MET Is a Potential Target across All Papillary Renal Cell Carcinomas: Result from a Large Molecular Study of pRCC with CGH Array and Matching Gene Expression Array

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Introduction

Papillary renal cell carcinomas (pRCC) are the second most common histologic subtype of renal carcinoma (1, 2).

Purpose: Papillary renal cell carcinomas (pRCC) are the most common nonclear cell RCC subtype. Germline mutations of the MET oncogene at 7q31 have been detected in patients with hereditary type I pRCC and in 13% of sporadic type I pRCC. Recent report of MET inhibition strengthened the role of c-Met inhibition across pRCC.

Experimental Design: We collected 220 frozen samples of sporadic pRCC through the French RCC Network and quality controlled for percentage of malignant cells >70%. Gene expression was assessed on 98 pRCC using human whole-genome Agilent 8 × 60K arrays. Copy number alterations were analyzed using Agilent Human 2 × 400K and 4 × 180K array for type II pRCC and comparative genomic microarray analysis method for type I pRCC. MET gene sequencing was performed on type I pRCC.

Results: MET expression level was high across all pRCC. We identified copy number alterations (gain) in 46% of type II pRCC and in 81% of type I pRCC. Correlation between DNA copy number alterations and mRNA expression level was highly significant. Eleven somatic mutations of MET gene were identified amongst 51 type I pRCC (21.6%), including 4 new mutations. We validated LRRK2 cokinase as highly correlated to MET expression.

Conclusion: The present report expands the role of MET activation as a potential target across all pRCC subtypes. These data support investigating MET inhibitors in pRCC in correlation with MET activation status. Clin Cancer Res; 20(13); 3411–21. ©2014 AACR.

Abstract

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-13-2173
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that MET-triggered pathway implicates several upstream regulators and coreceptors that are physically associated with MET either at the cell surface or intracytoplasmic domain that might consequently induce MET transactivation (15). For instance, leucine-rich repeat kinase 2 (LRRK2) has recently been reported as a required kinase for MET oncogenic signaling in pRCC (20). We therefore investigated the potential impact of both ligand and coactivators required for MET activation in pRCC.

Overall, we aimed at verifying the hypothesis that MET activation can be triggered in pRCC through different mechanisms, such as gene copy number gain alteration alone or in combination with known coactivators upregulation. We believe that this work is relevant to better understand the rationale and to optimize the ongoing development of MET inhibitors in this setting.

Materials and Methods

Collection, assessment of tumor samples, and frozen tissue control

The frozen tissue samples from patients with pRCC who underwent tumor radical surgical resection at eight French centers (St Joseph, Tenon, Foch, Necker in Paris, Gustave Roussy Institute in Villejuif, Henri Mondor in Creteil, Bordeaux and Rennes, France) between 1993 and 2011 were collected. Each diagnostic sample was independently reviewed by two specialized pathologists who were blinded to any clinical patient data. All patients had previously provided written informed consent for tumor collection and analysis. Pathologic data were collected and clinical data were retrospectively retrieved from urological and oncology files in each institution.

All frozen tissues had been stored under quality-controlled condition and retrieved. When collected, each frozen tissue has been controlled for tumor necrosis and percentage of tumor cells of at least 70%.

DNA and RNA extraction

DNA was extracted from frozen tumors using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer’s instructions. Quality and quantity were assessed both by NanoDrop spectrophotometer (Thermo Scientific) and Qubit (Life Technologies). RNA was extracted from frozen samples using Trizol’s protocol (Life Technologies). RNA purity was determined by using the LabOnChip (2100 Bioanalyzer System; Agilent Technologies) before labeling and hybridization.

CGH array profiling

High-resolution oligonucleotide comparative genomic hybridization array (CGH) arrays were performed following standard operating procedures from Agilent Technologies (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis G4410-90010).

