Papillary Renal Cell Carcinoma (PRCC): An Update

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Abstract: Papillary renal cell carcinoma (PRCC) is the second most common type of renal carcinoma following clear cell renal cell carcinoma. Papillary renal cell carcinoma is usually divided histologically into 2 types namely, type 1 and type 2. This classification, however, is unsatisfactory as many of papillary carcinoma are unclassifiable by the existing criteria. In recent years there has been a remarkable progress in our understanding of the molecular basis of PRCC. These studies have revealed that type 2 PRCCs represent a heterogenous group which may be subdivided into additional subtypes based on the genetic and molecular make up of these tumors and reflecting different clinical course and prognosis. Some of the molecular features such a hypermethylation of CPG islands in the promotor regions of genes and over expression of the antioxidant pathways within tumor cells have been recognized as markers of poor prognosis. Targeted therapies for papillary carcinoma in the past have been unsuccessful because of lack of clear understanding of the molecular basis of these tumors. It is hoped that recent progress in our understanding of the pathogenesis of various subtypes of PRCC, effective targeted therapies will eventually emerge in due course.

Key Words: renal carcinoma, papillary, familial, sporadic, c-Met, next generation sequencing, type 1, type 2

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Renal cell carcinoma is not a single disease but is a heterogenous group of various types of cancer. Each type has characteristic histologic features with corresponding genetic profile and a distinct clinical course and response to therapy.[1–3] Papillary renal cell carcinoma (PRCC) accounts for ~15% of kidney cancers. It is histologically characterized by the presence of fibrovascular cores with tumor cells arranged in a papillary configuration.[1–6]

In many of the early classifications of epithelial renal neoplasia, malignant tumors were grouped together regardless of their histologic architecture. In 1981 the first publication of WHO classification simply divided the renal malignant parenchymal tumors as *renal carcinoma and others*. This is even though in an earlier study in 1976 Mancilla-Jimenes and colleagues had documented a series of 34 cases of papillary carcinoma.[7,8] This was followed by recognition of several other renal parenchymal tumors such as oncocytoma and chromophobe carcinoma.[8]

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In recent years there has been a remarkable progress in our knowledge regarding the genetic profile of PRCC, which has markedly enhanced our understanding of the molecular basis of this tumor. These studies are providing valuable information for precise diagnosis and rational subtyping of these tumors with implications for clinical behavior and response to therapy.[9–12]

The purpose of this review is to trace the historical evolution of the concept of PRCC and to explore the progression of our knowledge and understanding of PRCC over last several decades. This, we hope, will provide perspective for interpretation of recent findings and will serve to highlight key observations relating to prognosis and development of new targeted therapies.

FORMAL RECOGNITION OF PAPILLARY RENAL CELL CARCINOMA

PRCC was formally recognized as a distinct entity in the Heidelberg classification of renal cell carcinoma almost 2 decades ago.[13] This was based on evidence from several genetic studies on familial PRCC which revealed aberrations of *MET* in most of these cases. In 1989, Kovacs proposed that PRCC should be considered as a separate entity of renal neoplasms and suggested that a renal tumor should be classified as such if at least 75% of the tumor consists of papillary structures.[14] Kovacs et al[15] found that PRCC is characterized by trisomy of chromosomes 3q, 7, 8, 12, 16, 17, or 20, and in men by loss of the Y chromosome. Furthermore, a combined trisomy of chromosomes 7 and 17 was the only karyotypic change found in several tumors, including some with size of <2 mm in diameter.[16] These observations strongly suggested that papillary renal carcinomas are characterized by duplication of genes on chromosomes 7 and 17 in early stage of development and are thus different from clear cell carcinomas which usually manifest 3p deletion.

HISTOLOGIC SUBCLASSIFICATION OF PAPILLARY RENAL CELL CARCINOMA

Amin and colleagues evaluated the histologic spectrum of 62 PRCCs and assessed significance of conventional pathologic prognostic parameters including, Fuhrman's nuclear grade, pathologic stage, tumor size, multifocality, necrosis, and foam cells, and correlated these with outcome. Grossly, most tumors were well circumscribed, averaged 6.7 cm in size and were predominantly localized to the renal poles. Microscopically, PRCCs were predominantly papillary or tubulopapillary, often with a thick fibrous capsule, foam cells, necrosis, hemorrhage, and multifocality. Thirty-five percent of these tumors were low grade (nuclear grade I and II) and 65% high grade (nuclear grade III and IV). Tumors were further distinguished by cytoplasmic features: eosinophilic (42%), basophilic (34%), and mixed (24%). Eosinophilic tumors were predominantly high grade, and basophilic tumors low grade.[5]

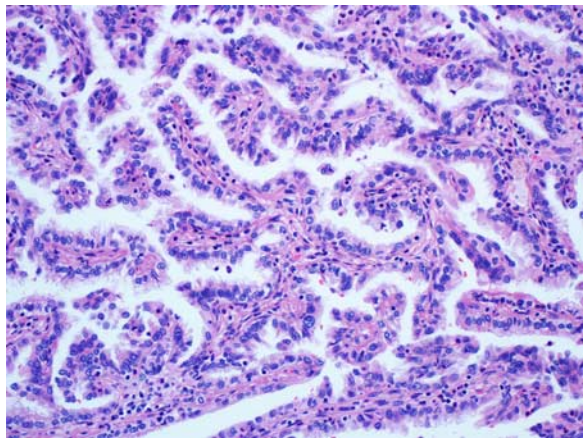

FIGURE 1. Photomicrograph featuring a type 1 papillary renal cell carcinoma. The cytoplasm is basophilic and the nuclei are dense without prominent nucleoli.

