Specific Genomic Aberrations Predict Survival, But Low Mutation Rate in Cancer Hot Spots, in Clear Cell Renal Cell Carcinoma

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Abstract: Detailed genetic profiling of clear cell renal cell carcinoma (ccRCC) has revealed genomic regions commonly affected by structural changes and a general genetic heterogeneity. VHL and PBRM1, both located at chromosome 3p, are 2 major genes mutated at high frequency but apart from these aberrations, the mutational landscape in ccRCC is largely undefined. Potential prognostic information given by the genomic changes appears to depend on the particular cohort studied. We analyzed a Swedish ccRCC cohort of 74 patients and found common changes (loss or gain occurring in >20% of the tumors) in 12 chromosomal regions (1p, 3p, 3q, 5q, 6q, 7p, 7q, 8p, 9p, 9q, 10q, and 14q). A poor outcome was associated with gain of 7q and losses on 9p, 9q, and 14q. These aberrations were more frequent in metastasized tumors, suggesting alterations of genes important for tumor progression. Sequencing of 48 genes implicated in cancer revealed that only VHL, TP53, and PTEN were mutated at a noticeable frequency (51%, 9%, and 9%, respectively). Shorter relative telomere length (RTL) has been associated with loss of specific chromosomal regions in ccRCC tumors, but we could not verify this finding. However, a significantly lower tumor/nontumor (T/N) RTL ratio was detected for tumors with losses in 4q or 9p.

Key Words: clear cell renal cell carcinoma, survival, genomic aberrations, VHL, TP53, PTEN

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correlated with poor survival. Moreover, loss of chromosomes 19, 20, and 22 were associated to poor survival in a study by Antonelli et al. Together, these studies indicate that the prognostic information given by different genomic alterations to a large extent appears to be cohort-dependent and more studies are needed to clarify genetic factors of more general importance for tumor progression.

To define the genetic constitution of our ccRCC cohort we genotyped the tumors using high-resolution genome-wide single-nucleotide polymorphism (SNP) arrays and detected alterations were correlated to stage and survival time. In a commercially available panel containing 48 genes, implicated to be altered in cancer, we aimed to find RCC-specific mutations by sequencing at high mean depths.

We and others have shown that telomeres in ccRCC are shorter compared with normal tissue. Short telomeres are known to cause genetic instability and have been reported in ccRCC to be coupled to loss on specific chromosome arms. We tested if relative telomere length (RTL) in our tumors was associated with loss of specific chromosomal regions.

**Materials and Methods**

**Patients**

The patients were nephrectomized with histologically verified ccRCC at the Department of Urology, Umeå University Hospital, Umeå, Sweden. In total, 74 patients with sporadic ccRCC, diagnosed between 2001 and 2008, were included in the study (see Table 1 for clinical characteristics). Routine staging procedures included physical examination and computerized tomography of the abdomen and chest. Staging was performed according to the 2002 tumor-node-metastasis (TNM) classification system. RCC type was defined according to the 2002 tumor-node-metastasis (TNM) classification system. 21 RCC type was defined according to the Heidelberg consensus conference. Follow-up data were available for all patients. Of these, 31 had died of the disease, 10 of other causes, and 33 patients were alive with a median survival of 80 months (range, 57 to 127 mo). The study was approved by the regional ethical review board in Umeå (Dnr 07-071M), and each patient participated after providing informed and signed consent.

**Samples**

Samples from tumors and tumor-free kidney cortex obtained immediately after extirpation were snap-frozen in liquid nitrogen. Blood samples were collected before therapy, and DNA was extracted from fresh-frozen tumor, kidney cortex, anduffy coats using a BioRobot M48 Workstation with MagAttract technology as described elsewhere (Qiagen Inc., Valencia, CA).

**SNP Array**

Genotyping of 74 tumors was performed using the HumanCytoSNP-12 v2.1 beadchip arrays, including approximately 300,000 SNPs, according to manufacturer’s protocol (Illumina Inc., San Diego, CA). For 22 of the 74 tumors, paired kidney cortex and peripheral blood samples were also genotyped. The signals were imaged on a BeadArray Reader and analyzed with Genome Studio v1.8 (Illumina Inc.). Log R ratios (LRR) and B allele frequencies (BAF) were used to identify regions of loss and gain. The LRR is a normalized measure of total signal intensity, meaning that LRR values decrease (LRR < 0) when a genomic region is lost and increase (LRR > 0) when a genomic region is gained. BAF is a measure of the allelic intensity ratio and BAF values cluster around 0, 0.5, and 1 for AA, AB, and BB genotypes, respectively. In heterogenous tumor tissues, a gain or loss will result in a split of the AB cluster. The SNP data set was deposited into the Gene Expression Omnibus database and is accessible through accession number GSE30460.