DNA was hybridized to Agilent Sure Print G3 Human CGH either to 2 × 400K (G4449A amadid 21850) or to 4 × 180K whole-genome Agilent arrays (G4448A amadid 22060) at the Genomic Unit of the Gustave Roussy Institute,
Villejuif, France. About respectively 2 × 400K and 4 × 180K arrays, for each sample, 1,500 and 500 ng of DNA were fragmented by a double enzymatic digestion (Alu I- Rsa I) and checked with LabOnChip (2100 Bioanalyzer System; Agilent Technologies) before labeling and hybridization. Tumor DNA and control DNA from Promega (Human Genomic DNA Female N° 30742202/male N° 30939901) were labeled by random priming with CY5-dCTPs and CY3-dCTP, respectively, and hybridized at 65°C for 48 or 24 hours depending on slides format at 20 rpm. The chips were scanned on an Agilent G2565BA DNA Microarray Scanner and checked with LabOnChip (2100 Bioanalyzer System; Agilent Technologies). Feature-Extraction software (Agilent Technologies). Feature-Extraction was used for the fluorescence signal acquisition from the scans. Raw data have been submitted to the ArrayExpress database with the accession number E-MTAB-1807.

A log2 intensity ratio of test to reference was used to define relative copy number alterations as subsequently: In a normal situation, the log2 ratio of normal (copy-neutral) clones is log2(2/2) = 0; a single copy loss is log2(1/2) = −1, and a single copy gain is log2(3/2) = 0.58. We defined amplification as a value of log2(5/2) ≥1.5 (meaning 3 additional copies of the gene for a diploid genome).

Gene expression array
Gene expression profiling was done with human whole-genome Agilent 28004, 8 × 60K v1 (G4851A) following standard operating procedures from Agilent Technologies (One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling G4851A). A total of 100 ng of each RNA was used to perform reverse transcription and one-color labeling steps. Amplified and labeled samples were purified using the RNeasy Mini Kit from Qiagen (74106). Purified samples were measured using Nanodrop ND2000 in microarray mode. The labeled RNA was hybridized at 65°C for 17 hours at 10 rpm. Raw data have been submitted to the ArrayExpress database with the accession number E-MTAB-1805.

Data analysis
For gene expression arrays, raw data files from Feature Extraction were imported into R with LIMMA (21), an R package from the Bioconductor project, and processed as follows: gMedianSignal data were imported, controls and replicates expressed as a value of log2 (5/2) was used for the fluorescence signal acquisition from the scans. Raw data have been submitted to the ArrayExpress database with the accession number E-MTAB-1807.

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Quantitative reverse transcriptase PCR analysis
Gene expression was assessed by quantitative reverse transcriptase (RT)-PCR, using total RNA from frozen tumors. The amplification of the cDNA was performed from 1 μg of total RNA with the Maxima cDNA Synthesis Kit (Thermo Scientific). A total of 20 ng cDNA was amplified in a 25-μl RT reaction using SYBR Green PCR master mix (Applied Biosystems) containing 0.3 μmol/L of specific primers. 18S primer sequences have been previously described by Schmittgen and Zakrajsek (23). To analyze Met transcript expression, we designed primers (exon 16 forward: 5' CATGCCGACAAGTGCAGTAT 3', and exon 16 reverse: 5' CACAACCAAATGCCCTCTT 3'). The total reaction was performed on the ABI Prism 7000 Detection System TaqMan (Applied Biosystems). Each sample was analyzed in duplicates in every run. Cycling conditions were as follows: 95°C for 5 minutes, and then 45 cycles of 30 seconds at 95°C and 1 minute at 62°C. Dissociation kinetic analyses of the amplification products were performed to ensure specific amplification by heating and cooling the samples at 95°C for 15 seconds, 60°C for 30 seconds, and 95°C for 15 seconds. MET expression was normalized to the 18S gene expression.

Sequencing and in silico mutation prediction
Mutations in the MET gene were screened by direct sequencing of exons 16 to 19 (primer sequences available upon request). PCR products were first purified using the enzyme ExoSAP-it (GE Healthcare Europe GmbH) for purification and then sequenced with the Big Dye Terminator v3.1 Kit (Applied Biosystems; Life technologies). Resin Sephadex G50 superfine (GE Healthcare Europe GmbH) was used for purification. Sequencing was performed on an ABI 3730 automatic DNA sequencer (Applied Biosystems) in 96-well plates. Variants and mutations were identified by visual inspection of the sequences using the Seqencher 4.10.1 Demo software (Gene Codes, Corporation). The NM_001127500 sequence corresponding to the short isoform (1408 amino acids) was used to determine the identified mutations. The NM_000245 sequence corresponding to the short isoform was used for in silico tools.
Results

