Molecular genetics and immunohistochemistry of renal tumours: translation into clinical practice

Fang-Ming Deng
Ming Zhou

Abstract

Renal cell carcinomas comprise a heterogeneous group of tumours with diverse clinicopathological and molecular characteristics as well as therapeutic options. Accurate diagnosis and classification are critical for patient management and prognosis prediction. They have traditionally been classified according to histopathological features. Recent advances in high throughput technologies, including next generation sequencing, have enabled us to search genome-wide for genomic, transcriptomic and proteomic changes in large number of renal cancer specimens. Studies have demonstrated that different histological subtypes harbour unique genomic and epigenetic alterations and gene expression and protein profile that can be integrated with clinicopathological features for diagnosis, classification, prognosis and individualized treatment and "n-of-1" trials. This review will discuss the immunoprofiles of major renal cell carcinoma subtypes, immunohistochemical markers that are commonly used in clinical laboratories, and recent genomic and epigenetic discoveries in renal cell carcinomas.

Keywords diagnosis; epigenetics; genomics; immunohistochemistry; kidney; next generation sequencing; proteomics; renal cell carcinoma; transcriptomics

Introductionintroduction

Renal cell carcinomas (RCC) comprise a heterogeneous group of tumours with diverse clinicopathological and molecular characteristics as well as therapeutic options. Accurate diagnosis and classification are critical for patient management and prognosis prediction. Recent emergence of small molecule inhibitors that target different molecular pathways makes precise histological classification of RCC even more imperative. For example, inhibitors of vascular endothelial growth factor and mTOR pathway are effective for clear cell RCC and show little effect for other RCC subtypes.

RCCs have traditionally been classified according to histopathological features. Recent explosive advances in high throughput technologies, including next generation sequencing (NGS), comparative genomic hybridization (CGH) and DNA methylation microarrays and gene expression profiling, have enabled us to analyse large number of RCC specimens to search genome-wide for genomic, transcriptomic and proteomic changes. Studies have demonstrated that different RCC subtypes harbour unique genomic and epigenetic alterations and protein expression profiles, suggesting that the genomic, epigenetic and proteomic characteristics can be integrated with clinicopathological features for diagnosis, classification, prognosis and treatment of RCC.

This review will discuss the immunoprofiles of major RCC subtypes and immunohistochemical markers that are commonly used in clinical laboratories. Recent discoveries of genomic and epigenetic changes in major renal tumours will also be briefly discussed.

Molecular genetics of renal cell carcinomas

1. Clear cell renal cell carcinoma (CCRCC)

The most common and characteristic genetic change in CCRCC is an alteration of chromosome 3p with 3p deletion in >90% tumours, followed by changes in other chromosomal regions, including 5q, 6q, 8p, 9p, 10p, and 14q.[1] Biallelic inactivation, by loss of one 3p arm and mutations involving the other chromosome 3, is a key event in the tumour development of CCRCC. Additional chromosomal aberrations are often associated with tumour progression. At least three regions harbouring several different genes on 3p have been implicated, including the von Hippel Lindau (*VHL*) gene on 3p25-26, the *FHIT* gene on 3p11-12, and the *RASSF1A* and *DRR1* genes on 3p21-22. VHL protein functions as a tumour suppressor and plays a crucial role in the hypoxia inducible factor (HIF) pathway by targeting HIF for proteosome mediated degradation and therefore maintaining HIF at low level. *VHL* gene inactivation, by gene mutation or promoter hypermethylation, leads to HIF accumulation and activation of the HIF pathway and its downstream target genes such as vascular endothelial growth factor (*VEGF*), platelet-derived growth factor (*PGDF*), epidermal growth factor (*EGF*), carbonic anhydrase IX (*CAIX*), *Glut-1* and *erythropoietin*. These genes act in concert to promote deregulated epithelial proliferation and angiogenesis and therefore contribute to the pathogenesis of CCRCC. The majority of CCRCC are sporadic, with <5% occurring in patients with inherited cancer syndromes such as von Hippel Lindau disease, tuberous sclerosis and constitutional chromosome 3 translocation syndrome.

