

## ORIGINAL ARTICLE

# Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma

The Cancer Genome Atlas Research Network\*

## ABSTRACT

**BACKGROUND**

Papillary renal-cell carcinoma, which accounts for 15 to 20% of renal-cell carcinomas, is a heterogeneous disease that consists of various types of renal cancer, including tumors with indolent, multifocal presentation and solitary tumors with an aggressive, highly lethal phenotype. Little is known about the genetic basis of sporadic papillary renal-cell carcinoma, and no effective forms of therapy for advanced disease exist.

**METHODS**

We performed comprehensive molecular characterization of 161 primary papillary renal-cell carcinomas, using whole-exome sequencing, copy-number analysis, messenger RNA and microRNA sequencing, DNA-methylation analysis, and proteomic analysis.

**RESULTS**

Type 1 and type 2 papillary renal-cell carcinomas were shown to be different types of renal cancer characterized by specific genetic alterations, with type 2 further classified into three individual subgroups on the basis of molecular differences associated with patient survival. Type 1 tumors were associated with *MET* alterations, whereas type 2 tumors were characterized by *CDKN2A* silencing, *SETD2* mutations, *TFE3* fusions, and increased expression of the NRF2-antioxidant response element (ARE) pathway. A CpG island methylator phenotype (CIMP) was observed in a distinct subgroup of type 2 papillary renal-cell carcinomas that was characterized by poor survival and mutation of the gene encoding fumarate hydratase (*FH*).

**CONCLUSIONS**

Type 1 and type 2 papillary renal-cell carcinomas were shown to be clinically and biologically distinct. Alterations in the *MET* pathway were associated with type 1, and activation of the NRF2-ARE pathway was associated with type 2; *CDKN2A* loss and CIMP in type 2 conveyed a poor prognosis. Furthermore, type 2 papillary renal-cell carcinoma consisted of at least three subtypes based on molecular and phenotypic features. (Funded by the National Institutes of Health.)

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**K**IDNEY CANCER, OR RENAL-CELL CARCINOMA, is not a single disease but is made up of various types of cancer that are characterized by different genetic drivers; each type has distinct histologic features and a distinct clinical course and response to therapy.<sup>1,2</sup> Papillary renal-cell carcinoma, which accounts for 15 to 20% of kidney cancers, is a heterogeneous disease with histologic subtypes and variations in both disease progression and patient outcomes. Papillary renal-cell carcinoma has two main subtypes: type 1, which is often multifocal, is characterized by papillae and tubular structures covered with small cells containing basophilic cytoplasm and small, uniform, oval nuclei,<sup>3</sup> whereas type 2, which is more heterogeneous, is characterized by papillae covered with large cells containing eosinophilic cytoplasm and large, spherical nuclei with prominent nucleoli.<sup>3,4</sup> Although in some patients papillary renal-cell carcinoma is indolent, bilateral, and multifocal, other patients present with solitary lesions that have an aggressive clinical course. Little is known about the genetic basis of the sporadic forms of papillary renal-cell carcinoma, and there are currently no effective forms of therapy for patients with advanced disease.

Much of our knowledge of the genetic basis of papillary renal-cell carcinoma has been based on the study of the inherited form of the disease. Hereditary papillary renal-cell carcinoma, a rare disorder that is associated with an increased risk of type 1 disease,<sup>4</sup> is characterized by activating germline mutations of *MET*.<sup>5</sup> Somatic *MET* mutations occur in 13 to 15% of nonhereditary papillary renal-cell carcinomas.<sup>6,7</sup> The hereditary leiomyomatosis and renal-cell cancer syndrome, which confers a predisposition to an aggressive form of type 2 papillary renal-cell carcinoma,<sup>8,9</sup> is caused by germline mutation of the gene encoding fumarate hydratase (*FH*), an enzyme of the tricarboxylic acid cycle.<sup>10</sup> These aggressive tumors are characterized by increased oxidative stress<sup>11</sup> and activation of the NRF2–antioxidant response element (ARE) pathway.<sup>12</sup> Mutations in the genes that regulate the NRF2–ARE pathway, such as *CUL3* and *NFE2L2* (which encodes NRF2), have also been observed in sporadic papillary renal-cell carcinoma.<sup>13</sup>

We performed an integrative genomic analysis of 161 papillary renal-cell carcinoma tumors

to provide molecular insights into tumor classification, inform clinical recommendations, and suggest paths to the development of mechanistically based therapies.

## METHODS

### PATIENTS

Tumors were selected from 161 patients. Pathological review was performed to classify the tumors as type 1, type 2, or uncharacterized papillary renal-cell carcinoma (see the Experimental Procedures section in Supplementary Appendix 1, available with the full text of this article at NEJM.org). The clinical and genetic characteristics of these patients are described in Supplementary Appendix 2.

### ANALYTIC PLATFORMS

We performed whole-exome sequencing and analyses to determine copy number, microRNA and messenger RNA (mRNA) expression, protein expression, and DNA methylation at CpG sites (Supplementary Appendix 3). Details of all the analyses are available in the Experimental Procedures section in Supplementary Appendix 1. All data sets are available at the Cancer Genome Atlas data portal (<https://tcga-data.nci.nih.gov/tcga>).