Overall, 220 frozen pRCCs were collected at eight French centers. Double pathologic review, including pRCC confirmation and control of percentage of tumor cells >70%, excluded 71 samples (32.3%). Reasons for sample exclusion were mostly insufficient number of tumor cells related to tumor necrosis \((n = 29)\), infiltration by predominant inflammatory cells \((n = 8)\), degradation related to frozen processing \((n = 8)\), or nonspecified causes of insufficient tumor cells \((n = 16)\), but also inadequate pRCC classification \((n = 3)\), inadequate sampling/labeling of nontumor kidney tissue \((n = 7)\). Characteristics of the cohort, including clinical features such as age and gender and pathologic stage and grade, are presented in Table 1.

To assess the various potential mechanisms of increased MET activity and its relevance in pRCC, we investigated the \(MET\) oncogene at (i) expression level, (ii) gene copy number alteration, (iii) screening for mutations, and (iv) ligand-dependent activation and coactivators in our cohort. Overall, all samples included in our cohort and different methods used in this report are presented in Fig. 1.

Gene expression across subtypes

Gene expression of the \(MET\) oncogene in pRCC was analyzed in 98 pRCC samples, including 47 pRCC type I, 45 pRCC type II, and six unclassified pRCC. For comparison purpose, gene expression in the same analysis profile was performed also for 6 ccRCC and 1 chomophobe tumor. For 10 patients, both tumor and normal kidney tissue were processed. \(MET\) expression was significantly higher in all RCC when compared with normal kidney: (fold change = 5.8/3.1/1.8; \(P = 1.4 \times 10^{-14}/P = 1.8 \times 10^{-4}/P = 0.1\) respectively for pRCC I/pRCC II/ccRCC when compared with normal kidney expression; Fig. 2A). \(MET\) expression was significantly higher in type I versus type II \((P = 1.23 \times 10^{-3})\) and both pRCC expression levels were higher than ccRCC.

To confirm our results from microarray studies, \(MET\) expression was verified by an independent gene expression profiling method. Using real-time qRT-PCR, \(MET\) expression level was analyzed in 36 pRCC I, 39 pRCC II, and 5 ccRCC (Fig. 2B). Upon analysis, the expression level of the target gene was computed relative to the expression level of the 18S reference gene. The results of real-time qRT-PCR experiments demonstrated that the relative expressions of the \(MET\) gene were significantly higher in RCC samples (fold change = 6.4/3.8/3.1; \(P = 9.64 \times 10^{-3}/P = 4.05 \times 10^{-4}/P = 9.99 \times 10^{-3}\) respectively for pRCC I/pRCCII/ccRCC) in comparison with normal kidney.

Copy number alterations

To further identify whether \(MET\) RNA expression was related to copy number alteration or other mechanisms, we performed a correlation analysis between copy number alteration from the CGH array results available from 37 type II pRCC and matching gene expression results. The correlation between copy number alterations of the \(MET\) gene located on 7q31 and gene expression was significant (correlation coefficient \(= 0.6; P = 8.2 \times 10^{-4}\); Fig. 3A and Supplementary Fig. S2). Seventeen tumors (46%) presented a gain in \(MET\) gene with a median value of \(\log_2\) (ratio) = 0.41 (0.11–0.83). No amplifications, as defined per a stringent threshold of \(\log_2\) (ratio) > 1.5, of \(MET\) gene were observed.

Table 1. Characteristics of the 220 pRCC population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall population (n = 220)</th>
<th>Type I (n = 85)</th>
<th>Type II (n = 80)</th>
<th>Non-specified (n = 54)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>175</td>
<td>64</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>21</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Median age at diagnosis year (min-max)</td>
<td>61 (26–86)</td>
<td>21</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1/pT2</td>
<td>129</td>
<td>62</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>pT3/pT4</td>
<td>60</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>25 (out of 108 informative patients)</td>
<td>6/44 (41 ukn)</td>
<td>18/40 (20 ukn)</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 + grade 2</td>
<td>9 (72) 81</td>
<td>4 (54) 78</td>
<td>0 (8) 8</td>
<td>10</td>
</tr>
<tr>
<td>Grade 3 + grade 4</td>
<td>100 (26) 126</td>
<td>22 (1) 28</td>
<td>50 (19) 69</td>
<td>90</td>
</tr>
</tbody>
</table>

Abbreviation: ukn, unknown.