**Mutation Analysis**

Mutation screening was performed on 36 of these RCCs using a TruSeq Amplicon cancer panel (Illumina Inc.) targeting 48 genes implicated in cancer (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A47). Sequencing libraries were made according to manufacturer’s protocol and sequenced on a MiSeq (Illumina Inc.) at an average depth of 218x. Corresponding kidney cortex from 5 tumors and peripheral blood from 2 geographically matched healthy volunteers were additionally included. The sequences were processed through MiSeq Reporter (Illumina Inc.) using a banded Smith-Waterman algorithm for alignment and Somatic Variant Caller for variant calling. The variants were subsequently visualized in Amplicon Viewer (Illumina Inc.) and the paired kidney cortex samples were filtered from the data set. The sequence data were uploaded to the Sequence Read Archive and is accessible through accession number...

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**Table 1. Clinical Characteristics of the Tumors, n = 74**

| Sex      | Male | Female |
|----------|------|--------|
| Age (y)  | 67 (44-87) | 69 (49-84) |
| Follow-up time (mo) | 65.5 (0-127) |
| Tumor diameter (mm) | 80 (16-180) |

| TNM stage | I | II | III | IV |
|-----------|---|----|-----|----|
|           | 23| 10 | 16  | 25 |

| Grade | 1 | 2 | 3 | 4 |
|-------|---|---|---|---|
|       | 3 | 27| 34| 10 |

For age, follow-up time, and tumor diameter median values are shown with range shown within brackets. Grade is represented by morphological grade.
number PRJEB3971. *VHL* sequencing of its 3 exons and adjacent intronic sequences was performed using primer pairs that have been previously published.\(^{24}\) Polymerase chain reaction (PCR) amplification (with annealing at 58°C for all amplicons) and sequence analysis were performed as described elsewhere.\(^{25}\) PCR reactions for exon 1 additionally contained 5% dimethyl sulfoxide.

**Telomere Length Measurements**

RTL was assessed by real-time PCR according to the method described by Cawthon\(^{26}\) and has been published previously by us.\(^{27}\) Telomere-to-Single copy gene (T/S) values for the samples were divided with the T/S value of a reference DNA included in each assay, generating RTL values. Samples were available for 70 tumors and corresponding kidney cortex tissues. Tumor/non-tumor (T/N) RTL ratios were generated by dividing tumor RTL with normal kidney cortex RTL. Tumors with loss of >10% of a chromosome arm were categorized as the “loss” group when testing for differences in distribution of RTL ratios. This categorization was carried out according to Chen and colleagues as our intention was to test if we could replicate their findings.

**Statistical Analyses**

PASW statistics 18 (IBM, New York, NY) was used for statistical analyses. Survival analysis was performed using the Kaplan-Meier with the log-rank test. Cancer specific survival was defined as the time (in months) between the date of diagnosis to ccRCC-specific death or to the date of last follow-up (March 2012). Cross tabulation with the Pearson \(\chi^2\) tests were used to evaluate differences in distribution between tumor groups with and without chromosomal changes in relation to TNM stage, Fuhrman grade, and metastatic stage. Pearson \(\chi^2\) tests were also used to test for difference between patients with and without *VHL* mutations in relation to metastatic stage and the Fuhrman grade. The Kruskal-Wallis test was used to check for differences in number of altered chromosomes and number of mutations between TNM stage groups I-III and IV. Multivariate analysis with the Cox regression model including the chromosomal aberrations, TNM stage, and Fuhrman grade used the backward conditional method to test if the chromosomal aberrations could prognosticate the patients. Differences in distribution of RTL ratios between tumor groups with and without chromosomal changes were analyzed with the Mann-Whitney \(U\) test.

**RESULTS**

**Characterization of Genomic Aberrations in ccRCC**

We observed large chromosomal aberrations in 70 of 74 ccRCCs. The breakpoints of the aberrations could be defined with great precision because of the high resolution of the SNP array. Three tumors showed no genomic changes (denoted by arrows in Fig. 1). In one tumor, a region of 2 Mbp demonstrated a gain in copy number on chromosome 7q, also seen in the corresponding kidney cortex sample. Apart from that, no genomic change was detected in the 22 paired kidney cortex tissues or blood samples, confirming the dissection of histologically non-tumorous kidney cortex tissues.