Delahunt and Eble proposed that PRCC can be morphologically classified into 2 subtypes. Type 1 is characterized by thin papillae and tubular structures covered by a single layer of small cells containing amphiphilic to basophilic cytoplasm and small, uniform, oval nuclei. Most of the familial PRCCs belong to this category. Type 2 which is more heterogenous, is characterized by papillae covered with large cells containing abundant eosinophilic cytoplasm and large, spherical nuclei with prominent nucleoli. In addition, frequent glomeruloid papillae, papillary edema, foamy macrophages in papillary cores, and psammoma bodies may also be present.[17,18] Generally, type 2 tumors have a poorer prognosis than type 1. In the recent WHO classification of renal tumors type 1 papillary carcinoma has been defined as a tumor composed of papillae covered by cells with nuclei arranged in a single layer on the papillary core, often with scanty pale cytoplasm (Fig. 1). Type 2 carcinomas are characterized by the presence of nuclear pseudostratification.[4] They are often of high nucleolar grade with cell containing abundant eosinophilic cytoplasm (Fig. 2).

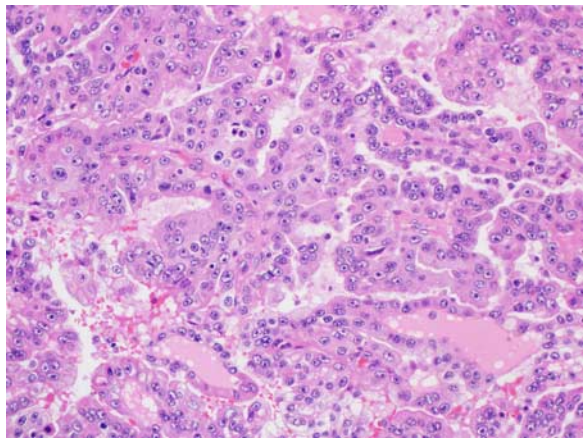

FIGURE 2. Photomicrograph depicting papillary renal cell carcinoma type 2. The tumor cells are large with abundant eosinophilic cytoplasm and large open nuclei with prominent nucleoli. Pseudo stratification of the nuclei is a prominent feature. Please see this image in color online.

The morphologic classification of PRCC, however, remains controversial, and there is limited molecular and biochemical evidence to support this morphologic classification. The relatively high incidence of mixed type 1 and 2 tumors poses additional difficulties for such a method of classification.[9] One study has suggested that immunostaining for MUC1 may be helpful in categorizing these tumors as MUC1 is usually positive in type1 PRCC while type 2 tumors are more likely to be nonreactive.[19] These findings, however, have not been validated by additional studies and are unlikely to provide reliable information for histologic classification of these tumors.

GENETIC BASIS OF TYPE 1 PAPILLARY RENAL CELL CARCINOMA

Much of our knowledge of the genetic basis of PRCC has been based on the study of the inherited form of the disease. Hereditary PRCC is a rare familial disorder that is associated with an increased risk of renal cancer. Individuals with hereditary PRCC usually have multiple kidney tumors and an increased risk of developing tumors in both kidneys. These tumors have morphology characteristic of papillary cancer type 1.[20–23] Currently, no other types of cancer or noncancerous health problems are known to be related to hereditary PRCC. The disease is characterized by activating germline mutations of *MET* (7q31).[20–22]

Several mechanisms can be involved in MET deregulation: overexpression, gene mutations, gene amplification, and epigenetic mechanisms. Germline missense mutations at 7q31, causing an alteration of MET tyrosine kinase domain with a consequently constitutive activation, have been found by Schmidt et al[23] in 6 of 7 families affected by hereditary PRCC. Fifteen different missense mutations have been identified in hereditary and sporadic PRCC. Bernues et al[24] described a partial duplication of chromosome 7q21-q35 in a hereditary PRCC family. Trisomy of chromosome 7, containing the MET locus, and trisomy of chromosome 17 are also commonly found in hereditary PRCC, and they are often associated with MET-activating mutations. Several neoplasms showed aberrant MET activation, amplification or overexpression. Overall, MET pathway is not the only mechanisms implicated in tumorigenesis of PRCC. Genetic analysis showed some chromosomal aberrations in all PRCC cases. Thus gains of chromosomes 7, 12, 17, 20 and loss of Y, 18, 9 are frequently found.[25–27]

Despite the evidence of the role of *MET* gene mutation in the pathogenesis of hereditary PRCC, *MET* mutations are quite uncommon in sporadic tumors. The analysis of *MET* proto-oncogene, performed by Schmidt and colleagues on 129 sporadic PRCC, showed a small proportion of mutations (13%). Moreover, 8 of these mutations (47%) were germline, although no familial history had been reported.[28] This finding suggests that the rate of sporadic c-*MET* mutations is lower. In a study performed by Lubensky and colleagues on 34 patients with papillary renal carcinoma, all cases (both sporadic and hereditary) with c-*MET* mutations showed a distinctive papillary type 1 histology.[29] Further studies analyzed c-MET protein expression in the cytoplasm and membrane of PRCC tumor cells. A strong MET expression (between 80% and 100%) has been described.