*pRCC reclassified to chRCC.
detected. Chromosome 7 alterations observed in the 37 CGH profiles are represented on Fig. 3B. Furthermore, to assess the copy number alteration frequency observed in type I pRCC, we performed a comparative genomic microarray analysis (CGMA; predefined $P$ value $5 \times 10^{-3}$ method (24) from the gene expression results of the 47 type I pRCCs and we identified a 81% incidence (40 cases of 47) of 7q copy number alterations (polysomy, gain) in type I pRCC (Fig. 3C).

**MET sequencing**

To determine whether MET activation relies on mutations, exons 16 to 19—corresponding to the intracellular tyrosine kinase domain of MET protein—were sequenced on 51 DNAs from type 1 pRCCs. Eleven missense mutations were identified out of 51 (21.6%) DNAs and confirmed by further sequencing (Table 2). Among these 11 MET mutations, 4 are novel variants: c.3476C>G, p.S1159W and c.3538C>T, p.H1180Y; about the 2 others, they affect the...
same nucleotide and codon: c.3637C>A, p. L1213I and c.3637C>T, p. L1213F. About mutations that were already reported, we detected 4 times the c.3803T>C, p. M1268T. Furthermore, as matched normal DNA was available for 4 tumors, we sequenced them, thus allowing confirmation of the somatic origin of the mutations.

We then performed an in silico analysis of the putative functional consequences of the 11 mutations. As shown in Table 2, all these missense mutations are predicted to be damaging by the two tools used.

To further investigate the relevance of the 4 newly identified mutations, we performed a correlation study of each mutated sample in comparison with the other mutated samples using the gene expression available dataset and focusing on the 36 genes involved in the MET pathway currently defined in biocarta_MET_pathway (as of access at http://www.broadinstitute.org/gsea/msigdb/cards/BIOCARTA_MET_PATHWAY on July 28, 2013). Only 10 of 11 mutated samples have been analyzed in Gene Expression data set. We performed a “one-to-one” comparison of each sample level of expression of the 36 genes with each one of the 9 other samples. Figure 4B exhibits both graphic representation and correlation coefficient for each comparison. Correlation coefficients between the 6 previously known mutations ranged from 0.81 to 0.96. Correlation coefficients between each one of the newly identified mutations and the 6 previously described ranged from 0.79 to 0.99. Finally, within the 4 newly diagnosed mutations, the correlation ranged between from 0.89 and 0.94. No sample seemed to be significantly different in terms of MET pathway gene expression level.

The correlation between the samples harboring a MET mutation and the 6 ccRCC samples is presented in Supplementary Fig. S1.