## RESULTS

### HISTOLOGIC SUBTYPING

Pathological review of the 161 tumors identified 75 type 1 tumors, 60 type 2 tumors, and 26 tumors that could not be classified as type 1 or type 2. The type 1 tumors were predominantly stage I, whereas the type 2 tumors were frequently stage III or IV (Fig. S1 in Supplementary Appendix 1); these findings were consistent with those of previous studies.<sup>3,14</sup>

### ROLE OF SOMATIC ALTERATIONS IN MOLECULAR DIFFERENCES BETWEEN TYPE 1 AND TYPE 2 TUMORS

#### *Copy-Number Alterations*

Single-nucleotide-polymorphism array–based profiling of somatic copy-number alterations revealed distinctive patterns across three main tumor subgroups. One subgroup, composed predominantly of type 1 and lower-grade tumors, was defined by multiple chromosomal gains (of at least one complete copy of the chromosome),

including nearly universal gain of chromosomes 7 and 17 and less frequent gain of chromosomes 2, 3, 12, 16, and 20 (Fig. 1A, and Fig. S2 in Supplementary Appendix 1). The other two subgroups were predominantly type 2 tumors; although one of these subgroups had few copy-number alterations, the other was characterized by a high degree of aneuploidy with multiple chromosomal losses, including frequent loss of chromosome 9p, and was associated with poorer survival ( $P < 0.001$ ) (Fig. 1A, and Fig. S2 in Supplementary Appendix 1).

#### *Whole-Exome Sequencing*

Whole-exome sequencing identified 10,380 putative somatic mutations in 157 tumors with an average of 1.45 nonsilent mutations per megabase (see the Experimental Procedures section in Supplementary Appendix 1). An initial screen for significantly mutated genes with  $q$  values of less than 0.1 ( $q$  values range from 0.0 to 1.0), with the use of MutSigCV, version 2.0, identified five such genes (*MET*, *SETD2*, *NF2*, *KDM6A*, and *SMARCB1*) that were recurrently mutated in papillary renal-cell carcinoma, representing 24% of cases (Fig. 1B). Further analysis, performed with restriction of multiple hypothesis testing to genes previously associated with cancer in the PanCan21 data set,<sup>15</sup> identified six additional significantly mutated genes (*FAT1*, *BAP1*, *PBRM1*, *STAG2*, *NFE2L2*, and *TP53*), with 36% of cases showing mutation of at least one of these genes (Fig. 1B). Mutation of these significantly mutated genes showed no evidence of subclonality (Supplementary Appendix 4).

#### *Hippo and Chromatin Modifier Pathways*

Several significantly mutated genes in papillary renal-cell carcinoma are components of well-known cancer-associated pathways or complexes, including *NF2* in the Hippo signaling pathway, *SMARCB1* and *PBRM1* in the SWI/SNF complex, and *SETD2*, *KDM6A*, and *BAP1* in several chromatin modifier pathways. Assessment of genes in these pathways (Supplementary Appendix 5) showed a high number of mutations in both type 1 and type 2 tumors involving the SWI/SNF complex (20% and 27%, respectively), chromatin modifier pathways (35% and 38%, respectively), and the Hippo signaling pathway (3% and 10%, respectively) (Fig. 1C).

