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Review

Genomics and epigenomics of renal cell carcinoma

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ABSTRACT

Kidney cancer accounts for about 2% of all cancers and worldwide >250,000 new cases of kidney cancer are diagnosed each year. Renal cell carcinoma (RCC) is the most common form of adult kidney cancer and this review describes our current knowledge of the genetic and epigenetic basis of sporadic RCC. Though to date major advances in understanding the underlying the molecular basis of renal cell carcinoma (RCC) have often been derived from studies of rare familial forms of renal cell carcinoma, large-scale genomic and epigenomic studies of sporadic tumours are beginning to provide clearer pictures of the genomic and epigenomic landscape of RCC and the key pathways implicated in the initiation and progression of the disease. Although current knowledge of the molecular pathogenesis of RCC is incomplete, and mostly relates to clear cell (conventional) RCC, the next five years will see an unprecedented flood of genomic and epigenomic data and the key future challenges will relate to the utilisation of this data to develop novel genetic and epigenetic markers for diagnosis and prognosis and to develop novel targeted therapies in order to enable an age of personalised medicine.

1. Background

Renal cell carcinoma (RCC) is a genetically and hisotopathologically heterogeneous disorder. As described in detail elsewhere in this issue, RCC can be subdivided into a number of different histopathological entities of which clear cell/conventional RCC is the most frequent (approximately 75% of all cases) followed by papillary RCC (~15%) [1]. The significance of considering the histopathological subtype for renal cancer biology is illustrated by the observation that mutations in the von Hippel–Lindau (*VHL*) tumor suppressor gene (TSG) is the most common genetic event in the evolution of sporadic clear cell RCC, but is not a feature of non-clear cell RCC subtypes [2–4]. Although a full description of the genomics and epigenomics of RCC would cover all the histopathological subtypes, often only very limited information is available for the rarer forms of RCC and so this review will inevitably will concentrate mostly on clear cell RCC.

2. Germline variants and predisposition to renal cell carcinoma

Inherited predisposition to RCC may be caused by rare high penetrance monogenic variants or common low penetrance polygenic variants. Though only a small fraction of all RCC is accounted for by rare inherited monogenic disorders (e.g. von Hippel–Lindau disease (caused by mutations in the *VHL* gene, Birt–Hogg–Dubé syndrome (*FLCN*), hereditary leiomyomatosis renal cell cancer (FH), hereditary papillary RCC (*MET*), succinate dehydrogenase subunit disorders (*SDHB*, *SDHD*) etc.) these syndromes have been critical for understanding the molecular pathogenesis of both familial and sporadic RCC [5]. The clinical and molecular features of high penetrance RCC predisposition genes are reviewed elsewhere in this issue and are not covered in detail here other than to highlight that a key functional consequence of the inactivation of the *VHL* TSG is stabilization of the hypoxia-inducible transcription factors HIF-1 and HIF-2 which leads to activation of a wide repertoire of hypoxia response genes [6,7].

A meta-analysis of epidemiological studies reported that a positive family history of kidney cancer conferred a 2.2-fold increased risk of the disease [8]. However, candidate gene association studies, including those for high penetrance inherited RCC genes, did not identify significant common susceptibility alleles and the first two such loci were identified by a genome-wide association study (GWAS). Thus Purdue et al. [9] found that two loci at 2p21 and 11q13.3 were associated with RCC susceptibility at genome-wide significance. The two statistically significant 2p21 SNPs mapped to intron 1 of the *HIF2/EPAS1* gene which encodes the α subunit of the HIF-2 transcription factor previously implicated in RCC associated with VHL inactivation [6,10,11]. The second locus at 11q13.3 did not map to a known gene but intriguingly was ~200 kb from the Cyclin D1 gene (*CCND1*) that is a downstream oncogenic target of HIF-2 and has also previously been implicated in VHL-related

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tumourigenesis [12]. Recently a further GWAS study identified two common variants in *ITPR2* on 12p11.23 that were significantly associated with RCC risk [13]. As one of the linked SNPs had previously been associated with waist-hip-ratio in a GWAS study it was speculated that *ITPR2* might, at least partially, be associated with RCC risk through obesity-related pathways. This is suggested by the finding that obesity is associated with increased RCC risk in epidemiological studies [14].

Germline methylation of the *KILLIN* gene has been associated with susceptibility to RCC in patients with Cowden syndrome or Cowden-like syndrome (without *PTEN* mutations) and in non-syndromic RCC patients, though further studies are required to fully define the role of germline *KILLIN* epimutations in RCC [15,16].

3. Somatic genetic and epigenetic alterations in renal cell carcinoma

3.1. Cytogenetic abnormalities and DNA copy number alterations aberrations

Following reports of recurrent chromosome 3 rearrangements in sporadic renal cell carcinoma and constitutional translocations involving chromosome 3p in two families with hereditary renal carcinoma, Zbar et al. [17] demonstrated frequent chromosome 3 allele loss in sporadic RCC. Subsequent cytogenetic and molecular studies revealed that the key region of 3p allele loss appeared to be distal to 3p13 [18] and the gene for von Hippel–Lindau disease was mapped to chromosome 3p25 [19]. Apart from chromosome 3p loss, losses of sex chromosomes and chromosome 14 and trisomy 7 were among the early cytogenetic abnormalities described in sporadic RCC [18,20]. Detailed mapping of chromosome 3p allele loss in RCC did not demonstrate a single area of critical allele loss. Thus evidence was found for tumour suppressor genes at 3p12-p14, 3p21 and 3p25 [21–24]. Though the finding of frequent somatic *VHL* gene mutations in sporadic clear cell RCC established the VHL TSG as a key 3p RCC TSG, there was also evidence that other, more centromeric 3p TSGs were also implicated [25,26] and subsequently several candidate 3p RCC TSGs (in addition to *VHL*) have been identified (see below).