Role of c-MET in type1 PRCC

c-Met, is a receptor protein that in humans is encoded by the *MET* gene. c-MET is a single pass tyrosine kinase receptor (Fig. 3). It is produced as a single-chain precursor

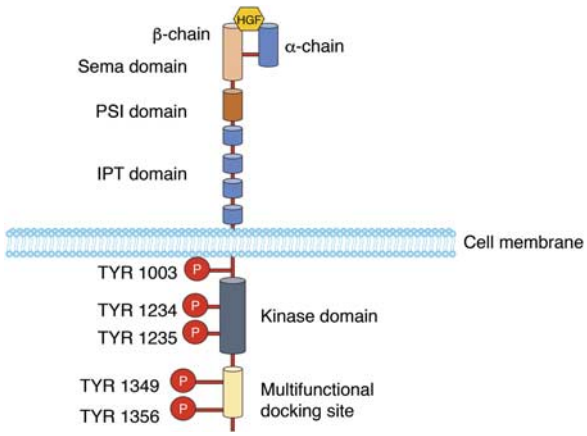

FIGURE 3. Diagram depicting domain structure of c-MET receptor composed of extracellular and intracellular parts. The extracellular portion of c-MET is composed of 3 domain types: a large semaphorin domain encompasses the whole α-subunit and part of the β-subunit. This is followed by plexin-semaphorin-integrin (PSI) domain which is connected to the transmembrane helix via 4 immunoglobulin-plexin-transcription (IPT) domains. The intracellular portion contains a tyrosine kinase catalytic domain flanked by distinctive juxtamembrane and carboxy-terminal sequences. This portion of c-MET contains the catalytic tyrosines Y1234 and Y1235, which positively modulate enzyme activity, while the juxta membrane tyrosine 1003 negatively regulates c-MET. The multifunctional docking site in the C-terminal tail contains tyrosines Y1349 and Y1356, which recruit several transducers and adaptors when c-MET is active. The c-MET ligand, hepatocyte growth factor (HGF), is secreted by mesenchymal cells and is the only known ligand for c-MET. Please see this image in color online.

that is proteolytically cleaved to yield a highly glycosylated extracellular α-subunit and a transmembrane β-subunit, which are linked together by a disulfide bridge. The extracellular portion of c-MET is composed of 3 domain types. The N-terminal 500 residues fold to form a large semaphorin domain, which encompasses the whole asubunit and part of the b-subunit. The PSI domain (found in plexins, semaphorins, and integrins) follows the semaphorin domain, spans ~50 residues and includes 4 disulfide bonds. This domain is connected to the transmembrane helix via 4 immunoglobulin plexin transcription domains, which are related to immunoglobulin-like domains and are found in integrins, plexins, and transcription factors. Intracellularly, the c-MET receptor contains a tyrosine kinase catalytic domain flanked by distinctive juxta membrane and carboxy-terminal sequences.[30,31] This cell surface receptor is usually expressed in epithelial cells of many organs, including the liver, pancreas, prostate, kidney but may also be expressed in muscle and bone marrow, during both embryogenesis and adulthood. c-Met is known to play a crucial role in embryonic development, organogenesis and wound healing. Hepatocyte growth factor (HGF) is the only known ligands of the MET receptor. MET is normally expressed by epithelial cells, while expression of HGF is restricted to cells of mesenchymal origin. When HGF binds its cognate receptor MET, it induces its dimerization leading to its activation. After binding with the ligand, HGF, a wide range of different cellular signaling pathways are activated, including those involved in proliferation, motility, migration, and invasion. Although c-MET is important in the control of tissue homeostasis under normal physiological conditions, it has also been found to be aberrantly activated in human cancers via mutation, amplification or protein

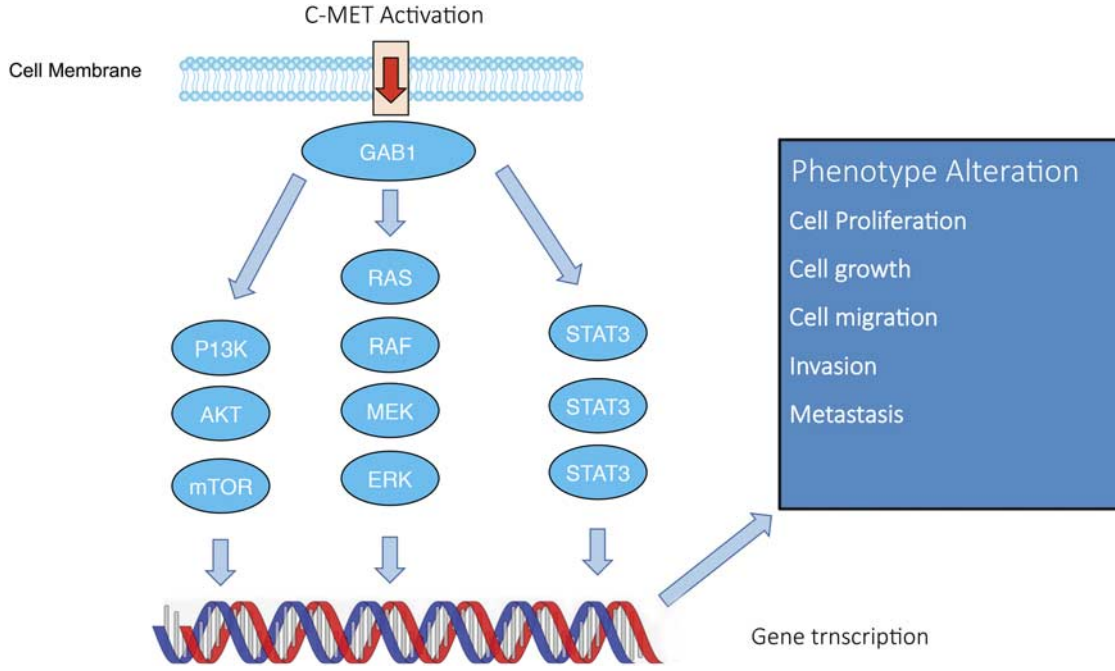

FIGURE 4. Activation of c-MET receptor leads to phosphorylation of the adaptor protein GRB2-associated binding protein 1 (GAB1). Once bound to and phosphorylated by c-MET, it creates binding sites for more downstream adaptors thus resulting in activation of several transduction pathways including MAPK, PI3K, and STAT3. Activation of these pathways leads to expression of a multitude of genes which cause a variety of potentially oncogenic phenotypic changes in the cells. Please see this image in color online.

