Chromosomal aberrations in renal cell carcinoma: An overview with implications for clinical practice

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Abstract

Chromosomal instability and aberrations are known in many cancers including renal cell carcinoma. Detailed understanding of these changes has led to an improved drug discovery and continued developments in other therapeutic options. Chromosomal aberrations have a potential to be used to monitor disease including prognostication. There has been a growing experience in cytogenetic techniques and gap between clinic and laboratory has narrowed significantly in the recent past. Nevertheless, more work on validation of these techniques, establishing threshold and interobserver agreement needs to be carried out for these diagnostic/prognostic tests before utilizing them in clinics as a part of “personalized medicine” care. The review presented here is a summary of common genetic disorders in renal cancer and some of acquired genetic changes which can be used as biomarkers. The review also describes basics of commonly used genetic techniques for wider clinical community involved in the management of renal cancer.

Keywords: Chromosome, clear-cell carcinoma, genetics, head and neck cancer, head and neck squamous cell carcinoma, intensity modulated radiotherapy, radiotherapy kidney, renal cell cancer, three-dimensional conformal radiotherapy, toxicity, xerostomia

INTRODUCTION

Kidney cancer is the twelfth most common cancer in the world (joint position with pancreatic cancer), with 338,000 new cases diagnosed in 2012 (Ferlay et al. 2012).[1] Renal cell cancer is the eighth most common cancer in the UK and is the second most common urological malignancy. It accounts for 2% of all the cancer deaths in the UK. It represents 3% of all new cancer cases in adults in the Western world (Landis et al., 1999).[2] Rising trends in renal cancer rates across all age groups have been observed. The widespread use of imaging resulted in an increased detection of asymptomatic renal tumors; however, it also coincides with a rise in the incidence of advanced renal cancer. These findings suggest that the detection of asymptomatic tumors by imaging alone cannot fully explain the increase seen for renal cancer overall (Tate et al., 2003).[3] The classification of renal cell tumors has recently been revised and published in the 2016 World Health Organization (WHO) classification (Inamura et al. 2017).[4,5]

Cancer cells are known to have a range of cytogenetic abnormalities and aberrations at chromosomal levels...
are being identified through genome-wide studies (Albertson et al. 2003). With improved detection of these changes, possibility of better understanding of carcinogenesis and treatment strategies is emerging in many cancers. Alterations in chromosomes in cancers can be structural changes or a numerical alteration (copy number variations [CNVs]). Specific focus is drawn on CNV which is a form of structural variation of the DNA sequence, including multiplication and deletions of a particular segment of DNA (>1 kb) (Stratton et al., 2009). These changes were notoriously difficult to study using conventional cytogenetic techniques, however, with combined genomic hybridization and fluorescent-labeled probes, possibility of detailed identification of both the changes has emerged (Albrecht et al. 2004). Latest technology of next-generation sequencing (NGS) has allowed these alterations to be studied at the resolution of a single nucleotide. With the arrival of NGS technologies (Metzker et al. 2010), sequence-based CNV detection has rapidly emerged as a viable option to identify CNVs with higher resolution and accuracy (Ku et al. 2010).

**GENETIC BASIS OF CANCER**

The genetic basis of cancer is a very complex process and involves a sheer number of genetic aberrations that have not been fully understood or characterized. Cancer is the clonal instability of genetically aberrant cells. Genetic instability innately affects cell birth or death (Lengauer et al., 1998). In renal cancer, the hereditary forms allowed to identify the gatekeeper genes that predispose to sporadic forms. However, the mechanisms by which a tumor becomes more aggressive and underlying genetic changes have not been fully understood. In general, the main mechanism involves either proto-oncogene or tumor suppressor genes, which control normal cell growth or programmed cell death. This is never straightforward and very few cancers can be linked to one particular gene dysfunctions. Chromosomal aberrations can vary from vast number of chromosomes seen in some tumor karyotypes on large scale to undetectable changes. These small changes can involve only a couple of base pairs, occurring as deletions or insertions (Lengauer et al., 1998). Aneuploidy is defined as chromosome number that is not an exact multiple of the usually haploid number. While polyploidy is defined as having a chromosome number that is a multiple greater than two of the haploid number. Segmental aneuploidy is the loss or gain of part of chromosome (Torres et al., 2008). Translocation of chromosomes occurs frequently, and parts of one chromosome can be found joined to another (Lengauer et al., 1998). These can be balanced or unbalanced, depending on the presence of all 23 pairs of homologous chromosomes even in different segments or loss of one part of a chromosome, respectively.