We identified 12 regions with common changes, defined as occurring in >20% of the ccRCCs. Loss of genetic material was found on chromosomes 1p (26%), 3p (88%), 3q (31%), 6q (28%), 8p (27%), 9p (24%), 9q (28%), 10q (22%), and 14q (39%). Gain of genetic material was detected on chromosome 5q (50%), 7p (26%), and 7q (26%). Minimum region of overlap ranged from 2.2 M base pairs to whole chromosomes (Table 2). A larger number of altered chromosomes was found in tumors with distant metastases (M1) compared with M0.

**FIGURE 1.** Heat map of tumors showing chromosomes affected by a genetic change. M0 (nonmetastatic) tumors are shown in the left pane and M1 (metastatic) in the right. The color gradients going from light green/red to dark green/red represents the extent of loss/gain on each chromosome. The darkest shading equals whole chromosome loss/gain and the palest shading represent more loss than gain/more gain than loss. Black color indicates undefined change. Arrows denote 3 tumors that showed no genomic alteration.
nonmetastatic (M0) cases ($P = 0.04$) (Figure 1). No tumor had $> 8$ common genomic changes.

### Association of the Chromosomal Aberrations to Metastatic Stage and Survival Time

The Kaplan-Meier analysis with disease-specific survival as endpoint showed that TNM stage group III had shorter survival time than stage group I and II ($P = 0.006$). Stage group IV had further shorter survival time than stage group III ($P = 1e^{-6}$) (Supplementary Figure 1a, Supplemental Digital Content 2, http://links.lww.com/AIMM/A48). Grade 4 tumors presented shorter survival time than grade 3 ($P = 0.0003$), whereas no difference was found between grade 3 and 2 or grade 2 and 1 (Supplementary Figure 1b, Supplemental Digital Content 2, http://links.lww.com/AIMM/A48). The Kaplan-Meier analysis further showed significantly shorter survival for patients with tumors containing gain of the 7q region, or loss of the 9p, 9q, or 14q regions (log-rank $P = 0.008$, 0.0004, 0.0003, and 0.001, respectively) compared with cases without these aberrations (Figs. 2A–D). Losses of 1p, 3p, 3q, 6q, 8p, or 10q or gains of the 5q or 7p regions were not coupled to survival time.

Pearson $\chi^2$ tests reached the level of significance for chromosomes 5q, 7p, 7q, 9p, 9q, and 14q when testing for differences in the distribution of genomic changes between M0 and M1 tumors (Table 3). The more advanced tumors (M1) demonstrated an accumulation of genetic changes compared with M0 tumors ($P < 0.00001$) (Table 3). Loss of genetic material on chromosome 3p is known to be an early event in ccRCC. Concordantly, we found no difference in distribution of 3p loss between M0 and M1 tumors ($P = 0.6$). The 7q, 9p, 9q, and 14q regions were also found more often in high TNM stage groups (III and IV) than in low stage groups (I and II) (Table 4). Furthermore, the 9p and 9q regions were found more often in grade 3 and 4 than in grade 1 and 2 tumors (Table 4).

The Multivariate Cox regression analysis using stepwise elimination showed that loss of the 9p region was an independent prognostic factor ($P = 0.04$) in patients without metastases, when including TNM stage, gain of 7q, losses of 9p, 9q, and 14q regions, respectively. When grade (1 + 2) versus (3 + 4) was included in the multivariate analysis (Table 5), loss on 9p presented borderline significance together with TNM stage. When including all patients, only TNM stage and tumor grade remained as independent significant factors ($P = 0.00002$ and 0.03, respectively), whereas loss of 9p lost its prognostic information ($P = 0.08$).

### Sequencing of Cancer Hot Spot Genes

Deep sequencing of 48 cancer-related genes revealed rather few mutations in the 36 ccRCCs tumors. One tumor was excluded from the analysis because of incorrect calling by the software. This could possibly be explained by poor DNA quality and/or the flexibility in the variant caller to allow for very low-frequency mutations. Non-synonymous mutations were detected in APC, CDKN2A, FGFR3, GNAS, IDH1, KIT, KRAS, PTEN, SMARCB1, SMO, TP53, and VHL (Fig. 3). Mutations in PTEN and TP53 were found in 3 (9%) tumors each, and mutations in VHL were found in 11 (31%) ccRCCs. No other gene showed mutations in $> 6$% of the tumors. Three tumors lacking large genomic alterations are marked with arrows in Figure 3. Two of them showed no mutations at all and the third presented a mutation in VHL. The VHL mutations detected with the cancer panel were verified by the Sanger sequencing (see further below). There was no difference in the number of mutations in the M0 group compared with the M1 RCCs ($P = 0.5$) detected by this commercial NGS gene panel.