After the identification of chromosome 3p loss in RCC it was established that there were significant correlations between cytogenetic alterations and histopathological subtypes of RCC: thus chromosome 3p loss was associated with clear cell RCC [27] and papillary RCC with trisomy 7, chromophobe RCC with polysomy 7 whilst oncocytomas often did not have cytogenetic abnormalities [24,28,29].

A specific somatic cytogenetic abnormality occurs in a rare subset of papillary RCC. The Xp11.2 translocation typically occurs in children or young adults. The translocation breakpoint involves the *TFE3* gene at Xp11.2 and the translocation is associated with the formation of a fusion gene between *TFE3* and a variety of partners including *PRCC* (papillary renal cell carcinoma (translocation-associated)) (1q21), *ASPSCR1* (*ASPL*, alveolar soft part sarcoma chromosome region, candidate 1) (17q25), *SFPQ* ((*PSF*, splicing factor proline/glutamine-rich) (1p34), *CLTC* (clathrin heavy chain) (17q23) and *NONO* (non-POU domain containing octamer-binding) (Xq12). Xp11.2 translocation positive cancers account for <1% of all RCC and have an aggressive course. The translocation results in overexpression of the fusion protein and although Xp11.2 translocations are rare, expression of *TFE3* is a more common finding and can result from *TFE3* amplification [30].

The development of high resolution microarrays has greatly enhanced the ability to define copy number abnormalities in RCC subtypes. Using 250K SNP arrays to analyse clear cell RCC (both VHL-associated and sporadic), Beroukhim et al. [31] identified 14 regions of nonrandom copy-number change: 7 regions of amplification (1q, 2q, 5q, 7q, 8q, 12p, and 20q) and 7 regions of deletion (1p, 3p, 4q, 6q, 8p, 9p, and 14q). Specific candidate genes were highlighted in different regions such as *VHL* and *CDKN2A/CDKN2B* in the chromosome 3p and 9p deletion regions and *MYC* in the 8q amplification region. Dalgliesh et al. [32] found, using Affymetrix 6.0 SNP arrays, recurrent losses on chromosomes 3p, 4, 6q, 8p, 9p and 14q and gains on chromosomes 1q, 2, 5q, 7 and 12 with the most frequent changes being losses of 3p (>80% of cases), 6q, 8p and 14q (>30% of cases) and gain of chromosome 5q (∼50% of cases).

Microarray analysis of papillary RCC using 100k SNP arrays revealed losses on chromosomes 1, 5p, 6p, 9, 11q, 14q, 19p, 21q, and 22q and copy number gains on chromosomes 2, 7, 12, and 17q [33]. In addition, differences between the copy number profiles of Type 1 and Type 2 papillary RCC were detected: Type 1 papillary RCC harboured more frequent gains on chromosomes 3 and 7 and losses on chromosome 21q and the poorer prognosis Type 2 had more frequent chromosome 8q gains and chromosomes 6p, 9q, and 13q losses [33].

Analysis of chromophobe RCCs and oncocytomas was undertaken by Yusenko et al. [34] using 250k SNP arrays. Chromophobe RCCs showed multiple abnormalities (most commonly loss of chromosomes 1, 2 and 10 but also 3, 5, 6, 9, 13, 17 and 21). A small number of tumours demonstrated homozygous loss at a variety of loci including chromosomes 1p22, 2q22.3-q23.2, 10q11.23-q22.3, 2q13 and 21q21.3-q22.11. Oncocytomas demonstrated a much lower frequency of copy number alterations though loss of the entire chromosome 1 occurred in a third of cases.

A small number of studies have investigated the copy number abnormalities in familial forms of RCC. Analysis of RCC from VHL disease patients with 250K SNP arrays reveals, as expected, that 3p loss is the most frequent change followed by 5q gain [31,35]. Most RCC in VHL disease patients are detected presymptomatically and removed when ∼3 cm in diameter whereas only a minority of sporadic RCC are detected <3 cm and so, on average, VHL-associated RCC are smaller and have fewer copy number abnormalities than reported in sporadic RCC. Nevertheless, in general, most of the copy number changes observed in VHL-associated clear cell RCC also occur in sporadic clear cell RCC with VHL inactivation [35]. In contrast, copy number analysis of RCC from patients with hereditary leiomyomatosis RCC syndrome associated with germline *FH* mutations were reported to demonstrate a distinct copy number profile (losses on chromosomes13, 14, 18, and X and gains on chromosomes 2, 7 and 17) compared with sporadic RCC tumours of the same histopathological subtype [36].

4. Somatic mutations

The frequent loss of chromosome 3p in clear cell RCC, the mapping of the VHL gene to 3p25 and the observation that statistical analysis of the age-at-onset of RCC in patients with VHL disease and sporadic cases was compatible with a one and two-hit model of tumourigenesis (as described for retinoblastoma) [17,19,37] paved the way for the finding (following the identification of the *VHL* tumour suppressor gene [90]) that somatic inactivation of *VHL* is frequent in sporadic clear cell RCC [2,3]. Since then a large repertoire of somatic VHL mutations have been identified in clear cell RCC (though not in other histopathological subtypes) and improvements in mutation detection methodologies have seen the reported frequency of VHL mutations in clear cell RCC increase to ∼75% (with ∼85% having biallelic inactivation by loss/mutation/promoter methylation) [38].

In the past 10 years multiple functions have been attributed to the *VHL* TSG product (pVHL) but the best characterised is its role as a substrate recognition component of an E3 ubiquitin protein ligase