In this review, we discuss chromosomal aberrations, that is, structural and copy number alterations, in renal cell carcinoma with focus on abilities of these changes to be used for effective diagnostic and prognostic investigations.

**CLASSIFICATION AND SUBTYPES**

Renal cancer occurs both in hereditary and sporadic forms. Although renal cancer has many genetic predispositions, the hereditary form accounts only for 3%–4% of cases. The current major subtypes of renal cell tumors in the 2016 WHO classification are briefly summarized in Table 1, with a focus on their molecular pathological epidemiology. Common genetic changes and hereditary syndromes are summarized in Table 2.

In addition to above syndromes, chromosome 3p translocation (Cohen et al.,) tuberous sclerosis, and succinate dehydrogenase gene mutation (Ricketts et al.,) predispose to rare forms of early-onset hereditary renal cancer. Five genes that have well-known associations with renal cancer were shown to be mutated in a substantial proportion of the clear-cell renal cell carcinoma (ccRCC) samples (Gwangwu et al. 2012), including promoters VHL (altered in 27% of the 98 ccRCCs), TP53 (altered in 6%), and genes involved in chromatin modification, such as polybromo-1 (PBRM1) (altered in 21%), lysine-(K-) specific demethylase 5C (KDM5C) (altered in 9%), and SET domain-containing 2 (SETD2) (altered in 4%), along with two tumor suppressor genes, BRCA1-associated protein-1 (BAP1) (mutated in 8% of the 98 ccRCCs) and TSC1 (mutated in 3%).

In ccRCC, the VHL tumor suppressor gene is the most frequently mutated gene (Creighton et al. 2013) and its complete loss through genetic (point mutations, insertions and deletions (in dels), and 3p25 loss) and/or epigenetic (promoter methylation) mechanisms constitute the earliest, truncal oncogenic driving event (Hakimi et al., 2013). VHL is the substrate recognition component of an E3 ligase complex that ubiquitylates HIF1α and HIF2α for proteasome-mediated degradation (Masson et al., 2014). Large-scale cancer genomic projects have been undertaken and have revealed several novel prevalent mutations in ccRCC, including PBRM1 (29%–41% of tumor samples), SETD2 (8%–12%), BAP1 (6%–10%), KDM5C (4%–7%), and MTOR (5%–6%) (Xu et al., 2016). Sporadic ccRCC
has been characterized by loss of chromosome 3p in 90% of cases (Junker et al., 2003). Papillary RCC is characterized by trisomy of chromosome 7 and 17 and loss of Y chromosome (Kovacs et al., 1997). Chromophobe RCC (chRCC) exhibits multiple numerical deletions of chromosomes 1, 2, 6, 10, and 17 (Brunelli et al., 2010). Collecting duct carcinoma shows a wide variety of aberrations involving chromosomes 1, X, Y with either translocations or deletions. Furthermore, chromosomes 13 and 22 are affected (Antonelli et al., 2003).

Much research in recent years investigated the role of molecular markers in predicting prognosis and response to treatment in renal cancer. However, none of these markers has been translated into clinical practice or been proven to improve the predictive accuracy of existing prognostic models. Therefore, none of them is recommended for use in routine clinical practice (Tan et al., 2013). This gap in research and clinical practice could be explained by reasons such as methodological differences introducing bias, poor study design including small samples, lack of standardization of the assay employed, and unsuitable statistical analysis (McShane et al., 2005). In the 2016 WHO classification, seven new subtypes were adopted as shown in Table 3. The features of their molecular pathological epidemiology are briefly summarized in Table 4.