### VHL Mutation Status

Tumors were defined as VHL-mutated if one allele was deleted and the second allele contained a non-synonymous or frame-shift mutation. Mutations in VHL were detected in 37 of 72 (51%) analyzed ccRCCs, all of which were VHL mutation negative in corresponding kidney cortex or blood samples by sequencing. Eleven tumors were VHL wild type. There was no difference in survival time, stage, or Fuhrman grade based on VHL mutation status ($P = 0.7, 0.4$, and $0.1$, respectively).

### Association to Telomere Length

No significant associations were observed between short tumor RTL and loss of chromosomal regions, although a borderline significant $P (0.06)$ was found for chromosome 3p. There were, however, significant differences for 2 of 13 regions when including tumor-to-normal tumor (T/N) RTL ratio as a parameter. Significantly, lower T/N RTL ratios were detected for patients with loss on 4q and 9p ($P = 0.02$ and 0.05, respectively).

### DISCUSSION

Gain of the q arm of chromosome 7 and losses on 9p, 9q, and 14q, each associated significantly to short survival in the present study. In total, we identified 12 common (occurring in $> 20\%$ of the investigated ccRCCs) genomic regions on chromosomes 1p, 3p, 3q, 5q, 6q, 7q, 8p, 9p, 9q, 10q, and 14q with structural changes. Alterations

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**TABLE 2.** Common Changes Detected in the ccRCC Tumors

| Chromosome, Type of Change | % of Tumors | Minimum Region | Size (Mb) |
|---------------------------|-------------|----------------|-----------|
| 1p, loss                   | 26          | 25,334,666-32,100,024 | 6.8       |
| 3p, loss                   | 88          | tel-46,765,401      | 46.8      |
| 3q, loss                   | 31          | cen-97,267,562      | 2.2       |
| 5q, gain                   | 50          | 172,012,494-tel     | 8.6       |
| 6q, loss                   | 28          | 152,878,353-tel     | 17.9      |
| 7p, gain                   | 26          | Whole arm           | 37.4      |
| 7q, gain                   | 26          | Whole arm           | 97.6      |
| 8p, loss                   | 27          | 12,653,559-27,797,474 | 15.1     |
| 9p, loss                   | 24          | tel-28,013,465      | 28.0      |
| 9q, loss                   | 28          | 130,719,998-138,443,132 | 7.7    |
| 10q, loss                  | 22          | 85,932,576-90,524,678 | 4.6      |
| 14q, loss                  | 39          | 69,560,017-91,189,113 | 21.6    |

ccRCC indicates clear cell renal cell carcinoma.
affecting these regions have been shown in previous investigations on ccRCC, but there is no overall consistent pattern in the literature. There was a large variation in our ccRCCs, showing a spectrum from no altered chromosome in 3 cases to 1 tumor with changes on 17 chromosomes.

The associations found for specific chromosomal aberrations are in line with previously published studies also using genome wide approaches, especially concerning chromosomes 9p and 14q (Table 6), indicating these regions to be consistently important for ccRCC progression. We also found the same regions to be more

**FIGURE 2.** The Kaplan-Meier analysis with disease-specific survival as endpoint in relation to genomic alterations in the 7q, 9p, 9q, and 14q regions. Survival time is shown in months and log-rank P-values are presented. A–D, Tumors containing the given genomic alteration showed a significantly decreased survival time.

**TABLE 3.** Distribution of Genomic Aberrations in Relation to Metastatic Stage, n = 74

|       | 5q | 7p | 7q | 9p | 9q | 14q | ΣAberrations |
|-------|----|----|----|----|----|-----|--------------|
| M0    | 53 | 22 | 31 | 10 | 43 | 8   | 73           |
| M1    | 21 | 15 | 6  | 9  | 12 | 11  | 68           |
|       |    |    |    |    |    |     |              |
|       | 8  | 45 | 10 | 43 | 15 | 38  |              |
|       | 10 | 11 | 10 | 11 | 14 | 7   |              |
|       | 0.02 | 0.03 | 0.003 | 0.003 | 0.004 | 0.002 | < 0.00001 |

Statistical analysis of differences between groups used the Pearson χ² analysis. Number of patients with genomic aberrations and without is showed as n+ versus n-.