overexpression. The RAS pathway mediates HGF-induced scattering and proliferation signals and induces sustained RAS activation, and thus prolonged MAPK activity (Fig. 4). Several additional pathways including PI3K, Stat, beta-catenin, and Notch pathways may also be activated. GAB1 is a key coordinator of the cellular responses to MET and binds the MET intracellular region. Upon interaction with MET, GAB1 becomes phosphorylated on several tyrosine residues which, in turn, recruit a number of signaling effectors.[30,31]

Abnormal MET activation in cancer triggers tumor growth, formation of new blood vessels (angiogenesis) and cancer spread to other organs. MET is deregulated in many types of human malignancies, including cancers of kidney, liver, stomach, breast, and brain. Normally, only stem cells and progenitor cells express MET, which allows these cells to grow invasively to generate new tissues in an embryo or regenerate damaged tissues in an adult. However, cancer stem cells are thought to acquire the ability of normal stem cells to express MET, thus causing persistence of cancer and leading to its spread to other sites in the body.[30,31]

HEREDITARY PAPILLARY RENAL CELL CARCINOMA WITH TYPE 2-LIKE MORPHOLOGY

Hereditary leiomyomatosis and renal cell cancer (HLRCC), is a rare autosomal cancer susceptibility syndrome characterized by the development of cutaneous and uterine leiomyomas and renal cancer. The lifetime risk of renal cancer is currently estimated to be 15%. These tumors tend to have an early age of onset with the mean age of 40 years at the time of diagnosis. Renal cancers associated with HLRCC have a characteristic pathologic appearance with large nuclei with inclusion like eosinophilic nucleoli surrounded by a clear halo. The pattern of renal cancer in HLRCC differs from other inherited renal cancer susceptibility syndromes in that the tumors tend to be solitary and unilateral and have a highly aggressive course of disease.[32–38]

The morphologic features of carcinomas associated with HLRCC frequently include papillary architecture with abundant eosinophilic cytoplasm, large nuclei, and very prominent nucleoli with perinucleolar clearing and thus resemble PRCC type 2. Papillary type 2 like component, however, is one of several morphologic features including collecting duct, solid, tubulocystic, cribriform, and cystic growth pattern.[33–37] In the 2 recently published studies by Chen and colleagues and Trpkov and colleagues papillary pattern was predominant in 33% and 57% of the cases, respectively.[36–37]

These tumors, however, have now been recognized as a distinct entity in the recent WHO classification.[4] Recognition of the clinical features of the HLRCC syndrome such as cutaneous and uterine leiomyomas may be helpful in establishing a definitive diagnosis. Immunohistochemical staining of the tumor may reveal lack of cytoplasmic staining for fumarate hydratase and overexpression of modified cysteine-s-(2-succino) cysteine, thus confirming the diagnosis.[4] Cases in which history or stigmata of HLRCC are not present the tumors are called FH deficient RCC.[35–37]

The gene mutated in HLRCC corresponds to fumarate hydratase gene located at 1q42.3-q43. It encodes a protein called fumarate hydratase, which is an enzyme of the Krebs cycle that catalyzes the conversion of fumarate to malate. Biallelic inactivation is detected in almost all HLRCC tumors. Lack of FH activity results in intracellular accumulation of fumarate leading to a pseudo hypoxic drive characterized by stabilization of hypoxia inducible factor HIF-a. This results in generation of several downstream oncogenic signals for angiogenesis, cell proliferation, increased glucose uptake, and chemotaxis.[33]

SPORADIC PAPILLARY RENAL CELL CARCINOMA WITH TYPE II MORPHOLOGY

Albiges and colleagues investigated the MET gene status in a large well-annotated cohort of 220 patients with sporadic PRCC. Each sample was independently reviewed by 2 specialized pathologists, both blinded to the clinical outcome. This robust dataset expands our knowledge about MET gene status for both type I and II PRCC subtypes by reporting on different mechanisms of MET activation: gene expression, copy-number alterations (CNAs) and mutational status. MET expression was significantly higher in both type I and type II PRCC than in clear-cell histology. However, type I PRCC presented a higher expression of MET when compared with the type II subtype. CNAs of MET were identified in 46% of type II PRCC and in 81% of type I PRCC. The correlation of copy number abnormality and MET mRNA expression was significantly high, which may provide a biological basis for enhanced MET signaling. Of note, 11 somatic mutations of the MET gene, including four new mutations, were identified in 51 type I PRCC (21.5%). However mutational analysis of type II PRCC was not performed.[39,40]

COMPREHENSIVE MOLECULAR CHARACTERIZATION OF PAPILLARY RENAL CELL CARCINOMA USING NEXT-GENERATION SEQUENCING

Previously published next-generation sequencing studies have identified several mutated genes associated with PRCC including: MET, NF2, SETD2, and Nrf2 pathway genes. However, these mutations were found in only ~10% to 15% of PRCC tumors in these studies.[41,42] The investigators of The Cancer Genome Atlas Research Network (TCGA) performed comprehensive molecular analysis, including whole-exome sequencing, identification of CNAs, micro RNA, and messenger RNA determination.[43]

On the basis of tumor histology, the authors identified 75 type 1 tumors, 60 type 2 tumors, and 26 tumors that could not be categorized as type 1 or type 2. Most of the type 1 tumors were localized (stage I), while the type 2 tumors were more frequently advanced or metastatic (stage III or IV). Analysis of chromosome alterations revealed 3 subgroups. One group, consisting mainly of type 1 and other low-grade tumors, showed chromosomal gains, particularly of chromosomes 7 and 17. The other 2 groups included predominantly type 2 tumors. One group revealed few genome copy number changes, while the other had multiple chromosome losses and was associated with poorer patient survival.[43]

The investigators sequenced the expressed regions of the genomes in 157 of the tumors to identify potential mutations. Significantly mutated genes included MET, SETD2, NF2, KDM6A, and SMARCB1, which were altered in 24% of tumors.