Several nomograms to predict the prognosis in RCC relying on clinical and pathological parameters have been
developed and externally validated. The integration of molecular or cytogenetic biomarker besides pathological and clinical parameters has been attempted to improve the prognostication of these nomograms (Karakiewicz et al., 2007, Kim et al., 2005, Klatte et al., 2009).[26–28]

The predictive accuracy of these nomograms ranged between 68% and 90%. The only cytogenetic marker added to a prognostic nomogram for all stages of ccRCC following nephrectomy, including TNM stage and Fuhrman grade, was the loss of chromosome 9p based on karyotyping, reaching predictive accuracy of 89% (Klatte et al., 2009a).[28]

Table 3: Classification of renal cell tumors according to the 2016 World Health Organization classification

<table>
<thead>
<tr>
<th>Current renal cell tumor subtypes</th>
<th>New renal cell tumor subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear-cell RCC</td>
<td>Multilocular cystic renal neoplasm</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>MIT family translocation RCC</td>
</tr>
<tr>
<td>Chromophobe RCC</td>
<td>Tubulocystic RCC</td>
</tr>
<tr>
<td>Collecting duct carcinoma</td>
<td>Acquired cystic disease–associated RCC</td>
</tr>
<tr>
<td>Renal medullary carcinoma</td>
<td>Clear-cell papillary RCC</td>
</tr>
<tr>
<td>Mucinous tubular and spindle cell carcinoma</td>
<td>Succinate dehydrogenase–deficient RCC</td>
</tr>
<tr>
<td>RCC, unclassified</td>
<td>Hereditary leiomyomatosis and RCC–associated RCC</td>
</tr>
<tr>
<td>Papillary adenoma</td>
<td>-</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>-</td>
</tr>
</tbody>
</table>

MiT: Microphthalmia transcription factor, RCC: Renal cell carcinoma

Table 4: New subtypes of renal cell tumors in the 2016 World Health Organization classification

<table>
<thead>
<tr>
<th>New renal cell tumor subtypes</th>
<th>Clinical features</th>
<th>Morphological/immunohistochemical features</th>
<th>Molecular features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilocular cystic renal neoplasm of low malignant potential</td>
<td>Excellent prognosis</td>
<td>Numerous cysts lined by clear cells; positive for CAIX and CK7</td>
<td>VHL mutation, chromosome 3p deletion</td>
</tr>
<tr>
<td>MIT family TRCC</td>
<td>Pediatric to young adult patients, mean age of 30 years</td>
<td>Papillary pattern, psammoma bodies, large epithelioid cells, and small cells; positive for TFE3 or TFEB</td>
<td>Xp11 TRCC: TFE3 rearrangement, t(6;12) RCC: TFE3 rearrangement</td>
</tr>
<tr>
<td>Tubulocystic RCC</td>
<td>Male predominance, mean age of 60 years, indolent</td>
<td>Dilated tubules with a single layer of cells</td>
<td>Gain of chromosomes 7 and 17; loss of chromosome Y and Y</td>
</tr>
<tr>
<td>ACD–associated RCC</td>
<td>End-stage renal disease or ACD, indolent</td>
<td>Eosinophilic cyttoplasm, sieve–like pattern, intratumoral oxalate crystals; positive for AMACR and CD10, negative for CK7</td>
<td>Gain of chromosomes 3, 16, and Y</td>
</tr>
<tr>
<td>CCPRCC</td>
<td>3%-4% of renal tumors, indolent, end-stage renal disease, VHL disease</td>
<td>Clear cyttoplasm, papillary pattern, apical–oriented nuclei; positive for CK7 and CAIX, negative for CD10</td>
<td>Lack of the genomic alterations observed in ccRCC/pRCC</td>
</tr>
<tr>
<td>SDH–deficient RCC</td>
<td>0.05%–0.2% of renal carcinomas, mean age of 37 years, good prognosis, germline mutation in one of the SDH genes</td>
<td>Cytoplasmic vacuoles and inclusion–like spaces; negative for SDHB, KIT, and CK7</td>
<td>Double-hit inactivation of one of the SDH genes, most commonly SDHB, no mutations in VHL, PIK3CA, AKT, MTOR, MET, or TP53</td>
</tr>
<tr>
<td>HLRCC–associated RCC</td>
<td>HLRCC syndrome, aggressive</td>
<td>Large nuclei with inclusion–like eosinophilic nucleioli and perinuclear clearing, abundant eosinophilic cyttoplasm, papillary/tubular pattern; positive for 2SC, negative for FH, CK19, 34betaE12, and CK7</td>
<td>Germline mutation in FH, metabolic shift to aerobic glycolysis, increased fumarate and Hif1A</td>
</tr>
</tbody>
</table>