M0 indicates nonmetastatic tumors; M1, metastatic tumors.
often affected in metastasized tumors, further strengthening this notion. For other genomic aberrations in ccRCC, the literature is heterogeneous with different regions implicated for survival in different cohorts. One contradictory finding is, for example, gain on 5q reported as associated with longer survival, whereas we found this aberration to be more frequent in the group of metastatic ccRCCs. The background for the various inconsistencies regarding survival is unclear but can be because of differences in study populations, treatment protocols, or the methodologies used for genomic analysis.

The VHL mutation status did not correlate to survival, TNM stage, or the Fuhrman grade. Our data confirm results in previous studies and further strengthens the belief that deletion of chromosome 3p and VHL inactivation is an initiation event in the tumorigenesis of ccRCC. VHL can also be silenced by methylation, which could explain why only 51% of the cases presented mutations compared with rates up to 80%, which has been published. Next to VHL, only PBRM1 has been shown to be frequently mutated in ccRCC, a gene not present in the cancer panel used in this study. We used a commercially available sequencing panel to investigate the presence of mutations in genes commonly reported in cancer. Of the genes analyzed only PTEN and TP53, beside VHL, showed mutations to an apparent extent with mutations in > 9% of the tumors. Mutations in PTEN and TP53 have been reported in ccRCC at rates of 7% and 4% to 14%, respectively.

An interesting finding in our ccRCCs with TP53 or PTEN mutations was that they also had the second allele deleted, implying that no functional protein was produced. This is likely to have been of importance for tumor progression in these particular patients.

Overall, the mutation rate was low in our screen of 48 cancer-associated genes. For instance, only one mutation was found in the gene encoding SMARCB1, which is a subunit of the SWI/SNF complex. As PBRM1 is a part of SWI/SNF, it could be speculated that SMARCB1 would also present mutations to a significant extent. CDKN2A, located at 9p21, was mutated in only one

### TABLE 4. Distribution of Genomic Aberrations in Relation to TNM Stage and Fuhrman Grade, n = 74

|       | 7q | 9p | 9q | 14q |
|-------|----|----|----|-----|
|       | n+ | n- | n+ | n- | n+ | n- | n+ | n- |
| TNM stage I+II | 37 | 5  | 32 | 5  | 32 | 6  | 31 | 7  | 30 |
| TNM stage III+IV| 37 | 13 | 24 | 13 | 24 | 24 | 22 | 22 | 15 |
| P      |     | 0.03 | 0.03 |     | 0.02 |     | 0.0004 |     |
| Grade 1 + 2 | 30 | 6  | 24 | 2  | 28 | 3  | 27 | 9  | 21 |
| Grade 3 + 4 | 44 | 12 | 32 | 16 | 28 | 18 | 26 | 20 | 24 |
| P      |     | 0.5 |     | 0.003 |     | 0.004 |     | 0.2 |

Statistical analysis of differences between groups used the Pearson χ² analysis. Number of patients with genomic aberrations and without is showed as n+ versus n-.

### TABLE 5. Multivariate Cox Analyses of the 7q, 9p, 9q, and 14q Regions in Nonmetastatic Patients, n = 52

|       | 7q | 9p | 9q | 14q |
|-------|----|----|----|-----|
|       | n+ | n- | n+ | n- | n+ | n- | n+ | n- |
| TNM stage I+II vs. (III+IV) | 0.02 | 5.17 | 1.26 | 21.12 |
| Grade (1+2) vs. (3+4) | 0.05 | 0.19 | 0.04 | 1.00 |
| Gain 7q | 0.3 | 2.61 | 0.48 | 14.16 |
| Loss 9p | 0.3 | 2.58 | 0.39 | 17.22 |
| Loss 9q | 0.7 | 1.53 | 0.22 | 10.62 |
| Loss 14q | 0.4 | 0.53 | 0.12 | 2.46 |
| Step 4 |     |     |     |     |
| TNM stage I+II vs. (III+IV) | 0.02 | 4.20 | 1.22 | 14.40 |
| Grade (1+2) vs. (3+4) | 0.07 | 0.24 | 0.05 | 1.14 |
| Loss 9p | 0.05 | 3.58 | 1.00 | 12.86 |

Backward conditional Cox regression analysis of factors predicting survival in patients without distant metastases (M0, n = 52) including (a) and (b).
ccRCC, despite that lack of p16 expression has been reported in 53% of RCCs in another study.\(^3^9\) For FGFR3, in contrast, we found mutations in 2 of 35, whereas no mutation was detected in a screen of 101 ccRCCs.\(^4^0\) Thirty-six of the 48 genes screened did not show any nonsynonymous or frame-shift mutations at all, and the mutations detected were comparable between the M0 and M1 groups, suggesting that genes associated with tumor progression in ccRCC were absent in this panel.