Evaluation of genes previously associated with cancer revealed 6 additional significantly mutated genes, FAT1, BAP1, PBRM1, STAG2, NFE2L2, and TP53, that increased the number of tumors with alterations to 36%.

Analysis of CNAs resulted in the identification of 3 patterns: predominantly type 1 tumors with frequent gain of

chromosomes 7 and 17; type 2 tumors with few CNAs; and type 2 tumors with aneuploidy, including frequent loss of chromosome 9p. Most of type1 PRCC tumors (81%) had gains of chromosome 7 or altered MET status (mutation, gene fusion or splice variant of MET). While these findings support the hypothesis of MET as a driver mutation in type 1 PRCC, it cannot be concluded from this evidence alone. Further supporting this theory, however, is the finding that levels of MET mRNA expression were significantly higher in type 1 tumors than type 2 tumors.[10]

Whole-exome sequencing identified 11 significantly mutated genes, including previously identified genes such as MET, SETD2, NF2, and BAP1, among others. These mutations, many of which are part of known cancer-associated pathways, were present in a higher percentage of tumors than was reported by previous studies. CDKN2A alterations were found in 21 tumors (13%) and included 25% of type 2 tumors. These alterations included focal loss of 9p21, mutation, or promotor hypermethylation of CDKN2A. In addition, increased expression of miR-10b-5p was correlated with decreased expression of CDKN2A. CDKN2A altered tumors were found, on univariate analysis, to be associated with lower overall survival when compared to tumors without CDKN2A alterations.[10]

A novel CpG island methylator phenotype (CIMP) was identified in nine tumors, all of which also had hypermethylation of the CDKN2A promoter. Eight of 9 of these tumors were papillary type 2. CIMP-associated tumors were noted to have poor survival.

The CpG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5′→3′ direction.

Cytosines in CpG dinucleotides can be methylated to form 5-methylcytosine. In humans, about 70% of promoters located near the transcription start site of a gene (proximal promoters) contain a CpG island. Regulation of gene expression is a general key mechanism that is operative in normal tissues and has an important role in the preservation of genomic stability, embryonic development, and tissue differentiation. CpG islands are common in promoter sites rich in CpG dinucleotides. More than 50% of human genes have been found to be regulated in this way, by promoters including CpG islands. In cancer cells, CpG islands may also be aberrantly hypermethylated, causing inappropriate silencing of gene expression (Fig. 5). Aberrant genomic methylation is thought to result in tumorigenesis by deregulating gene expression of key (tumor suppressor) genes.[43] This phenotype has been reported in several other tumor types, including gastric, lung, liver, ovarian, glioblastomas, endometrial, breast, leukemias, and colorectal carcinoma.

A cluster-of-clusters analysis was performed using the various data types to identify PRCC subgroups. Four subgroups were identified (C1, C2a, C2b, and C2c) and were associated with progressively worse overall survival. C1 included primarily papillary type 1 tumors, while C2a and C2b included primarily papillary type 2. Subgroup C2c included only type 2 PRCC with CIMP-associated tumors, which had the lowest overall survival. This analysis, which elucidated the complexity of PRCC and the heterogeneity of type 2 PRCC specifically, has significant implications for the design of future clinical trials and the development of targeted therapies for PRCC.[10]

Examination of these changes according to PRCC subtypes, revealed several alterations that were specific to each. For example, most of MET mutations were identified in type 1 tumors. In contrast, alterations in CDKN2A, due to loss of chromosome 9p21, as well as mutations in SETD2, BAP1, and PBRM1 were associated with type 2 tumors.

One distinguishing feature of type 2 tumors that emerged from the combined analysis was increased expression of the Nrf2-antioxidant response element pathway, that is exemplified by the expression of the well-known NRF2-ARE gene NQO1.[10] Nrf2 antioxidant response element signaling is a major mechanism in the cellular defense against oxidative stress (Fig. 6). Activation of this pathway controls the expression of genes whose protein products are involved in the detoxication and elimination of reactive oxidants through conjugative reactions and by enhancing cellular antioxidant capacity. NQO1 expression was lowest in cluster C1 tumors, intermediate in cluster C2a and C2b tumors, and highest in cluster C2c tumors.[44,45] Interestingly, increased NQO1expression was associated with decreased survival.[10]

Taken together, the findings from this comprehensive study revealed that type 1 and type 2 PRCC are 2 distinct