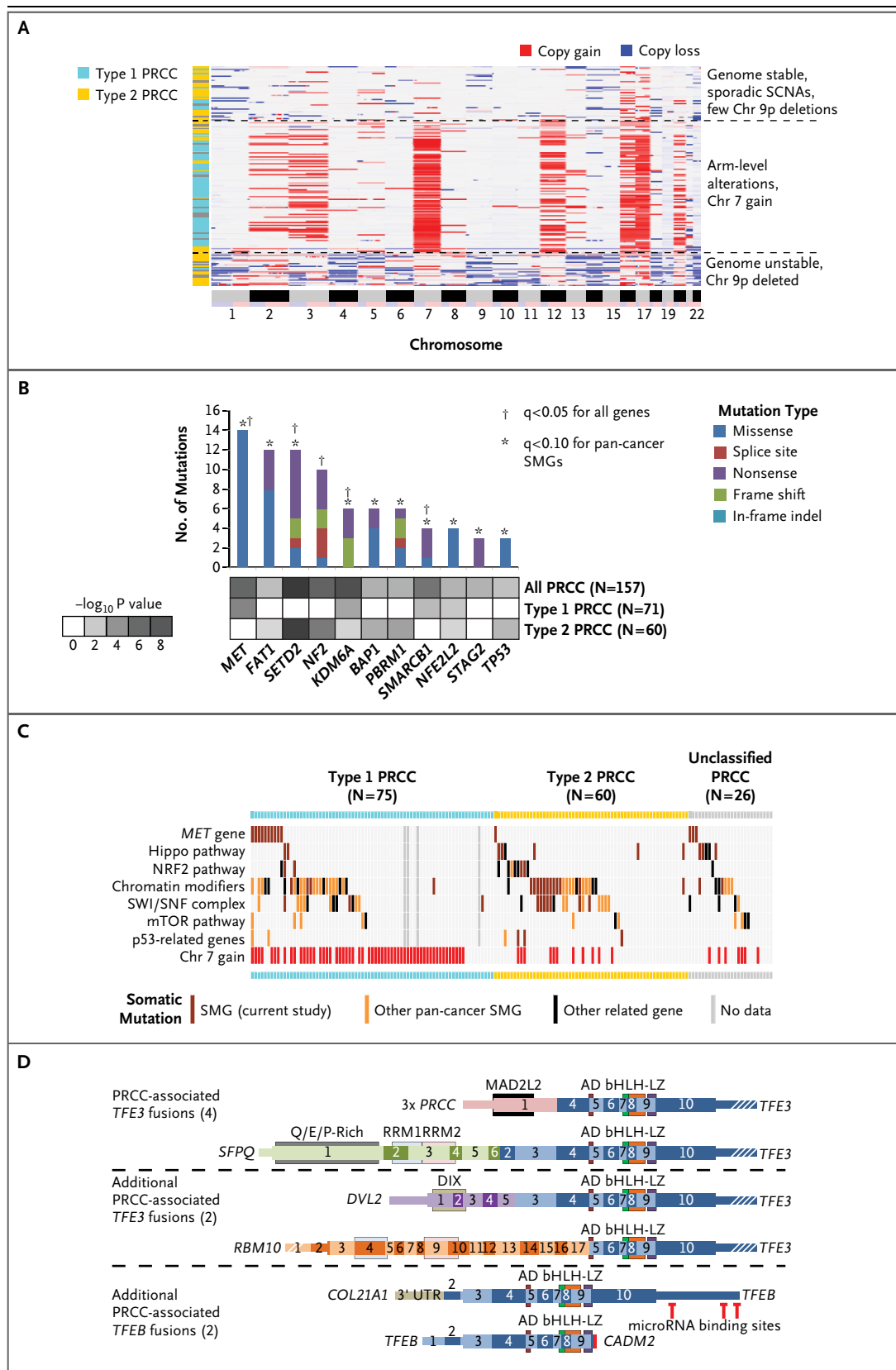
#### *TFE3 and TFEB Gene Fusions*

Gene fusions involving *TFE3* or *TFEB* have previously been associated with papillary renal-cell carcinoma (reviewed in Kauffman et al.<sup>16</sup>). We identified gene fusions in 17 tumors (10.6%), including 8 involving *TFE3* or *TFEB* (Supplementary Appendix 6). Four of the *TFE3* fusions involved known fusion partners, *PRCC* and *SFPQ*, and 2 involved novel fusion partners, *RBM10* and *DVL2* (Fig. 1D). The tumors with *TFE3* fusions showed varying degrees of increased mRNA expression for known *TFE3* transcriptional targets, including *CTSK*, *BIRC7*, *DIAPH1*, and *HIF1A* (Fig. S3 in Supplementary Appendix 1). The two *TFEB* fusions involved novel fusion partners, *COL21A1* and *CADM2*, with the *COL21A1*-*TFEB* fusion resulting in a construct similar to the known *MALAT1*-*TFEB* fusions<sup>16</sup> and the *TFEB*-*CADM2* fusion resulting in a novel truncated version of *TFEB* that had lost several microRNA binding sites (Fig. 1D). The tumors with *TFEB* fusions showed high mRNA expression of the *TFEB* transcription factor and a known target gene, *CTSK* (Fig. S4 in Supplementary Appendix 1). Seven of the fusions involving *TFE3* or *TFEB* were identified in the type 2 tumors (7 of 60 [12%]).

#### **ALTERATIONS SPECIFIC TO TYPES OF PAPILLARY RENAL-CELL CARCINOMA**

##### *MET Mutation in Type 1 Tumors*

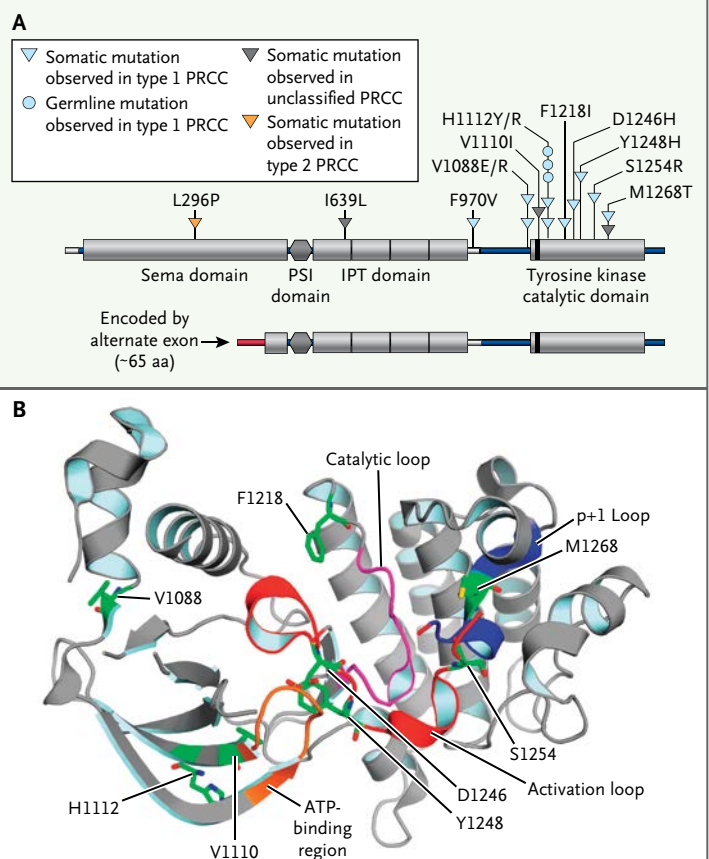
We found mutation of *MET* in 17 tumors, including germline mutation in 3 tumors. A total of 14 of the 17 *MET* mutations were in the tyrosine kinase domain, and 13 of these mutations were observed in type 1 tumors (17% of the 75 type 1 tumors) (Fig. 2A and 2B). In addition, an alternate *MET* RNA transcript that replaces canonical exons 1 and 2 with a novel exon 1 spliced to canonical exon 3 (Fig. 2A) was identified in 8 tumors (4 type 1 tumors, 3 type 2 tumors, and 1 unclassified tumor). This isoform represented the majority of transcripts in 2 tumors and a fraction in the remaining 6 tumors and was recently observed to produce a stable, shortened protein in gastric-cancer cell lines (Fig. S5A in Supplementary Appendix 1).<sup>19</sup> Exons 1 and 2 of *MET* encode the ligand-binding domain of hepatocyte growth factor receptor; this isoform, analogous to the epidermal growth factor receptor variant III isoform,<sup>20</sup> may result in ligand-independent *MET* activation. In addition, gene