### Coactivators

**Ligand expression.** We analyzed both HGF and HGFAC expressions to identify potential ligand for MET activation.

### Table 2. Mutations identified in the MET oncogene in a series of 51 pRCC type I and in silico analysis

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino acid Long isoform NM_001127500</th>
<th>Amino acid Short isoform NM_000245</th>
<th>Mutation origin</th>
<th>PolyPhen (score)</th>
<th>SIFT (score)</th>
<th>Previous report</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>3328G&gt;A</td>
<td>V1110I</td>
<td>V1092I</td>
<td>ND</td>
<td>Possibly damaging (0.938)</td>
<td>Damaging (0)</td>
<td>(9, 10, 11, 25)</td>
</tr>
<tr>
<td>17</td>
<td>3476C&gt;G</td>
<td>S1159W</td>
<td>S1141W</td>
<td>Somatic</td>
<td>Probably damaging (1)</td>
<td>Damaging (0)</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>3538C&gt;T</td>
<td>H1180Y</td>
<td>H1162Y</td>
<td>ND</td>
<td>Probably damaging (0.999)</td>
<td>Damaging (0)</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>3616G&gt;T</td>
<td>V1206L</td>
<td>V1188L</td>
<td>ND</td>
<td>Probably damaging (0.994)</td>
<td>Damaging (0)</td>
<td>(8, 11)</td>
</tr>
<tr>
<td>18</td>
<td>3637C&gt;A</td>
<td>L1213I</td>
<td>L1195I</td>
<td>Somatic</td>
<td>Probably damaging (0.996)</td>
<td>Damaging (0)</td>
<td>L1213V (8)</td>
</tr>
<tr>
<td>18</td>
<td>3637C&gt;T</td>
<td>L1213F</td>
<td>L1195F</td>
<td>ND</td>
<td>Probably damaging (1)</td>
<td>Damaging (0)</td>
<td>L1213V (8)</td>
</tr>
<tr>
<td>19</td>
<td>3803T&gt;C</td>
<td>M1268T</td>
<td>M1250T</td>
<td>Somatic</td>
<td>Probably damaging (0.999)</td>
<td>Damaging (0)</td>
<td>(8, 10, 11)</td>
</tr>
<tr>
<td>19</td>
<td>3803T&gt;C</td>
<td>M1268T</td>
<td>M1250T</td>
<td>ND</td>
<td>Probably damaging (0.999)</td>
<td>Damaging (0)</td>
<td>(8, 10, 11, 12)</td>
</tr>
<tr>
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<td>3803T&gt;C</td>
<td>M1268T</td>
<td>M1250T</td>
<td>Somatic</td>
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<td>Damaging (0)</td>
<td>(8, 10, 11, 12)</td>
</tr>
<tr>
<td>19</td>
<td>3803T&gt;C</td>
<td>M1268T</td>
<td>M1250T</td>
<td>ND</td>
<td>Probably damaging (0.999)</td>
<td>Damaging (0)</td>
<td>(8, 10, 11, 12)</td>
</tr>
<tr>
<td>19</td>
<td>3742T&gt;C</td>
<td>Y1248H</td>
<td>Y1230H</td>
<td>ND</td>
<td>Probably damaging (0.998)</td>
<td>Damaging (0)</td>
<td>(8)</td>
</tr>
</tbody>
</table>
HGF expression level was very low across pRCC with no significant difference in terms of fold change. Unlike HGF, HGFAC was upregulated in pRCC with median fold change of \( +3.8 \) (\( P = 6.25 \times 10^{-8} \)) and \( +1.9 \) (\( P = 3.03 \times 10^{-2} \)) when compared with normal renal tissue, amongst type I and type II pRCC, respectively. HGF and HGFAC expression levels in correlation with \( \text{MET} \) expression level are presented in Fig. 5A and B.

**Co-required kinase.** We further investigated the relevance of LRRK2 as a cokinase for \( \text{MET} \), reported to be required for \( \text{MET} \) oncogenic signaling in pRCC. Gene expression analysis demonstrated higher expression of LRRK2 RNA across all pRCCs: fold change \( = 7.4/3.6/2.8 \); \( P = 7.38 \times 10^{-11} \); \( P = 5.82 \times 10^{-2} \) for pRCC I/pRCCII/ccRCC, respectively. Expression level was higher in type I than in type II tumors (Fig. 5D). These results were subsequently confirmed on the qRT-PCR performed in 73 pRCC samples (Fig. 5E). Its expression was higher in RCC samples, compared with nontumor tissues (fold changes \( = 6.4/3.3/2.8 \); \( P = 1.70 \times 10^{-3} \); \( P = 4.07 \times 10^{-4} \); \( P = 5.28 \times 10^{-3} \) for pRCC I/pRCCII/ccRCC, respectively).

Finally, we assessed correlation between LRRK2 and \( \text{MET} \) level (Fig. 5C): correlation coefficient \( = 0.63 \); \( P = 1.3 \times 10^{-13} \).