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frozen, or formalin-fixed paraffin embedded), touch preps, or cell cultures

- Comparative genomic hybridization (CGH) [Figure 3] allows genome-wide screening for CNVs in solid tumors. Conventional CGH relied on two genomes, a test and a control, which are differentially labeled and competitively hybridized to metaphase chromosomes. In an attempt to improve the resolution of traditional CGH, scientists have developed a more advanced technique that combines CGH with microarrays technology. Array CGH relies on slides arrayed with small segments of DNA called probes as the targets of analysis instead of using metaphase chromosomes (Lucito et al., 2003). The main advantage of array CGH (aCGH) is its ability to identify aneuploidies, deletions, gains including duplications or amplifications of any locus represented on an array simultaneously.

- Microsatellites [Figure 4] are short tandem repeats of DNA sequences that are made of units of 1–4 nucleotides. The units can be repeated at variable rates at a given microsatellite leading to genetic polymorphism. Microsatellite analysis has been widely employed for mapping, to trace allelic inheritance, and to investigate the somatic loss of heterozygosity (LOH). Using paired control (blood or normal renal tissue) and tumor DNA, microsatellite analysis is a sensitive technique for detecting LOH in tumors. It is fast and inexpensive and can be performed on degraded DNA extracted from formalin-fixed paraffin-embedded tissue. Moreover, in comparison to aCGH and I-FISH, microsatellite typing can detect copy number neutral LOH.

- NGS: As mentioned previously, the high demand for low-cost sequencing has driven the development of high-throughput sequencing, which also goes by the term NGS. In image-guided biopsy of renal masses, performance of a custom NGS panel was evaluated for diagnostic and prognostic utility and it was found that targeted NGS can robustly detect genomic alterations requiring only limited DNA (Gowrishankar et al., 2016).

CYTOGENETICS IN SPORADIC RENAL CELL CARCINOMA-DISCUSSION

Many cytogenetic studies have investigated aberrations of chromosomes in relation to pathological parameters and clinical outcomes. The focus was mainly the most common subtypes of RCC: clear cell and papillary. Loss
of short arm of chromosome 3 is the most frequent chromosomal CNV in ccRCC, reported in more than 70% of sporadic cases. It distinguishes ccRCC from other subtypes and is associated with better survival in patients affected with it (Kroeger et al., 2013). The gain of region 5q31 was associated with prolonged survival in high-grade ccRCC (Gunawan et al., 2001). On the other hand, loss of chromosomes 4p, 14q, and 9p are associated with poor prognosis. However, 9p deletion was the only aberration that retained its prognostic significance in multivariate analysis (Klatte et al., 2009a). In addition, deletion of Y chromosome is a common nonrandom CNV observed in ccRCC (Kovač and Frisch, 1989). The loss of Y chromosome was associated with distant metastasis in ccRCC and some other adverse histological features. Trisomy of chromosome 7 is a frequent aberration in ccRCC (Klatte et al., 2009a). It has no known prognostic value in ccRCC. The gain of chromosome 8q which harbors the c-MYC oncogene was observed in 28 out of 336 tumors studied by karyotyping. This aberration was found to be associated with metastatic disease and risk of cancer-specific death, and on multivariate analysis was confirmed to be an independent prognostic factor (Klatte et al., 2012), summarized in Table 5.