**Figure 1 (facing page). Somatic Alterations in Papillary Renal-Cell Carcinoma and Molecular Differences between Type 1 and Type 2 Cancers.**

Unsupervised clustering of DNA copy profiles of 161 papillary renal-cell carcinomas (PRCCs) (Panel A) revealed three molecular subtypes, one of which was highly enriched for type 1 tumors and the other two for type 2 tumors. SCNA denotes somatic copy-number alterations. Significantly mutated genes (SMGs) in PRCC (Panel B) were determined by considering all genes ( $q < 0.1$  [range, 0.0 to 1.0]) or focusing on the set of 260 genes previously implicated in cancer by large-scale, pan-cancer exome analyses<sup>15</sup> ( $q < 0.1$ ). P values were calculated with the MutSigCV algorithm, version 2.0. A pathway-centric view of gene mutations in PRCC (Panel C) shows key pathways and genes implicated in cancer, either in the current study or elsewhere.<sup>15</sup> The tumors were classified according to histologic type (from left to right) and according to gene or pathway altered (from top to bottom). Pathways and genes represented include *MET*, the Hippo pathway (*NF2*, *SAV1*, and *WWC1*), the NRF2 pathway (*NFE2L2*, *KEAP1*, *CUL3*, *SIRT1*, and *FH*), chromatin modification (*CREBBP*, *DOT1L*, *EHMT1/2*, *EP300*, *EZH1/2*, *KAT2A/B*, *KDM1A/B*, *KDM4A/B*, *KDM5A/B/C*, *KDM6A/B*, *MLL1/2/3/4/5*, *NSD1*, *SETD2*, *SMYD4*, and *SRAP*), the SWI/SNF complex (*ACTB*, *ACTL6A/B*, *ARID1A/B*, *ARID2*, *BCL6A/B/C*, *BCL11A/B*, *BRD7/9*, *DPF1/2/3*, *PHF10*, *PBRM1*, *SMARCA2/4*, *SMARCB1*, *SMARCC1/2*, *SMARCD1/2/3*, and *SMARCE1*), the mammalian target of rapamycin (mTOR) pathway (*MTOR*, *PIK3CA*, *PTEN*, *STK11*, *TSC1*, and *TSC2*), and the p53 pathway (*ATM*, *CDKN1A*, *CDKN2A*, *FBXW7*, *RB1*, and *TP53*). Fusion gene analysis (Panel D) identified *TFE3* or *TFEB* fusions in eight PRCC tumors, including two novel gene-fusion partners for *TFE3* (*DVL2* and *RBM10*) and two novel gene-fusion partners for *TFEB* (*COL21A1* and *CADM2*). Schematic versions of these fusions show the exons and functional domains that are present in the different gene fusions and the position of potential microRNA binding sites in *TFEB*. The retained exons of *TFE3* or *TFEB* are colored in shades of blue. Thin regions represent noncoding sequence, thick regions represent the translated reading frame, and white strips indicate that the region is no longer to scale. AD denotes strong transcription activation domain, bHLH basic helix-loop-helix domain, DIX dishevelled and axin domain, LZ leucine zipper domain, MAD2L2 mitotic arrest deficient-like 2 interaction domain, and RRM RNA-recognition motif.

fusions involving *MET* were observed in 3 tumors (Supplementary Appendix 6). Levels of *MET* mRNA expression and of protein phosphorylation (pY1235) were significantly higher in type 1 tumors than in type 2 tumors ( $P < 1 \times 10^{-9}$  and  $P = 0.007$ , respectively, by t-test) (Fig. S5B in Supplementary Appendix 1) — a finding potentially driven in part by trisomy of chromosome 7



**Figure 2. Alterations in Papillary Renal-Cell Carcinoma Involving the *MET* Oncogene.**

Panel A is a schematic representation of somatic mutations in *MET*, along with germline variant H1112R, which was previously implicated in hereditary papillary renal-cell carcinoma,<sup>17</sup> and the novel RNA transcript variant of *MET* lacking the canonical exons 1 and 2 but containing a novel exon 1 that splices to the canonical exon 3. IPT denotes immunoglobulin-like, plexins, and transcription factors, and PSI plexins, semaphorins, and integrins. Panel B shows the crystal structure for the *MET* tyrosine kinase catalytic domain (RCSB-PDB 3I5 N<sup>18</sup>), on which are mapped the residues that are altered in papillary renal-cell carcinoma. All numbering of amino acids is based on the *MET* protein sequences.

in type 1 tumors. Altered *MET* status (defined as mutation, splice variant, or gene fusion) or increased chromosome 7 copy number (which encodes *MET* but may also involve other genes) was identified in 81% of type 1 papillary renal-cell carcinomas. Analysis by means of Genomic Identification of Significant Targets in Cancer (GISTIC), version 2.0, determined that the loss of 1p36 observed in 18 papillary renal-cell carcinomas (11.2%) included the candidate tumor suppressor *ERF1*, a negative regulator of *EGFR*



(Fig. S6 in Supplementary Appendix 1). Deletions of 1p36 co-occurred significantly with gain of chromosome 7 and *EGFR* amplification ( $P=0.02$  by Fisher's exact test).