**Discussion**

In the metastatic setting, pRCC prognosis is worse than ccRCC prognosis, especially in type II subpopulation (6, 26, 27). Currently, there is no standard of care for systemic treatment of metastatic pRCC. Progression-free survival observed in first line treatment with current available VEGF receptor tyrosine kinase inhibitor agents and mTOR inhibitors ranges from 2 to 7 months (28–30) with an overall survival being around 1 year. Therefore, there is a need to develop specific targeted agents in pRCC. Knowledge from hereditary syndrome biology and first reports on \( \text{MET} \) inhibitors in this tumor subtype may represent the first breakthrough in terms of therapeutic compound available for pRCC. Choueiri and colleagues reported a phase II study investigating foretinib (16; GSK 1363089 and XL880), a dual \( \text{MET} \) and VEGFR2 inhibitor in patients with pRCC with either \( \text{MET} \) mutation or \( \text{MET} \) gene amplification or no \( \text{MET} \) alteration. Very interestingly, besides substantial benefit in \( \text{MET} \) germline-mutated tumors, median progression-free survival was also promising in both mutated and nonmutated pRCC patients, and furthermore irrespectively of subtypes I and II, which were not assessed in this study.

In our report, we explored several variables about the \( \text{MET} \) gene status to provide a preclinical rationale for targeting \( \text{MET} \) in all subtypes of pRCCs. As any proto-oncogene, malignant transformation occurs when \( \text{MET} \) activity is increased inappropriately and/or constitutively activated. The aim of the present work was to assess the differential \( \text{MET} \) gene status and expression across different pRCC tumors that may lead to \( \text{MET} \) activation using...
combined (i) gene expression, (ii) matching copy number alterations, (iii) gene sequencing, (iv) and finally assessment of potential ligand-dependent or cofactors activation of MET protein.

Our first set of results is about the magnitude of MET RNA upregulation (through gene expression profiling and qRT-PCR validation) across pRCC when compared with ccRCC or normal kidney tissue. This information is consistent with the MET staining that has previously been reported by Gibney and colleagues (31). Indeed, they used automated quantitative analysis to characterize the expression of MET protein levels in 317 RCC tumors including 45 pRCC. Protein expression level was not only higher in RCC tissue than in adjacent paired normal tissue but also significantly higher in papillary than in clear cell subtypes \( (P < 0.0001) \). MET protein expression has been reported in up to 90% of pRCC, including both type I and type II in a pathology report from Choi and colleagues (32).

Copy number alterations were detected in 81% of type I and 46% of type II, where CGH array identified 17 gains and no amplification of MET. Copy number alterations highly correlate with upregulation in RNA level. Interestingly, the first report from Glokhova and colleagues (33) demonstrated that gene copy number increase was associated with upregulation of MET expression. This observation is not limited to kidney cancer: similar correlations between MET gene copy number and protein expression level measured by immunohistochemistry have also been reported in non-small cell lung cancer (34). Previous cytologic reports identified chromosome 7 trisomy as a common feature of pRCC (11). Further reports, using both CGH arrays and SNP arrays, did confirm that either chromosome 7 trisomy or partial gain is more likely to be seen in pRCC (35, 36). Noteworthy, some authors considered the chromosome 7 alterations more likely to be seen in type I pRCC (37), as 17q gain and 9p loss are specific features of type I and II, respectively (38). On the contrary, other reports considered chromosome 7 trisomy as shared by all pRCC subtypes irrespectively of grade, size, and hereditary versus sporadic presentation (39–42). More interestingly, patients with MET mutations, either inherited or somatic, were reported to present allelic imbalanced duplication on 7q of the mutated allele (43, 44), drawing the hypothesis that both events are required for oncogenic transformation in hereditary and somatic MET mutant pRCC. Our results represent the largest CGH array cohort of type II pRCC with copy
number alteration assessment and matching gene expression levels while underlining in both pRCC subtypes the relevance of copy number alteration in relation with gene expression. Interestingly, no amplification was identified in type II pRCC. These results are consistent with penile squamous cell carcinoma where MET expression is detected in 87% of tumors and correlates with polysomy-7 and without gene amplification (45), but are in contrast with various tumor types, such as gastroesophageal, gastric, ovarian, and non–small cell lung cancers (17, 46) where amplifications of the MET gene have been reported.

