffuse positive cytoplasmic immunoreactivity for CK7. In addition, there can be a diffuse membranous expression of CAIX as in ccRCC; however, a unique "cup-like" pattern may be identified on basolateral tumor cells [8]. AMACR is not usually expressed in ccpRCC. There can be variable expression for HMWCK using 34βE12 antibody [7]. In contrast to ccRCCs, this subtype is negative for CD10 and positive for CK7. Unlike pRCC, this subtype is negative for AMACR and CD10.

Microphthalmia-associated transcription (MiT) family translocation carcinomas warrant careful discrimination from their morphologically similar counterparts, namely ccRCC and pRCC, because of the differences in disease outcome, prognosis, and management [56]. MiT family translocation carcinomas do not express CKs or EMA but do express CD10 and RCCM. Although the primary diagnostic modality is a fluorescence in situ hybridization assay, IHC markers may be used to distinguish MiT family translocation RCCs [57]. Translocation RCCs involving chromosome Xp11.2 are negative for CK7 and CAIX, but positive for AMACR [29]. A specific IHC marker, TFE3, may be used to identify this transcription factor that is overexpressed in this particular translocation [8]. A similar translocation-related marker, TFEB, may be used to distinguish cells affected by a translocation at chromosome 6p21 [58]. In relation to both chromosomal loci, an additional marker, cathepsin-K, may also be overexpressed and detectable in both Xp11.2 and 6p21 translocation tumors. Cathepsin-K is expressed in TFEB translocation RCCs, and in up to 60% of TFE3 RCCs [59,60]. IHC markers can be used as a supportive aid to diagnosis, but must be cautiously supplemented by other diagnostic tools, because of the occurrence of false positive and false negative results [61].

Acquired cystic disease-associated RCCs are slow-growing tumors that occur within cysts. These tumors can occur multi-focally and bilaterally [14,62]. Their diagnosis can often be made according to their characteristic morphological features; however, IHC markers may assist with diagnosis. This particular subtype of RCC is often immunoreactive to AMACR, AE1/AE3 antibody, CAM 5.2 antibody, vimentin, CD10, and RCCM. They have been reported to demonstrate variable expression for CAIX and CK7 [62,63].

Tubulocystic RCCs, although similar to pRCCs morphologically, may be distinguished using IHC markers. These tumors stain positive for CK7, CD10, PAX2, PAX8, and diffusely for AMACR [29]. In a study of 3 tubulocystic RCC specimens, all 3 cases stained positive for CK19, as well as vimentin, while being negative for HMWCK [15].

IHC markers for succinate dehydrogenase-deficient RCCs have been characterized in limited reports. These tumors have variable CK expression but have positivity for PAX8, EMA, and Ksp-cadherin. Typically, these tumors stain negative for vimentin, CD117, RCCM, and CAIX [64,65]. These tumors require a loss of the SDHB gene, which codes a subunit for succinate dehydrogenase enzyme [64]. A negative stain for SDHB can be useful to confirm this diagnosis.

It is important to differentiate collecting duct carcinomas from urothelial carcinomas. These tumors stain positive for CK5/6, CK7, CK8, CK19, as well as for HMWCK [15] and are immunoreactive for vimentin, PAX2, and PAX8 [10,15]. They usually stain negative for CD10. In addition to PAX8, urothelial markers p63 and GATA3 may be used to rule out a urothelial tumor [30].

Renal medullary carcinoma is closely related to collecting duct carcinomas and may be identified in individuals with sickle cell trait or anemia [66,67]. This subtype demonstrates immunoreactivity for CK7 and AE1/AE3 antibody but not to 34βE12 [15]. There is variable EMA expression. These tumors stain positive for PAX2 and PAX8. SMARCB1, a nuclear transcriptional regulator, can be used to distinguish renal medullary carcinoma from collecting duct carcinoma [68]. Another transcription factor, OCT3/4, can be used as a marker to distinguish this tumor from urothelial or collecting duct carcinomas [69].

Multilocular cystic renal cell neoplasms of low malignant potential demonstrate IHC features that are identical to those found in low-grade ccRCCs. This subtype is immunoreactive to CAM 5.2 antibody, EMA, CK7, CAIX, and PAX2 [70,71]. Variable expression has been reported for CD10 [70,71].