#### *CDKN2A Mutation in Type 2 Tumors*

Analysis by GISTIC, version 2.0, identified focal loss of 9p21 in 13 papillary renal-cell carcinomas (8.1%), resulting in loss of *CDKN2A* (Fig. S7A in Supplementary Appendix 1). We found mutation or promoter hypermethylation of *CDKN2A* in 11 tumors (Fig. S7B in Supplementary Appendix 1), including 3 of the tumors with focal loss of 9p21, resulting in 21 tumors (13.0%) defined as having *CDKN2A* alteration (Fig. S7C in Supplementary Appendix 1). *CDKN2A* alteration was strongly associated with type 2 histologic features, with 25% of type 2 tumors (15 of 60) showing alterations. *CDKN2A*-altered tumors showed both increased levels of phosphorylated retinoblastoma protein (Rb) and increased expression of cell-cycle-related genes, findings consistent with the predicted consequences of *CDKN2A* loss (Fig. S7D in Supplementary Appendix 1). In a univariate analysis, patients with *CDKN2A*-altered tumors had a significantly lower rate of overall survival than those without *CDKN2A*-altered tumors ( $P<1\times 10^{-10}$ ) (Fig. S7E in Supplementary Appendix 1). The findings were similar when the analysis was limited to patients with type 2 tumors ( $P<0.001$ ) (Fig. S7F in Supplementary Appendix 1). In addition, increased expression of microRNA miR-10b-5p correlated with decreased expression of its target, *CDKN2A* (Fig. S8 in Supplementary Appendix 1).

#### *SETD2, BAP1, and PBRM1 Mutation in Type 2 Tumors*

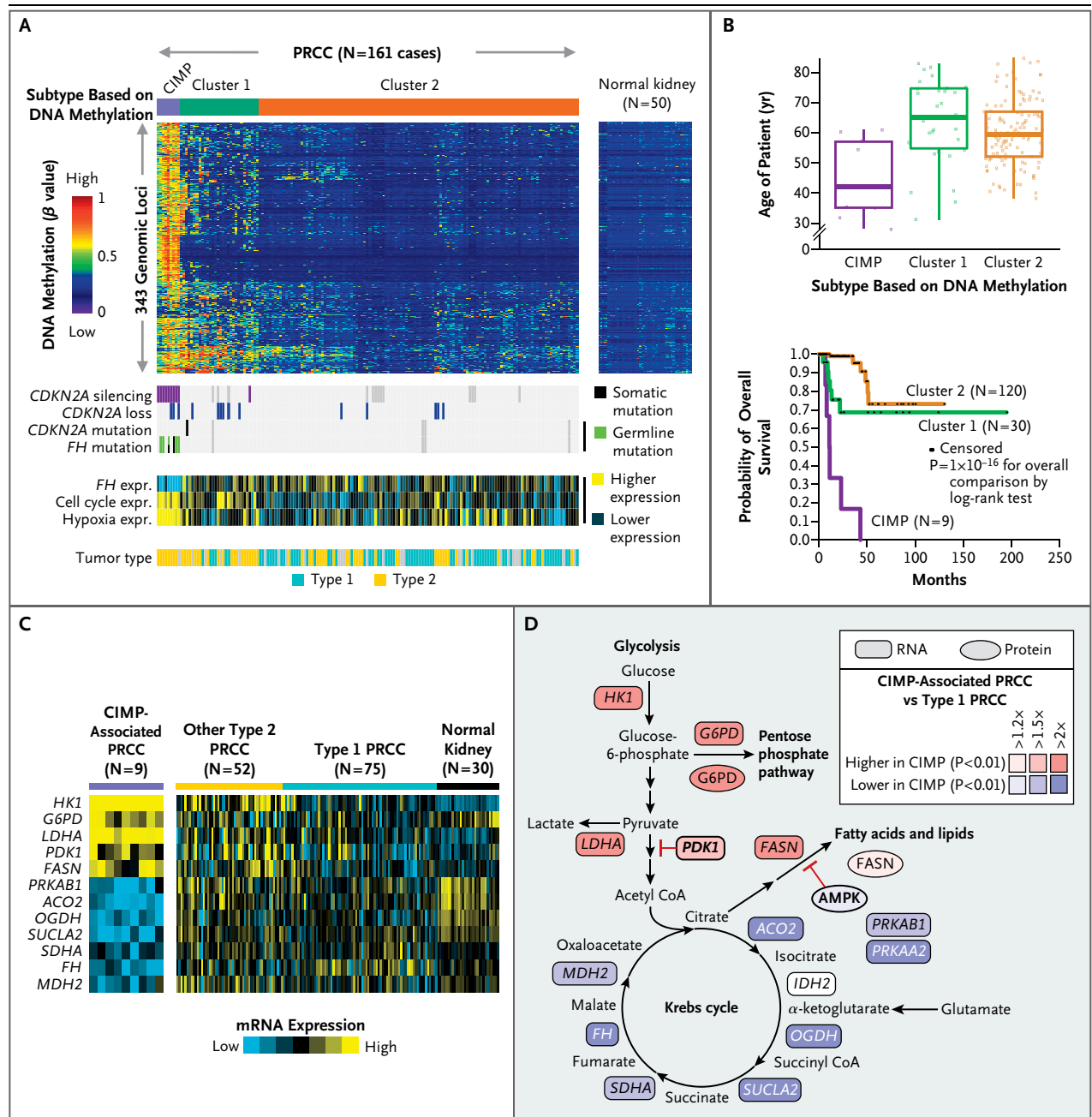
Type 2 tumors were associated with mutations in the chromatin-modifying genes *SETD2*, *BAP1*, and *PBRM1*, which are frequently mutated in clear-cell kidney tumors in combination with loss of chromosome 3p.<sup>21</sup> Mutations of *BAP1* and *PBRM1* were mutually exclusive, but *PBRM1* mutations were frequently concurrent with *SETD2* mutations (Fig. S9 in Supplementary Appendix 1). Although loss of chromosome 3p was also associated with type 2 papillary renal-cell carcinoma, only 3 of 13 type 2 tumors with *SETD2*, *BAP1*, or *PBRM1* mutation showed such loss, and no promoter hypermethylation was observed (Fig. S9 in Supplementary Appendix 1).