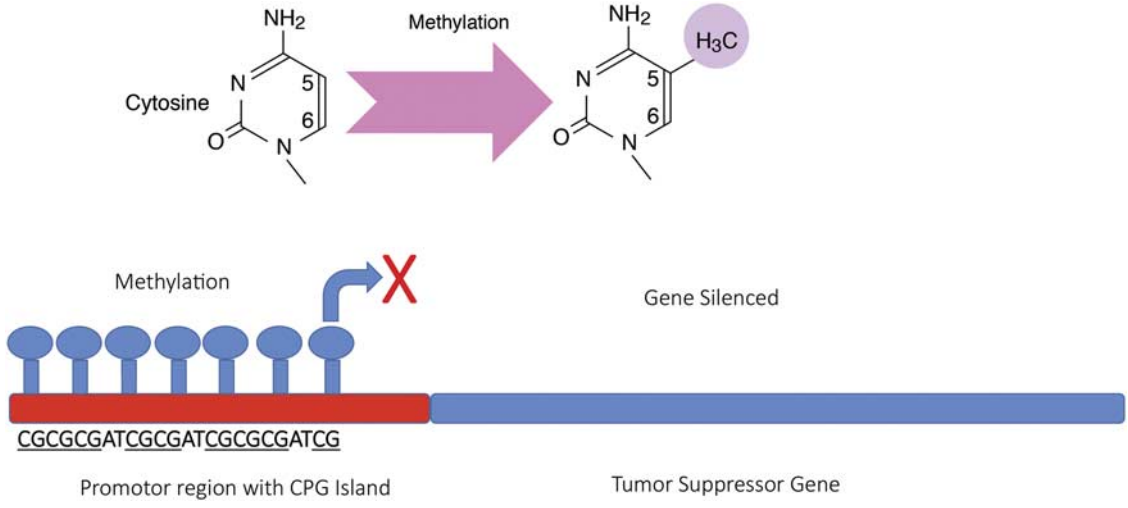

FIGURE 5. Increased methylation of CPG islands in several tumor suppressor and other genes results in gene silencing which may have impact on prognosis. Please see this image in color online.

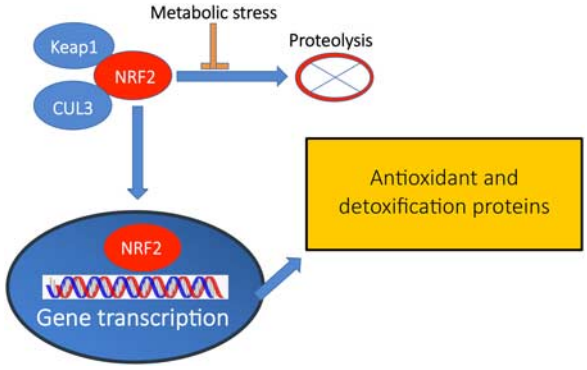

FIGURE 6. The nuclear factor erythroid 2–related factor 2 (Nrf2) is regulator of cellular resistance to oxidants. Under normal or unstressed conditions, Nrf2 is kept in the cytoplasm by a cluster of proteins (KEAP1, CUL3) that degrade it quickly by proteolysis. Under oxidative stress, Nrf2 is not degraded, but instead travels to the nucleus where it binds to a DNA promoter and initiates transcription of antioxidative genes and their proteins. Please see this image in color online.

diseases and that type 2 can be further stratified into 3 subgroups. This improved classification of PRCC may lead to the development of more specific, targeted therapies as well as improved disease management and design of clinical trials.[10]

In a more recent study Pal and colleagues performed molecular characterization of advanced PRCC via targeted sequencing of 315 genes in tumors from 169 patients, of whom 60% had stage IV disease. Most of their findings are in line with TCGA highlights, but the study has also shed light on pivotal issues that characterize metastatic disease.[11] MET is yet again a central oncogenic alteration in type 1 PRCCs, with mutations in 20% and amplifications in 13% of cases. The frequency of MET alterations in this study is onethird of >80% with MET alterations in the TCGA dataset. Indeed, this is because Pal and colleagues chose to consider CNAs with only high-level MET amplifications (>6). This study also revealed that the ontogeny of type 2 PRCC might share similarities with type 1 PRCC. Indeed, both subtypes exhibit alterations in cell cycle genes CDKN2A/B, TERT, RAS/RAF signaling, the DNA damage pathway, and the mTOR pathway with comparable frequency. Above all, we still must understand whether type 2 PRCCs encompass similar entities or multiple diseases. The answers might come from dedicated studies of specific type 2 PRCC alterations, including FH mutations, NF2 mutations, and SWI/SNF chromatin remodeling complex alterations that might induce genome-wide reprogramming.

NEW PROPOSED SUBCLASSIFICATION OF PAPILLARY RENAL CELL CARCINOMA

In a recent study Saleeb et al[12] have proposed a new classification system of PRCC integrating morphologic, immunophenotypical, and molecular analysis. In a study of cohort of 108 cases of PRCC it was shown that these cases may be divided into 3 categories, each with its characteristic immunohistochemical staining profile (Table 1). A panel of 3 potentially distinguishing markers (CA9, ABCC2, and GATA3) was assessed by immunohistochemistry. The panel exhibited distinct staining patterns between the 2 classic PRCC subtypes (PRCC1&2), sub classified 30% of the histologically unclassified group into either PRCC1 or PRCC2; and additionally, recognized another group of tumors (PRCC3) that accounted for 35% of the total cohort. Molecular testing using miRNA expression and copy number variation analysis confirmed the presence of 3 distinct molecular signatures corresponding to the 3 subtypes. Disease-free survival was significantly enhanced in PRCC1 versus 2 and 3 ($P=0.047$) on univariate analysis. The newly described PRCC3 has overlapping morphologic features between PRCC1 and pRCC2 and would therefore be difficult to classify on histologic appearance alone but can be diagnosed by using the immunohistochemical panel (Table 1). Molecularly PRCC3 has a distinct signature although clinically it behaves like PRCC2. Thus, it seems that the new classification stratifies PRCC patients into subgroups, which may have significant implications on the management of PRCC.[12]

TABLE 1. Immunohistochemical Staining Pattern in Different Types of PRCC Using a Panel of 3 Antibodies[12]

Antibody	PRCC1	PRCC2	PRCC3	PRCC4
ABCC2	Negative	Strong diffuse positivity	Weaker patchy positivity	Strong diffuse positivity
CA9	Negative	Perinuclear dot-like positivity	Negative	Negative
GATA3	Negative	Negative	Negative	Positive

PRCC indicates papillary renal cell carcinoma.