The vast majority of papillary RCCs are sporadic with two recognized, histologically and cytogenetically different subtypes. The sporadic forms show frequently trisomy of chromosome 7 and loss of Y (Brown et al., 1997) which are commonly occurring also in the clear-cell subtype. Trisomy of chromosome 17 is present in 80% of papillary RCC, predominantly in Type I (Corless et al., 1996). Furthermore, loss of chromosome 9p has been reported and was associated with the more aggressive type II papillary RCC (Klatte et al., 2009, Sanders et al., 2002), summarized in Table 5.

CGH was used to detect specific alterations in each of RCC subtypes, in which clear-cell RCC showed −3p, +5/5q, −8p, −9, −14, −18; papillary (chromophilic) RCC gains of chromosomes 7, 17, 16, 3, 12; chRCC loss of chromosomes 1, 2, 6, 10, 13, 17, 21; renal oncocytes (Ros) loss of chromosomes 1/1p and 14. Furthermore, for clear-cell RCC, it was possible to define alterations which are associated with metastatic disease: Loss of 9, 10, 14 (Junker et al., 2003). Microphthalmia-associated transcription (MiT) family translocation RCC is an RCC subtype characterized by early onset. The MiT family of transcription factors—including MiTF, TFE3, TFEB, and TFEC—shares a basic helix-loop-helix DNA-binding domain and similar target genes (Kentarū et al. 2017).

The discovery of the VHL gene in familial and sporadic ccRCC has revolutionized treatment for advanced RCC. Targeted therapy aiming at suppressing angiogenesis through vascular endothelial growth factor (VEGF) or platelet-derived growth factor-mediated pathways has replaced immunotherapy such as interferon alpha and interleukin-2 as treatment for metastatic RCC. The current Food and Drug Administration approved targeted therapy drugs for RCC which are the tyrosine kinase inhibitors (sunitinib, sorafenib, pazopanib, and axitinib), monoclonal antibody to VEGF (bevacizumab), and the MTOR inhibitors (temsirolimus and everolimus) (Yap et al., 2015, Fishman et al., 2013). Targeted therapy has improved treatment outcome as the overall and cancer-specific survival of metastatic RCC patients has improved in the targeted therapy era compared to the immunotherapy era (Yap et al., 2015, Soerenson et al. 2014, Nelson et al. 2013).

NGS or exome sequencing studies have discovered several novel genes involved in chromatin modification which are mutated in ccRCC (Duns et al., 2012). The newly identified genes are PBRM1, AT-rich interactive domain-containing protein 1A, BAP1, SETD2, and KDM5C. PBRM1 mutations are found in up to 41% of ccRCC, making it the second most mutated gene after VHL (Veral et al. 2011). The roles of these chromatin modification genes and their proteins products are not fully understood yet, but various studies have shown that the mutational status of these genes may possess prognostic influence on ccRCC. Other genetic aberrations of interest, such as changes at chromosome regions 5q, 8p, 9p, and 14, may affect the prognosis of ccRCC. Copy number gains at 5q conferred a favorable

### Table 5: Chromosomal aberrations found in renal cell carcinoma subtypes

<table>
<thead>
<tr>
<th>RCC subtype</th>
<th>Chromosome</th>
<th>Aberration</th>
<th>Prognosis</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>ccRCC</td>
<td>3p21</td>
<td>Missense</td>
<td>Worse</td>
<td>Hakimi et al. 2013</td>
</tr>
<tr>
<td></td>
<td>1p36</td>
<td>Copy number loss</td>
<td>Worse</td>
<td>Lichner et al. 2013</td>
</tr>
<tr>
<td></td>
<td>5q31</td>
<td>Copy number gain</td>
<td>Better</td>
<td>Gunawan et al. 2001</td>
</tr>
<tr>
<td></td>
<td>9p</td>
<td>LOH</td>
<td>Worse</td>
<td>de Oliveira et al. 2014</td>
</tr>
<tr>
<td>pRCC</td>
<td>17</td>
<td>Polysomy</td>
<td>Better</td>
<td>Klatte et al. 2009</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>Loss</td>
<td>Worse</td>
<td>Jiang et al. 1998</td>
</tr>
<tr>
<td></td>
<td>3p</td>
<td>Loss</td>
<td>Worse</td>
<td>Klatte et al. 2009</td>
</tr>
<tr>
<td>chRCC</td>
<td>1, 2, 6, 10, 13, 17, 21</td>
<td>Loss</td>
<td>Worse</td>
<td>Yap et al. 2015</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>1/1p, 14</td>
<td>Loss</td>
<td>No change</td>
<td>Yap et al. 2015</td>
</tr>
</tbody>
</table>