#### *CpG Island Methylator Phenotype (CIMP) in Type 2 Tumors*

Nine tumors (5.6%) had increased DNA methylation at loci that were unmethylated in matched normal tissue. This represents a novel kidney-associated CIMP<sup>22</sup> that included universal hypermethylation of the *CDKN2A* promoter (Fig. 3A). Eight of the nine tumors were type 2 papillary renal-cell carcinomas. In five tumors, we found germline or somatic mutation of *FH* (56%). We found decreased expression of *FH* mRNA and increased expression of genes associated with cell-cycle progression and response to hypoxia in all nine tumors (Fig. 3A). Patients with CIMP-associated tumors were younger at the time of presentation and had a lower probability of overall survival than other patients with papillary renal-cell carcinoma (Fig. 3B). Fumarate hydratase-deficient type 2 tumors in patients with the hereditary leiomyomatosis and renal-cell cancer syndrome are characterized by a Warburg-like metabolic shift to glycolysis-dependent metabolism and an increased expression of hypoxia-related genes.<sup>25,26</sup> Similarly, CIMP-associated tumors showed increased expression of key genes involved in glycolysis (*HK1*, *LDHA*, and *PDK1*), the pentose phosphate pathway (*G6PD*), and fatty-acid synthesis (*FASN*) (Fig. 3C, and Fig. S10 in Supplementary Appendix 1). In addition, there was decreased expression of the majority of genes involved in the Krebs cycle and the adenosine monophosphate-activated protein kinase (AMPK) complex, a suppressor of fatty-acid synthesis (Fig. 3D). Data on the expression of proteins G6PD, *FASN*, and AMPK correlated with the data on mRNA expression (Fig. 3D).

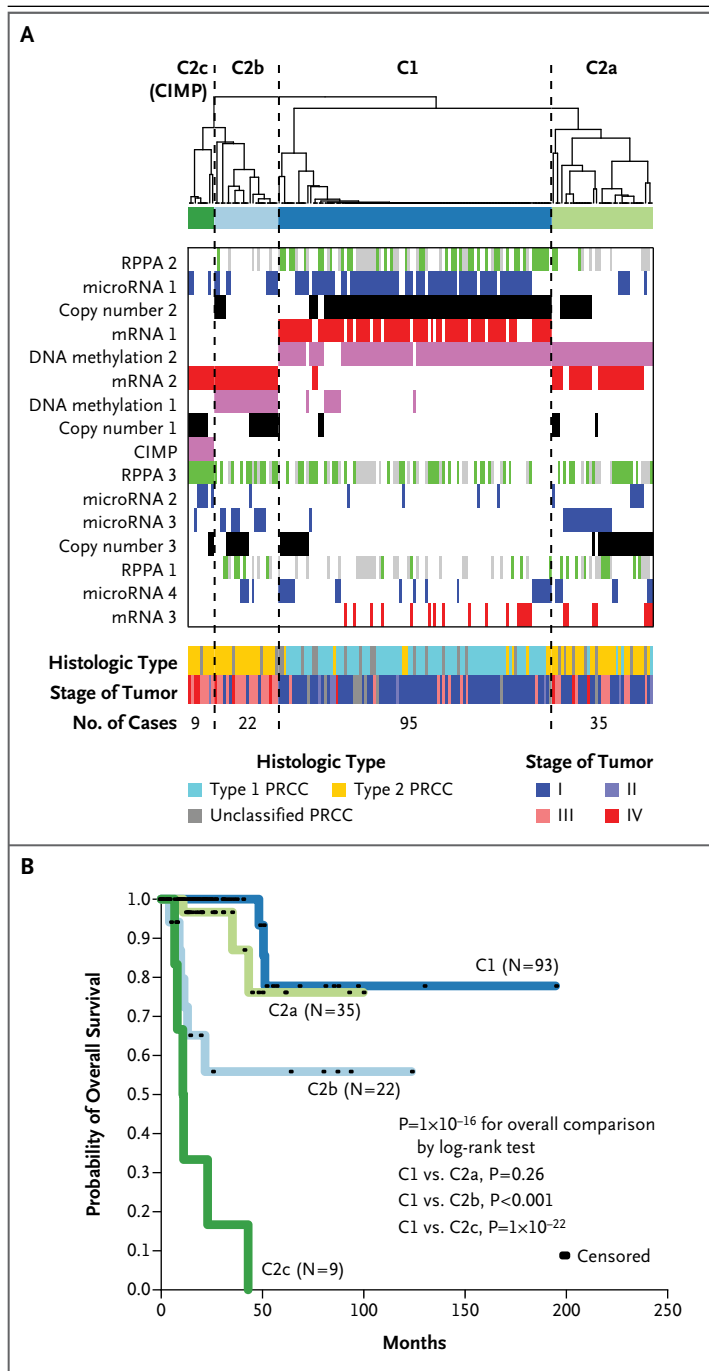
#### **IDENTIFICATION OF PAPILLARY RENAL-CELL CARCINOMA SUBGROUPS BY MULTIPLATFORM ANALYSIS** *Cluster-of-Clusters Analysis*