Loss on 9p is the most common structural change with impact on survival in ccRCC. The 28 Mbp region on 9p deleted in our cohort contains PTPRD, CDKN2B, and TUSC1, all of which have been reported mutated and/or inactivated in several tumor types.\(^4^1\)\(^-\)\(^4^4\) Screening of these genes may reveal inactivating mutations also in ccRCC. Preliminary gene expression data for our tumors further showed that TUSC1 mRNA is down-regulated in tumors with allelic loss of TUSC1, \(P = 0.00002\) (data not shown).

Two genes (JAK2 and CDKN2A) in the cancer panel are located within the altered 9p region showing prognostic significance in multivariate analysis. However, only 1 mutation was demonstrated (in CDKN2A), indicating that aberrant expression of these genes did not contribute to the outcome of the patients. However, hypermethylation of CDKN2A is known to occur in RCC\(^3^9\) showing that other regulatory mechanisms for gene expression might occur. Three tumors, with typical ccRCC appearance by histology, showed no large genomic alterations in the SNP arrays and only 1 harbored a VHL mutation. These 3 ccRCCs represent different grades (3 to 4), stages (I to IV), and had different survival times (26, 80, and 117 mo). It is likely that these RCCs contain genomic aberrations that remained undetected with the approaches used. DNA methylation of genes located within chromosome 7, the 9p, and 9q regions are reported in RCC\(^4^5\) and gain in copy number per se is known to increase the expression of genes in ccRCC and copy-number loss decreases the expression.\(^9\)\(^,\)\(^1^3\) Together, this suggests that more factors besides mutations have impact on the survival seen for our patients.

Short telomeres cause genetic instability and may inflate the amount of chromosome losses or gains. Chen et al\(^2^0\) reported shorter telomeres in tumors with loss on chromosome arms 1p, 2q, 3p, 4q, 6p, 6q, 9p, 9q, 10q, 17p, 18p, and 22q.

### TABLE 6. Genome Wide Studies Presenting Association of Specific Chromosomal Changes to Survival in ccRCC

| References         | Study Size (n) | Method           | Chromosomal Change Associated With Shorter Survival Time | Chromosomal Change Associated With Longer Survival Time |
|--------------------|----------------|------------------|----------------------------------------------------------|--------------------------------------------------------|
| This study         | 74             | SNP array        | Gain 7q                                                  |                                                        |
| Girgis et al\(^2^8\) | 154            | SNP array (meta-analysis) | Loss 9p, 9q, 14q                                           | Gain chr12                                              |
| Monzon et al\(^1^1\) | 85             | SNP array        | Loss 1p, 4, 9, 13q, 14q, 18                               |                                                        |
| Sanjmyatav et al\(^1^5\) | 53              | CGH array        | Gain 8q                                                  | Gain 7q, 20q                                           |
| Antonelli et al\(^1^6\) | 131            | G-banding        | Loss 14q                                                  |                                                        |
| Klatt et al\(^1^4\)  | 246            | G-banding        |                                                        | Gain/loss 12, 16                                       |
| Arai et al\(^8\)    | 51             | CGH array        |                                                        |                                                        |
| Gunawan et al\(^2^9\) | 104            | G-banding        |                                                        |                                                        |
| Moch et al\(^3^0\)  | 37             | CGH array        |                                                        |                                                        |

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18p, 18q, and 22q. It can be hypothesized that deletions on 4q and 9p affect genes of importance for telomere maintenance as loss of these regions were found to be associated with short RTL by Chen and colleagues and with low T/N RTL ratio in the present study.

In conclusion, our study showed an association between structural chromosomal changes and the clinical course in patients with ccRCC. In total, 12 chromosomal regions, commonly affected by genetic changes, were identified in the tumors. Decreased survival time for patients with ccRCC was significantly associated to gain of 7q and loss on 9p, 9q, or 14q, respectively, highlighting the inconsistent findings between different ccRCC cohorts. Of 48 cancer implicated genes evaluated, only VHL, PTEN, and TP53 showed mutations to an apparent extent.