The markers also further characterized the oncocytic PRCC (PRCC4) as a distinct subtype.[12] PRCCs with eosinophilic (oncocytic) cytoplasm and oncocytoma-like low-grade nuclei have been called oncocytic PRCCs (Fig. 7). Because tumors with this morphology have not yet been fully characterized, they are not considered a distinct WHO entity. The Vancouver consensus conference has recommended diagnosing such tumors as type 2 PRCC for the time being.[3]

Most reported cases are clinically indolent and showed no disease progression. Despite having an immunophenotype more comparable with PRCC2 (strong diffuse positivity for ABCC2), the authors suggest that PRCC4 may be closer to PRCC1 molecularly and clinically based on similar gains of chromosomes 7 and 17 and a good prognosis.[12]

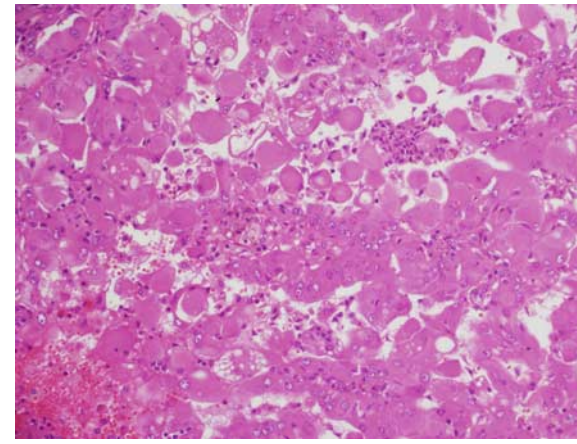

FIGURE 7. Photomicrograph depicting an oncocytic variant of papillary carcinoma. The tumor cells have abundant eosinophilic cytoplasm and round to oval relatively small nuclei, some containing nucleoli. Please see this image in color online.

UNCOMMON HISTOLOGIC VARIANTS OF PAPILLARY RENAL CELL CARCINOMA

Several categories of apparent PRCCs with unusual morphology features have been reported in the literature.

Solid Variant of Papillary Renal Cell Carcinoma

Argani et al[46] described 5 cases of PRCC with low-grade spindle cell component, which they characterized as solid variant of PRCC. All patients were male, and ranged in age from 17 to 68 years. All tumors were predominantly solid, featuring compact areas of low-grade spindle cells lining thin, angulated tubules. Mucinous stroma was not appreciated in any case. All cases were diffusely immunoreactive for cytokeratin 7, and focally CD10 positive. All 5 cases showed trisomy of chromosome 7, and 3 of 5 showed trisomy of chromosome 17 by fluorescence in situ hybridization (FISH), supporting classification as PRCC. Zhang et al[47] documented 2 additional cases of solid variant of PRCC by light microscopy, special staining, immunohistochemical staining and FISH and compared their findings with 2 cases of mucinous tubular spindle cell tumors. They found that morphologic and immunophenotyping features showed more overlap between these 2 types of tumor. In addition, gains of chromosomes 7 and 17 and loss of Y, which are characteristic of PRCC, were observed in 2 cases of solid PRCC and one case of mucinous tubular spindle cell tumor. Ren et al[48] compared the genome-wide CNAs in tumors displaying classic histologic features of MTSCC in comparison to the solid variant of type 1 PRCC and indeterminate cases with overlapping histologic features. The study included 11 histologically typical MTSCC, 9 tumors with overlapping features between MTSCC and PRCC, and 6 cases of solid variant of type PRCC. DNA samples extracted from macrodissected or microdissected tumor areas were analyzed for genome-wide CNAs using an SNP array platform suitable for clinical archival material. All cases in the MTSCC group exhibited multiple chromosomal losses, most frequently involving chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22, while lacking trisomy 7 or 17. In contrast, cases with overlapping morphologic features of MTSCC and PRCC predominantly showed multiple chromosomal gains, most frequently involving chromosomes 7, 16, 17, and 20, similar to the chromosomal alteration pattern that was seen in the solid variant of type 1 PRCC cases. Morphologic comparison of these molecularly characterized tumors identified histologic features that help to distinguish MTSCC from PRCC, but immunohistochemical profiles of these tumors remained overlapping. The study concluded that characteristic patterns of genome-wide CNAs strongly support mucinous tubular spindle cell carcinoma and solid variant of PRCC as distinct entities despite their immunohistochemical and certain morphologic overlap.

Another type of solid variant of PRCC has also been recognized where distinct papillary structures are not easily discernable. Renshaw et al[49] identified 6 tumors composed of solid sheets of cells without true papillae but that otherwise resembled PRCCs. Four of 4 tumors tested showed trisomies for chromosome 7, chromosome 17, or both by either cytogenetic analysis or FISH. Four cases were composed of solid sheets of cells containing distinct micronodules that in some cases resembled abortive papillae. The cells composing the micronodules had abundant eosinophilic cytoplasm, open chromatin, and in some cases prominent nucleoli. The intervening cells had similar nuclei, but the amount of cytoplasm was variable. This tumor may have strong morphologic resemblance to metanephric adenoma. Mantoan Padhilla et al[50] studied the immunohistochemical profile of a series of solid variant of PRCC and metanephric adenoma and found overlapping immunoreactivity for S100, CD57, and CK7. Metanephric adenomas however were positive for WT1 and negative for epithelial membrane antigen (EMA) and alpha-methylacyl-CoA racemase (AMACR). By contrast the tumor cells in solid variant of PRCC were positive for EMA and AMACR and negative for WT1. Ulamec and colleagues studied the immunohistochemical profile of 10 cases of solid variant of PRCC. All 10 cases were strongly and diffusely positive for CK7 and negative for WT-1.[51]