As was the case with the copy-number analysis and DNA-methylation analysis, the profiles of mRNA expression and microRNA expression and the data on protein expression clustered the cases of papillary renal-cell carcinoma into separate groups with distinct overall outcomes (Fig. S11, S12, and S13 in Supplementary Appendix 1). The five data types were combined to perform a cluster-of-clusters analysis<sup>27,28</sup> that identified four tumor clusters: C1 (enriched for type 1 tumors), C2a and C2b (both enriched for type 2 tumors), and C2c (consisting solely of



**Figure 3. A Subgroup of Papillary Renal-Cell Carcinoma That Manifests a CpG Island Methylator Phenotype (CIMP).**

As depicted in Panel A, molecular subtyping by means of a DNA methylation platform revealed three subtypes of papillary renal-cell carcinoma (PRCC), one of which showed widespread DNA hypermethylation patterns characteristic of CIMP-associated tumors (the other subtypes are identified as cluster 1 and cluster 2). Corresponding data tracks highlight molecular features associated with CIMP tumors (nine cases), including *CDKN2A* silencing, germline or somatic mutations of *FH*, type 2 histologic status, and expression of both cell-cycle-related genes<sup>23</sup> and hypoxia-related genes.<sup>24</sup> Panel B shows differences in patient age and overall survival among the three subtypes. Data on survival were not available for two patients in the cluster 2 group. Panel C shows differential messenger RNA (mRNA) expression patterns for key genes involved in metabolism among CIMP-associated PRCC, type 1 PRCC, non-CIMP-associated type 2 PRCC, and normal kidney. Panel D shows differential expression patterns of CIMP-associated tumors versus type 1 tumors in metabolism-related pathways, with a focus on gene-expression and protein-expression patterns previously associated with Warburg-like effects in kidney cancer.<sup>21</sup> P values were calculated with the use of a t-test.



**Figure 4. Multiplatform-Based Subtype Discovery in Papillary Renal-Cell Carcinoma.**

As shown in Panel A, integration of subtype classifications from five genomic data platforms with the use of a cluster-of-clusters analysis identified four major groups of papillary renal-cell carcinoma: C1 (enriched for type 1), C2a and C2b (enriched for type 2), and C2c (representing the CIMP-associated papillary renal-cell carcinomas). The heat map (center of panel) displays the subtypes defined independently by DNA methylation (pink), chromosomal copy number (black), microRNA expression (blue), mRNA expression (red), and protein (RPPA) expression (green); samples with missing data for protein expression are shown in gray. Clinical features associated with the multiplatform-based subtypes are also shown. Panel B shows differences in overall survival according to subtype. Data on survival were not available for two patients in the C1 group.

cell carcinoma and was associated with an early stage of tumor development and DNA methylation cluster 2. Cluster C2b consisted exclusively of type 2 and unclassified papillary renal-cell carcinomas and was associated with DNA methylation cluster 1, a later stage of tumor development (stage III or IV), and mutation of *SETD2*. The CIMP-associated tumor subtype that was observed previously in DNA-methylation analysis was preserved as cluster C2c. Patients with cluster C1 or cluster C2a tumors had the highest probability of overall survival, patients with cluster C2b tumors had a lower probability, and patients with cluster C2c tumors had the lowest probability (Fig. 4B).

#### *NRF2-ARE Pathway in Type 2 Tumors*

Pathway analysis was performed to compare the microRNA and mRNA signatures of type 1 tumors with those of type 2 tumors (Fig. S15, S16, and S17 in Supplementary Appendix 1, and Supplementary Appendixes 7, 8, and 9), and data on mRNA expression highlighted the NRF2-ARE pathway as a distinguishing feature of type 2 tumors (Fig. S17A in Supplementary Appendix 1). Expression of *NQO1*, a gene activated by the NRF2-ARE pathway,<sup>29</sup> was lowest in cluster C1, intermediate in clusters C2a and C2b, and highest in the CIMP cluster C2c ( $P=1 \times 10^{-18}$  by analysis of variance) (Fig. S18A in Supplementary Appendix 1), and increased *NQO1* expression was associated with decreased survival ( $P=0.001$ ) (Fig. S18C in Supplementary Appendix 1). These findings are consistent with those of studies showing increased activation of the NRF2-ARE

CIMP-associated papillary renal-cell carcinoma) (Fig. 4A).

Cluster C1 was predominantly type 1 papillary renal-cell carcinoma and was strongly associated with gain of chromosome 7, *MET* mutation, mRNA cluster 1, and an early stage of tumor development (stage I or II) (Fig. 4A, and Fig. S14 in Supplementary Appendix 1). Cluster C2a was predominantly type 2 papillary renal-



pathway in type 2 tumors and mutations in NRF2-ARE pathway genes (*NFE2L2*, *CUL3*, *KEAP1*, and *SIRT1*).<sup>12,13</sup> Four *NFE2L2* (*NRF2*) mutations in known activating hotspots were identified, as well as mutations in both *CUL3* (five mutations) and *KEAP1* (one). These mutations in *NFE2L2*, *CUL3*, and *KEAP1* correlated with high levels of *NQO1* expression ( $P < 1 \times 10^{-6}$  by t-test) but did not solely account for the observed differences in *NQO1* expression among subtypes (Fig. S18A in Supplementary Appendix 1).