LOH: Loss of heterozygosity, RCC: Renal cell carcinoma, ccRCC: Clear-cell RCC, pRCC: Papillary RCC, chRCC: Chromophobe RCC
prognosis whereas a loss had an adverse effect (Nagao et al. 2002).[^98] LOH in 8p, 9p, and 14q has been associated with higher grade, stage, unfavorable prognosis, and tumor recurrence (Presti et al. 2002).[^31] Potential candidate genes include CDK2NA (cyclin-dependent kinase inhibitor 2A) at 9p21 and HIF1A at 14q23.2 (Yap et al. 2015, Grady et al. 2001).[^40,^52]

Papillary RCCs frequently display gains of chromosomes 7 and 17 (Balint et al. 2009).[^33] Trisomies 7 and 17 discovered in small papillary renal cell neoplasia indicate that these genetic alterations may be involved in initial tumor development (Brunelli et al. 2003).[^54] At present, only one gene on chromosome 7 has been positively identified and linked to papillary RCC (pRCC). Hereditary pRCC associated with Type 1 tumors is caused by the mutation of the MET proto-oncogene at 7q31. An activating missense mutation of the MET gene and duplication of chromosome 7 along with the mutated MET gene were postulated to increase the oncogenic effect of MET (Fischer et al. 1998).[^83] MET mutation associated hereditary pRCC and sporadic pRCC are typically low grade, bilateral tumors with multiple lesions (Yap et al. 2015, Duns et al. 2012).[^40,^48]

Hereditary chRCC is found in individuals with Birt–Hogg–Dubé syndrome (BHD). Renal tumors of different histologies such as ccRCC, pRCC, chRCC, and oncocytoma have been reported in BHD sufferers with chRCC and oncocytomas being the predominant types (Pavlovich et al. 2005).[^54] Germline mutation of the BHD or folliculin gene was discovered and mapped to chromosome 17p11.2 in families with BHD syndrome (Schmidt et al. 2005).[^77] Common genetic alterations found in sporadic chRCC are the LOH at chromosomes 1, 2, 6, 10, 13, 17, and 21 (Brunelli et al. 2010).[^22] There is no difference in chromosomal loss pattern between eosinophilic and classic variants of chRCC Brunelli et al. 2005).[^88] One frequently mutated candidate gene identified in sporadic chRCC is TP53 at 17p13.1 (Gad et al. 2007).[^99] chRCC and RO pose a diagnostic challenge as both tumors have morphological overlaps. Correct diagnosis is important because RO is largely benign while chRCC is malignant. Losses of chromosomes 2, 6, 10, 13, 17, and 21, found in up to 93% of chRCCs, are not features of ROs and could be used to differentiate the two tumor types (Yap et al. 2015, Yusenko et al. 2009, and Tan et al. 2010).[^40,^50,^51]

**CONCLUSION**

Each RCC subtype has a distinctive pattern of genetic aberrations, although there are some overlaps in chromosomal and genetic changes. These genetic changes may play an important role in tumorigenesis and affect the progression or prognosis of the tumor. Hence, detection of genetic or chromosomal changes could be a useful diagnostic or prognostic tool as adjunct to conventional immunohistochemistry and histology. Identification of frequently mutated genes and affected signaling pathways also allows for the development of new therapeutic targets or personalized-targeted therapy for better management of advanced RCC.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**


40. Balint I, Szporar A, Jauch A, Kovacs G. Trisomy 7 and 17 mark
Quddus, et al.: Chromosomal aberrations in RCC