Biphasic Squamoid Alveolar Renal Cell Carcinoma

Biphasic squamoid alveolar renal cell carcinoma is another rare morphologic variant of PRCC that has been recently described as a distinct neoplasm. The largest series consists of 21 cases from 12 institutions that were analyzed using routine histology, immunohistochemistry, array comparative genomic hybridization and FISH. The size of tumors ranged from 1.5 to 16 cm. Follow-up information was available for 14 patients (range, 1 to 96 mo), and metastatic spread was found in 5 cases. All tumors comprised 2 cell populations arranged in organoid structures: small, low-grade neoplastic cells with scant cytoplasm usually lining the inside of alveolar structures, and larger squamoid cells with more prominent cytoplasm and larger vesicular nuclei arranged in compact nests. In 9 of 21 tumors there was a visible transition from such solid and alveolar areas into papillary components. Areas composed of large squamoid cells comprised 10% to 80% of total tumor volume. Emperipolesis was present in all (21/21) tumors. Immunohistochemically, all cases were positive for cytokeratin 7, EMA, vimentin, and cyclin D1. The authors concluded that tumors show a morphologic spectrum ranging from RCC with papillary architecture and large squamoid cells to fully developed BSARCC. Emperipolesis in squamoid cells was a constant finding. All carcinomas expressed CK7, EMA, vimentin, and cyclin D1. Multiple chromosomal aberrations were identified in all analyzable cases including gains of chromosomes 7 and 17, indicating that they are akin to PRCC. Thus, available microscopic, immunohistochemical, and molecular genetic data strongly support the view that biphasic squamoid renal cell carcinoma is a distinctive and peculiar morphologic variant of PRCC.[52]

PRCC With Oncocytic Morphology

Cases of PRCC with distinct eosinophilic cytoplasm named oncocytic PRCC have been described by several authors. However, owing to the rarity of oncocytic PRCC, the clinicopathologic and genetic features of the tumor have still not been well elucidated and whether it should be regarded as an independent subtype of PRCC remains controversial. Microscopically, typical oncocytic PRCC possessed fine papillary structures with delicate fibrovascular cores, lined with a single layer cell with large, deeply eosinophilic granular cytoplasm and round or polygonal-shaped nucleus exhibiting low nuclear grade. Furthermore, solid oncocytoma-like pattern as well as cases with sarcomatoid differentiation have also been recognized.

Immunohistochemically, the majority of tumors presented high expression rates of AMACR, CD10 and vimentin, similar to type 2 PRCC. Genetically, FISH analysis reveals trisomy of chromosome 7 in 7 OPRCCs and

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trisomy of chromosome 17. Among male patients, loss of chromosome Y may also be seen. Prognosis is generally favorable, although occasional tumors may behave aggressively.[53] As mentioned earlier, since tumors with this morphology have not yet been fully characterized, they are not considered a distinct WHO entity. The Vancouver consensus conference has recommended diagnosing such tumors as type 2 PRCC for the time being.[3]

Warthin-like Papillary Renal Cell Carcinoma

Warthin-like PRCC is morphologically very close to oncocytic PRCC, from which it differs by the presence of dense lymphoid stroma. In a recent study Skenderi and colleagues analyzed clinicopathologic, morphologic, immunohistochemical, and molecular-genetic characteristics of 11 oncocytic PRCCs with prominent tumor lymphocytic infiltrate, morphologically resembling Warthin's tumor. Papillary growth pattern was predominant, comprising > 60% of tumor volume. Tubular and solid components were present in 5 and 3 cases, respectively. Uniform immunohistochemical positivity was found for AMACR, PAX-8, MIA, vimentin, and OSCAR. Tumors were mostly negative for carboanhydrase 9, CD117, CK20, and TTF-1. Tumor infiltrating lymphocytes consisted of both B and T cells. Chromosomal copy number variation analysis showed great variability in 5 cases, ranging from a loss of one single chromosome to complex genome rearrangements. Only one case showed gains of chromosomes 7 and 17, among other aberrations. In 6 patients no lethal progression was noted, while 3 died of disease indicating that Warthin-like PRCC is a potentially aggressive tumor.[54]

MISCELLNEOUS RENAL CARCINOMAS WITH VARIABLE PAPILLARY COMPONENT

Minor papillary carcinoma components have been seen in a variety of renal carcinomas. These include tubulocystic carcinoma, mucinous tubular spindle cell carcinoma, clear cell PRCC, collecting duct carcinoma, medullary carcinoma, MiT family translocation renal cell carcinoma, HLRCC and other fumarate hydratase deficient tumors.[1–4] Whether the presence of papillary component in these tumors indicates a histogenetic relationship with PRCC is a matter of debate.

THE WAY FORWARD

Efforts aimed at the development of effective forms of therapy for PRCC have been hindered over the past decades by a lack of understanding of the molecular basis of sporadic PRCC. For many years, what was known about PRCC was based on the insight gained from hereditary forms of the disease. However, recent genomic profiling studies, have refined our understanding of the heterogeneity within PRCC. These efforts identified a multitude of key dysregulated pathways beyond the MET pathway that may prove to be promising targets for development of new therapeutic agents.

Clinical trials targeting MET in type 1 PRCC with MET alterations are ongoing. However, given the wide range of genomic alterations reported in type 2 PRCC, the integration of molecular profiling into clinical routine is of utmost importance. Clear understanding of the molecular basis of the various subtypes of type 2 papillary renal carcinoma as well as separately recognized entities with PRCC2-like morphologic features will go a long way toward finding appropriate therapies for these cancers. As multiple oncogenic mutations might co-occur in the same tumor, advanced models are needed to identify the most important oncogenic drivers. This it is hoped will avoid the disappointments encountered in the earlier trials using targeted therapies such as MTOR inhibition in PRCC.[55,56] It will also be necessary to closely study tumor heterogeneity especially in relation to the various categories of type 2 PRCC encompassing indolent local tumor to aggressive phenotype. Indeed, new integrative models for histologic and molecular analysis of PRCCs will be needed to define distinct phenotypic and molecular portraits of PRCC subgroups for potential targeted therapies.[57]

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