Recent studies identified in CCRCC several frequent mutations in histone modifying and chromatin remodelling genes, including *PBRM1*, a subunit of the PBAF AWI/SNF chromatin remodelling complex,[2] *ARID1A*, a subunit of the BAF AWI/SNF chromatin remodelling complex,[3] histodeubiquitinase *BAP1*,[4,5] histone deubiquitinase *KDM5C*,[6] and histone methyltransferase *SETD2*.[7] Most mutations in these chromatin modulators in CCRCC are loss-of function, implicating major roles for epigenic regulation of additional functional pathways participating in the development and progression of this tumour. Clinical data has shown these mutations are associated with advanced stage, grade and tumour invasion.[8,9]

The comprehensive molecular characterization of CCRCC by the Cancer Genome Atlas (TCGA) research network[8] has

Fang-Ming Deng *MD PhD, Assistant Professor of Pathology, Department of Pathology, New York University Langone Medical Center, New York, USA. Conflicts of interest: none declared.*

Ming Zhou *MD PhD, Professor of Pathology, Department of Pathology, New York University Langone Medical Center, New York, USA. Conflicts of interest: none declared.*

confirmed these findings. It has also uncovered in aggressive CCRCC a metabolic shift, including down regulation of genes involved in the TCA cycle, decreased AMPK and PTEN protein level, up regulation of the pentose phosphate pathway and the glutamine transporter genes, increased acetyl-CoA carboxylase protein, and altered promoter methylation of miR-21 and GRB10, suggesting that metabolic pathways are potentially therapeutic targets.

2. Papillary renal cell carcinoma (PRCC)

Gain of chromosomes 7 and 17 and loss of Y chromosome are the most common cytogenetic changes in PRCC. Gains of additional chromosomes 3, 12, 16, 20 and others are often associated with tumour progression. Hereditary papillary renal cell carcinoma syndrome (HPRCC) harbours mutations in *c-Met* gene (7q31). Gain-of-function mutations in *c-MET* result in altered cellular processes related to renal papillary carcinogenesis, although these mutations are uncommon in sporadic PRCC.

Hereditary leiomyomatosis/renal cell carcinoma syndrome (HLRCC) harbours mutations in fumarate hydratase (*FH*) (1q42) gene. FH functions as an enzyme that converts fumarate to malate in the tricarboxylic acid cycle. Recent studies suggest that FH also regulates the stability of HIF, and may therefore play a role in renal carcinogenesis. High grade RCC with morphology reminiscent of type 2 PRCC occurs in about 1/4 of patients with HLRCC syndrome. Lack of functional FH protein results in accumulation of a high level of fumarate in tumour cells which then leads to aberrant succination of cellular proteins. Resultant S-(2-succino)-cysteine (2SC) proteins can be detected by immunohistochemistry as the surrogate marker for lack of *FH* gene activity due to mutations.[10] Immunostains for 2SC proteins can therefore be a useful ancillary tool in the differentiation of HLRCC renal tumours from other high-grade renal cell carcinomas. Suspected cases should be confirmed by *FH* gene sequencing.

A comprehensive genomic analysis of 161 PRCCs, carried out by the TCGA Research Network, shed light on the molecular basis of this tumour.[11] The findings confirmed that type 1 PRCC is characterized by alterations in the cell signalling involving *MET* gene. *MET* gene mutations or other alterations were identified in 81% of the type 1 PRCCs. These findings raise the possibility to treat type 1 PRCCs with inhibitors of the MET signalling pathway, including the MET/VEGFR inhibitor foretinib, which is currently in phase II clinical trials for PRCC and other cancer types. Type 2 PRCC, however, was more heterogeneous genomically and was characterized by *CDKN2A* silencing, *SETD2* mutation, and increased expression of the NRF2-antioxidant response element pathway. A CpG island methylation phenotype (CIMP) was found in a distinct subgroup of type 2 PRCC with the least favourable outcome. Of all type 2 PRCCs, 25% demonstrated decreased expression of *CDKN2A*, a tumour suppressor gene that regulates cell cycle progression. Loss of *CDKN2A* expression was also associated with a less favourable outcome.