Histologically, hereditary leiomyomatosis renal cell carcinoma-associated RCC (HLRCC) was historically reported as being similar to type 2 pRCC or collecting duct carcinomas. In these tumors, there can be positive staining for CK7, CAM 5.2 antibody, and CD10. The stroma is negative for CD117. Loss of the fumarate hydratase gene is specific for HLRCC [72]. Because of increasing fumarate, IHC staining may result from accumulating S-(2-succinyl cysteine) (S2C), where strong nuclear and cytoplasmic expression has been described. S2C can, however, be found in type 2 pRCCs [72].

Mucinous tubular and spindle cell carcinoma (MTSCC) displays several IHC similarities to pRCC, despite being genetically dissimilar. These tumors are immunoreactive for AMACR, CK7, PAX2, E-cadherin and EMA, but stain negative for RCCM, 34βE12, and CD117 [15,73,74]. There can be variable staining for vimentin [15]. MTSCC differs from pRCC, with a lower

level of staining for CD10 (15% versus 100%) [73]. Reports have also described the in situ hybridization expression of *VSTM2A*. *VSTM2A* is expressed in moderate to high levels in MSTCC, with a reported diagnostic area under receiver operating characteristics curve of 99.2% [75].

Other Tissue Markers

Other useful markers may help distinguish RCC from non-renal cell tumor origins. PAX2 and PAX8 are transcription factors implicated in kidney and Müllerian organ development [30,76]. PAX2 and PAX8 are usually expressed and found diffusely in normal kidney tissue; however, they are also present in up to 90% of renal neoplasms. PAX2 differs from PAX8 in that it is not usually expressed in ROs or chRCCs. In addition, urothelial carcinomas do not express PAX2 or PAX8, thereby demonstrating their utility in determining tumor origin [77].

GATA3, an endothelial cell transcription factor, is a marker for urothelial carcinoma that consistently stains negative in RCC specimens [78]. CA-125, a marker classically associated with ovarian cancer, may also be used as a negative marker for RCCs, distinguishing them from other tumors of clear cell morphology [26]. CK20 is a useful negative marker for ruling out renal cell neoplasms [30]. Most RCCs typically stain negative for CK20, with the exception of previously reported eosinophilic solid cystic RCCs [79]. These may be useful in ruling out other CK20+ tumors, such as urothelial, ovarian, or colorectal carcinomas. Other markers useful in work-up for ruling out non-renal neoplasms include RCCM, CD10, vimentin and CKs.

Additional tissue-based markers are being investigated, as guided by the recent advances in the study of RCC genomic alterations. Commonly reported altered genes for ccRCC include *VHL*, *PBRM1*, and *SETD2* [80]. pRCC may demonstrate *MET* mutation, while chRCC may exhibit *TP53* or *PTEN* mutations [81]. Some genomic alterations may also aid differentiation of chRCC and RO [81]. Although genomic alterations have largely been studied using next-generation sequencing techniques, some have been adapted to IHC. *VHL*, a tumor suppressor gene, is commonly inactivated in both hereditary and sporadic ccRCCs [82]. The IHC detection of its gene product is expressed in up to 90% of primary renal tumors and 86% of metastatic RCC specimens [83]. However, it may also be identified in non-renal tumors such as clear cell carcinomas of the ovary or uterus [83]. *PBRM1* has also been studied as an IHC marker; however, its application is mainly to aid prognostication. A loss of *PBRM1* expression is associated with late tumor stage and poor differentiation [84]. Similarly, IHC *SETD2* expression has been identified in metastatic RCC, and demonstrates utility in determining likely prognostic outcomes [85]. Future studies should continue to investigate the adaptation of altered gene products to the field of diagnostic tissue markers.

Conclusion

The characterization of RCC subtypes using tissue biomarkers must undergo ongoing review as new markers and techniques are developed and described. IHC staining remains a useful method to subtype RCCs and to distinguish them from non-renal tumors, especially in small tissue volume specimens, such as in metastatic tissue biopsies. More study is required to further characterize the subtypes of RCC to further delineate them and improve the accuracy of diagnosis, treatment, and prognostication.

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