Genetic evidence that MET plays a crucial role in pRCC came from the identification of families carrying autosomal dominant missense mutations in the tyrosine kinase encoding sequence of MET gene (8–12, 25). Some mutations were subsequently identified in somatic tumors with a 13% incidence (9, 10). We obtained a mutation rate of 21.5% for the MET gene in type I pRCC (11/51), which is higher than in previous studies. As our cohort only includes sporadic cases, we may postulate that these mutations are somatic, as shown for 4 cases. We identified 4 new variants in this study and, interestingly, two are linked to a same position, which is the leucine in 1213, already known to be mutated in a Valine (8). In our cases, the mutations lead to an Isoleucine, which is similar to leucine, and Phenylalanine, which is an aromatic residue probably leading to a different conformation of the tyrosine kinase domain of the HGF receptor. In silico tools were used to evaluate the potential effect of these mutations and because mutated residues belong to conserved domains, we can expect a damaging consequence. We also detected 4 already described mutations including M126ST, which is the most frequently reported mutation (8), suggesting a hot-spot or a position which is highly critical for the protein function and may be selected specifically through tumor progression. We chose to sequence only type I pRCCs, whereas frequently the two types of pRCCs are considered together. For example in COSMIC website, a mutation rate of 4% is reported for the MET gene in 40 pRCCs. However, as we chose to sequence the 4 exons of the tyrosine kinase domain only, our mutation rate might probably be underestimated, even if it is difficult to evaluate the functional consequence of mutations detected in other domains than the tyrosine kinase one.

Our ligand gene expression analysis did not identify HGF upregulation as a potential mechanism for MET activation in pRCC, but it is of interest to point out that MET upregulation was correlated to HGFAC upregulation, therefore suggesting that the serine protease is more relevant than the ligand in this setting of MET activation and signaling.

Looyenga and colleagues (20) identified LRRK2 as a mechanism of signaling cross-talk that promotes MET activation, tumor cell growth, and survival in papillary renal and thyroid carcinomas. We confirmed that LRRK2 is amplified and upregulated across pRCC and highly correlates with MET expression.

There are obviously some limitations in our results. The first main limits of the present report rely on the challenge of investigating the expression and activation of downstream molecules of MET pathways, mainly due to the fact that effectors are highly shared with other growth factor receptors and none may be specifically used as a surrogate of MET activity (AKT, ERK, etc.). The second major concern is the use of primary tumor samples of heterogeneous population (localized, locally advanced, and metastatic tumors). Nevertheless, we believe that this report provides new insights on MET gene status across pRCC.

Conclusion

This report, to our knowledge, is the largest study on both gene expression and cytogenetic analysis of sporadic pRCC; it emphasizes the upregulation of MET gene across pRCC. We confirmed the occurrence of MET mutation in type I pRCC and describe 4 newly identified somatic mutations in MET gene. Copy number analysis identified MET gain as another potential mechanism of increased MET activity relevant in pRCC. Finally, the upregulation of cokinase required for MET signaling has also been identified in pRCC and may therefore not only be a potential predictive marker of MET inhibitors activity but also a therapeutic target in itself.

Disclosure of Potential Conflicts of Interest

L. Albiges is a consultant/advisory board member for Angen, Novartis, Pfizer, and Sanofi. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The authors thank all patients and their families for their contributions to this study, and all the urologists, pathologists, and biobanks from 8 institutions who helped to make this study, especially Rennes Tumorothèque led by Dr. B. Turlin, Necker Hospital Tumorothèque led by Professors N. Brousse, Bordeaux CHU Tumorothèque led by Professor JP Merlio, Tenon hospital Tumorothèque led by Professor P. Callard, Foch Hospital Tumorothèque led by Dr. Y. Denoux, Mondor Hospital Tumorothèque led by Professor K. Leroy, and IGR CRB led by Dr. J. Bosq. They also thank C. Machavoine for her help in frozen tissue processing.

Grant Support

This work was supported by the ARTuR grant (to L. Albiges) and the Taxe d’apprentissage grant (to L. Albiges) of the Functional Genomics Unit from the University of Paris-Sud and Gustave Roussy Institute, Villejuif.

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Received August 7, 2013; revised January 30, 2014; accepted February 13, 2014; published OnlineFirst March 21, 2014.
References


Clinical Cancer Research

MET Is a Potential Target across All Papillary Renal Cell Carcinomas: Result from a Large Molecular Study of pRCC with CGH Array and Matching Gene Expression Array

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