3. Chromophobe renal cell carcinoma (ChRCC)

ChRCC frequently has multiple complex chromosomal losses, including Y, 1, 2, 6, 10, 13, 17 and 21. Renal oncocytoma, a benign tumour that may bear morphological resemblance to chromophobe RCC, is characterized by alterations involving chromosome 11q, partial or complete loss of chromosomes 1 or 14, or a sex chromosome (Y or X). ChRCC and renal oncocytoma share some cytogenetic similarity, although the former typically demonstrates more complex karyotypic alterations. Patients with Birt–Hogg–Dube syndrome, the gene for which is folliculin (*fln*) and is mapped to 17p11.2, often develop ChRCC, oncocytoma, and hybrid tumours with features of both ChRCC and oncocytoma. Recently, a TCGA project studying ChRCC found some metabolic pathways related to energy production in mitochondria were enriched in ChRCC, while the same pathways were suppressed in CCRCC. Pathways that lead to altered mitochondrial function, mutations in mitochondrial DNA, and recurrent structural rearrangements within the *TERT* promoter region have been identified in ChRCC.[12]

4. MiT family translocation renal cell carcinoma

TFE3 translocation RCC is defined by the chromosomal translocation involving *TFE3* gene on chromosome Xp11.2, and one of the partner genes including *PRCC* on 1q21, *ASPL* on 17q25, *PSL* on 1p34, *NonO* on Xq12 and *CLTC* on 17q23, resulting in the over-expression of the TFE3 protein, a member of the MITF/TFE transcriptional factor family. Immunohistochemical stains for the TFE3 protein on formalin-fixed and paraffin-embedded tissues offer a simple, sensitive and specific assay for the TFE3 translocation RCC. However, molecular genetic analysis for the chromosomal translocation involving *TFE3* gene provides the most definitive evidence.

Another variant of RCC harbours a t(6;11)(p21;q12) translocation that results in over-expression of TFEB transcriptional factor gene on 6p21, another member of MITF/TFE family. Both TFE3 and TFEB translocation RCCs predominantly affect children and young adults.

Recently, Malouf et al[13] demonstrated genomic heterogeneity in translocation RCCs which also harboured alterations common in CCRCC (e.g., 3p loss) and PRCC (e.g., trisomy 7 and/or 17). When compared with tumours in young patients (<18 years of age), adults translocation RCCs displayed distinct genomic and epigenetic aberrations, exemplified by lower LINE-1 methylation and frequent 17q partial gain, which were consistent with a large-scale dosage effect affecting RCC carcinogenesis. The results show that genetic alterations commonly present in other RCC subtypes can also been seen in translocation RCCs and are associated with worse outcomes.

5. Succinate dehydrogenase (SDH) deficient renal cell carcinoma

SDH is a multiprotein Krebs cycle/transport chain enzymatic complex composed of four subunits (SDHA, B, C, and D) which are located in the cristate of mitochondria, but are encoded by the nuclear (autosomal) genome. Patients with germline mutations in one of the SDH subunits are prone to develop pheochromocytoma/paraganglioma, gastrointestinal stromal tumour, and rarely RCC.[14,15] SDHB gene is most commonly mutated and is detected in about 80% the tumours. Mutations in other subunits are much less common. Tumour cells usually have abundant eosinophilic flocculent cytoplasm and striking intracytoplasmic vacuoles. These vacuoles are not empty; rather they are pale and greyish and represent giant mitochondria. This finding can be diffuse or focal. Loss of SDHB protein detected on immunohistochemical analysis is reported to be a sensitive and specific marker for these neoplasms and indicates germline mutation in one of the subunits of the mitochondrial complex 2.