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REFERENCES

1. Kaelin WG Jr. The von Hippel-Lindau tumor suppressor gene and kidney cancer. Clin Cancer Res. 2004;10(pt 2):6290S–6295S.

2. Latif F, Tory K, Gnarra J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. 1993;260:1317–1320.

3. Varela I, Tarpey P, Raine K, et al. Exome sequencing identifies genomic alterations associated with metastasis and cancer specific survival in clear cell renal cell carcinoma. J Urol. 2011;186:2078–2083.

4. Antonelli A, Arrighi N, Tardanico R, et al. Prognostic value of cytogenetic analysis in clear cell renal cell carcinoma: a study on 131 patients with long-term follow-up. Anticancer Res. 2010;30:4705–4709.

5. Mehel C, Liungberg B, Roos G. Telomere shortening in renal cell carcinoma. Cancer Res. 1994;54:236–241.

6. Gisselsson D, Gorunova L, Högland M, et al. Telomere shortening and mitotic dysfunction generate cytogenetic heterogeneity in a subgroup of renal cell carcinomas. Br J Cancer. 2004;91:327–332.

7. Nordfjall K, Svenson U, Norrback KF, et al. The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet. 2009;5:e1000375.

8. Kirchgesner K, Ebert F, Huttner B, et al. Telomere length and mitotic delay in renal cell carcinoma. Br J Cancer. 2009;100:1564–1569.

9. Young AC, Craven RA, Cohen D, et al. Analysis of VHL gene alterations and their relationship to clinical parameters in sporadic conventional renal cell carcinoma. Clin Cancer Res. 2009;15:7582–7592.

10. Kohn L, Kazdhaev K, Burstedt MS, et al. Mutation in the PYK2 binding domain of PITPNM3 causes autosomal dominant cone dysrophy (CORD5) in two Swedish families. Eur J Hum Genet. 2007;15:664–671.

11. Sawtell KN, Bandi V, Smith SR, et al. Prognostic impacts of telomere length on survival and metastasis in renal cell carcinoma. Clin Cancer Res. 2011;17:4423–4431.

12. Sawtell KN, Bandi V, Smith SR, et al. Prognostic impact of telomere length in renal cell carcinoma. Clin Cancer Res. 2012;18:107–116.

13. Sawtell KN, Bandi V, Smith SR, et al. Prognostic impact of telomere length in renal cell carcinoma. Clin Cancer Res. 2012;18:107–116.

14. Klatte T, Rao PN, de Martino M, et al. Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. J Clin Oncol. 2009;27:746–753.

15. Sanjmyatav J, Junker K, Matthews S, et al. Identification of genomic alterations associated with metastasis and cancer specific survival in clear cell renal cell carcinoma. J Urol. 2011;186:2078–2083.

16. Antonelli A, Arrighi N, Tardanico R, et al. Prognostic value of cytogenetic analysis in clear cell renal cell carcinoma: a study on 131 patients with long-term follow-up. Anticancer Res. 2010;30:4705–4709.

17. Mehle C, Liungberg B, Roos G. Telomere shortening in renal cell carcinoma. Cancer Res. 1994;54:236–241.

18. Gisselsson D, Gorunova L, Högland M, et al. Telomere shortening and mitotic dysfunction generate cytogenetic heterogeneity in a subgroup of renal cell carcinomas. Br J Cancer. 2004;91:327–332.

19. Svensson U, Liungberg B, Roos G. Telomere length in peripheral blood predicts survival in clear cell renal cell carcinoma. Cancer Res. 2009;69:2896–2901.

20. Chen M, Ye Y, Yang H, et al. Genome-wide profiling of chromosomal alterations in renal cell carcinoma using high-density single nucleotide polymorphism arrays. Int J Cancer. 2009;125:2342–2348.

21. Sobin L, Wittekind C. International Union Against Cancer (UICC). TNM Classification of Malignant Tumors. 6th ed. New York: Wiley-Liss; 2002:193–195.

22. Kovacs G, Akhtar M, Beckwith BJ, et al. The Heidelberg classification of renal cell tumours. J Pathol. 1997;183:131–133.

23. R Core Team. R: A Language and Environment for Statistical Computing; 2013. Available at: http://www.R-project.org/. Accessed March 31, 2014.

24. Young AC, Craven RA, Cohen D, et al. Analysis of VHL gene alterations and their relationship to clinical parameters in sporadic conventional renal cell carcinoma. Clin Cancer Res. 2009;15:7582–7592.