#### INTEGRATED ANALYSIS OF LOW-FREQUENCY CANDIDATE DRIVER MUTATIONS

Some tumors (most relatively small) lacked high-confidence candidate cancer-driving events. Manual pathway analysis identified candidate driver mutations in known cancer-associated genes, such as *PTEN*, *NRAS*, *KRAS*, *TP53*, *TSC2*, and those in the *MLL* and *ARID* families, in an additional 27% of the cases (Fig. S19A in Supplementary Appendix 1, and Supplementary Appendix 10). For the remaining 37 tumors (23%), low-frequency somatic events in genes identified by HotNet2 analysis (Fig. S19 in Supplementary Appendix 1) or associated with cancer in either the PanCan21 data set<sup>15</sup> or the Catalogue of Somatic Mutations in Cancer database were proposed as potential drivers and are listed in Supplementary Appendix 10. In comparison with the tumors with candidate cancer-driving events, the remaining 37 papillary renal-cell carcinomas showed a higher percentage of type 1 tumors (26 of 37 [70%]) ( $P = 0.001$  by Fisher's exact test), and most (21 of 26 [81%]) showed a gain of chromosome 7, which includes *MET*. This gain of chromosome 7, which is seen in a number of tumors (e.g., Wilms' tumor and papillary thyroid cancer), could be considered a driver event, but it does not identify a specific driver. Although gain of chromosome 7 was associated with increased *MET* expression in papillary renal-cell carcinoma ( $P < 0.001$  by two-factor analysis of variance) (Fig. S20 in Supplementary Appendix 1), other potential driver genes on chromosome 7, such as *EGFR*, could influence tumorigenesis.

#### DISCUSSION

We used a comprehensive genomics approach to characterize the biologic foundation of papillary renal-cell carcinoma and found that type 1 and

type 2 papillary renal-cell carcinoma are distinctly different diseases and that type 2 papillary renal-cell carcinoma is a heterogeneous disease with multiple distinct subgroups. Common driver mutations among the different subtypes were relatively rare, as had been observed in two recent studies.<sup>7,30</sup> Molecular and phenotypic differences between type 1 and type 2 papillary renal-cell carcinoma were reflected in individual and combined analyses of various data platforms. The usefulness of *CDKN2A* alterations as an independent prognostic marker associated with type 2 tumors requires validation. This study suggests that gene fusions involving *TFE3* or *TFEB* are underappreciated in type 2 tumors in adults and should be considered in any patient with type 2 disease. Although papillary renal-cell carcinomas with fusions involving *TFE3* or *TFEB* are generally considered to be diseases of children and young adults,<sup>16</sup> the mean age in our study was 52 years, and we found tumors with *TFEB* fusions in patients 64 and 71 years of age.

The most distinct of the three type 2 subgroups was the subgroup defined by the CIMP, which was associated with the worst overall survival. CIMP hypermethylation patterns have been observed in a number of other cancer subtypes, including glioblastoma,<sup>31</sup> lung adenocarcinoma,<sup>32</sup> and gastric adenocarcinoma.<sup>33</sup> The CIMP-associated tumors showed low levels of *FH* mRNA expression, and five had germline or somatic mutation of *FH*. Germline mutation of *FH* has been observed in the aggressive type 2 tumor associated with the hereditary leiomyomatosis and renal-cell cancer syndrome.<sup>9,34</sup> In this syndrome, the high levels of fumarate accumulating from loss of fumarate hydratase enzyme activity result in impaired function of enzymes such as the TET family of enzymes, which play a role in maintaining appropriate DNA methylation within the genome.<sup>35</sup> The subgrouping of type 2 tumors according to molecular features and the presence of specific subsets of type 2 tumors, such as those with *TFE3* fusions or CIMP, suggest that substratification of type 2 papillary renal-cell carcinoma according to specific molecular markers may allow more accurate diagnosis that could lead to the development of mechanistic, disease-specific targeted therapies.

This classification of papillary renal-cell carcinoma could potentially have a substantial effect on clinical and therapeutic management and on

the design of clinical trials. Alteration of *MET* or gain of chromosome 7 was observed in a large percentage (81%) of type 1 tumors. Antitumor activity of an agent targeting the *MET* and *VEGFR2* pathways has been shown in a phase 2 trial involving patients with papillary renal-cell carcinoma, with a particularly high response rate among patients who had tumors with *MET* mutations.<sup>36</sup> Mutation of the Hippo pathway tumor suppressor, *NF2*, was observed in a number of papillary renal-cell carcinomas. This pathway has been targeted in other cancers with agents such as dasatinib, an inhibitor of the *YES1* kinase that interacts with the *YAP* transcription factor that is up-regulated with Hippo pathway dysregulation.<sup>37</sup> The *CIMP*-associated tumors showed a Warburg-like metabolic shift, similar to that observed in fumarate hydratase-deficient tumors in patients with the hereditary leiomyomatosis and renal-cell cancer syndrome.<sup>11,25,26</sup> A clinical trial targeting this metabolic shift in papillary renal-cell carcinoma is currently under way (ClinicalTrials.gov number, NCT01130519). Increased expression of the *NRF2-ARE* pathway

has been observed in both hereditary and sporadic type 2 papillary renal-cell carcinomas.<sup>12</sup> Immunohistochemical analysis for *NQO1* could provide a valuable marker of activation of the *NRF2-ARE* pathway. Currently, there is intense interest in the *NRF2-ARE* pathway in cancer,<sup>38</sup> and novel strategies have recently been developed to target this pathway.<sup>39</sup>

The identification of altered genes and pathways provides a comprehensive foundation for an understanding of the molecular basis of papillary renal-cell carcinoma. This refined classification more accurately reflects the genotypic and phenotypic differences among the various types of these tumors and may lead to more appropriate clinical management and development of more effective forms of therapy.

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#### APPENDIX

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