25. Kohn L, Kazdhaev K, Burstedt MS, et al. Mutation in the PYK2 binding domain of PITPNM3 causes autosomal dominant cone dysrophy (CORD5) in two Swedish families. Eur J Hum Genet. 2007;15:664–671.

26. Sawtell KN, Bandi V, Smith SR, et al. Prognostic impacts of telomere length on survival and metastasis in renal cell carcinoma. Clin Cancer Res. 2011;17:4423–4431.

27. Nordfjall K, Svensson U, Norrback KF, et al. The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet. 2009;5:e1000375.

28. Girgis AH, Iakovlev VV, Behebchi B, et al. Multilevel whole-genome analysis reveals candidate biomarkers in clear cell renal cell carcinoma. Cancer Res. 2012;72:5273–5284.

29. Gunawan B, Huber W, Holtrup M, et al. Prognostic impacts of cytogenetic findings in clear cell renal cell carcinoma: gain of 5q31-qter predicts a distinct clinical phenotype with favorable prognosis. Cancer Res. 2001;61:7731–7738.

30. Moch H, Presti JC Jr, Sauter G, et al. Genetic aberrations detected by comparative genomic hybridization are associated with clinical outcome in renal cell carcinoma. Cancer Res. 1996;56:27–30.

31. Nagao K, Yamaguchi S, Matsuyama H, et al. Allelic loss of 3p25 associated with alterations of 5q22.3 approximately q23.2 may affect the prognosis of conventional renal cell carcinoma. Cancer Genet Cytogenet. 2005;160:43–48.

32. Kondo K, Yao M, Yoshida M, et al. Comprehensive mutational analysis of the VHL gene in sporadic renal cell carcinoma: relationship to clinicopathological parameters. Genes Chromosomes Cancer. 2004;34:58–62.

33. Baldeiwins MM, van Vlodrop JJ, Smits KM, et al. Different angiogenic potential in low and high grade sporadic clear cell renal cell carcinoma is not related to alterations in the von Hippel-Lindau gene. Cell Oncol. 2009;31:371–382.

34. Smits KM, Schouten LJ, van Dijk BA, et al. Genetic and epigenetic alterations in the von hippel-lindau gene: the influence on renal cancer prognosis. Clin Cancer Res. 2008;14:782–787.
35. Jonasch E, Futreal PA, Davis II, et al. State of the science: an update on renal cell carcinoma. Mol Cancer Res. 2012;10:859–880.
36. Kondo K, Yao M, Kobayashi K, et al. PTEN/MMAC1/TEP1 mutations in human primary renal-cell carcinomas and renal carcinoma cell lines. Int J Cancer. 2001;91:219–224.
37. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat. 2007;28:622–629.
38. Szymanska K, Moore LE, Rothman N, et al. TP53, EGFR, and KRAS mutations in relation to VHL inactivation and lifestyle risk factors in renal-cell carcinoma from central and eastern Europe. Cancer Lett. 2010;293:92–98.
39. Vidaurreta M, Maestro ML, Sanz-Casla MT, et al. Inactivation of p16 by CpG hypermethylation in renal cell carcinoma. Urol Oncol. 2008;26:239–245.
40. Stoehr CG, Stoehr R, Hartmann A, et al. Mutational activation of FGFR3: no involvement in the development of renal cell carcinoma. J Cancer Res Clin Oncol. 2012;138:359–361.
41. Solomon DA, Kim JS, Cronin JC, et al. Mutational inactivation of PTPRD in glioblastoma multiforme and malignant melanoma. Cancer Res. 2008;68:10300–10306.
42. Veeriah S, Brennan C, Meng S, et al. The tyrosine phosphatase PTPRD is a tumor suppressor that is frequently inactivated and mutated in glioblastoma and other human cancers. Proc Natl Acad Sci USA. 2009;106:9435–9440.
43. Costa-Guda J, Soong CP, Parekh VI, et al. Germline and somatic mutations in cyclin-dependent kinase inhibitor genes CDKN1A, CDKN2B, and CDKN2C in sporadic parathyroid adenomas. Horm Cancer. 2013;4:301–307.
44. Shan Z, Parker T, Wiest JS. Identifying novel homozygous deletions by microsatellite analysis and characterization of tumor suppressor candidate 1 gene, TUSC1, on chromosome 9p in human lung cancer. Oncogene. 2004;23:6612–6620.
45. Morris MR, Maher ER. Epigenetics of renal cell carcinoma: the path towards new diagnostics and therapeutics. Genome Med. 2010;2:59.