The Importance of Pathology and Genetics for the Diagnosis and Therapy of Renal Cell Carcinoma

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Abstract

Recent efforts in cytogenetic and molecular genetic research have brought insights and correlations of phenotypes and genotypes in renal cell carcinoma (RCC). Based on the knowledge of these different cytogenetic pathways, an intensive research investigated molecular changes that were associated with these chromosomal aberrations. This finally led to the discovery of important tumour suppressor genes such as the von Hippel-Lindau gene (VHL) in clear-cell RCC and oncogenes that are involved in cell cycle regulation and differentiation. Our refined understanding of the molecular pathways involved in renal carcinogenesis has provided a rationale for novel therapeutics that specifically target molecules of aberrantly activated pathways. This article provides an overview of pathology and genetics of renal neoplasms, insights into the pivotal role of the VHL protein, a description of techniques to identify genes that might play a role in tumour biology or represent candidate diagnostic markers, and a summary of molecular targets for the therapy of RCC.

1. Pathology and genetics of renal cell carcinoma

Progress in pathology and genetics during the past 20 yr has led to a tremendous multiplication of our knowledge of renal cell carcinoma (RCC) and adenoma [1]. Up to that time RCC was understood as a single entity, whereas today we are dealing with a broad spectrum of different tumours with various phenotypes and at least genotypes. The basis of the morphologic classification of renal tumours published by the World Health Organization (WHO) in 2004 was the systematic pathologic analysis by Thoenes and coworkers [1]. Table 1 provides an overview of the most often found renal tumour types with respect to the International Classification of Diseases for Oncology (ICDO) classification and expected incidence [2–4]. Some of these tumours, such as the Xp1.2 translocation carcinoma and the metanephric adenofibroma, predominantly affect children and young adults, whereas papillary adenomas are usually found in patients older than 70 yr. Each tumour type presents a special histologic spectrum of phenotypes that is accompanied by the

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expression of a certain spectrum of antigens that can be detected immunohistologically (among others for differential diagnostic purposes). As a rule, the tumour types no.1, 2, and 8 from Table 1 share antigens with the proximal tubular system of the kidney, whereas tumour types no 3, 4, 5, and 12 share antigens with the distal tubular system and collecting duct of the kidney. Furthermore, there are some clues to the histogenesis of these tumours because for some clear-cell RCC and for some oncocytic adenomas their related nephron segments are involved in a special chemical carcinogenesis [4]. This has been proven for the trichloroethylene exposure, which results in typical clear-cell RCC.

Generally speaking, nearly each renal cell tumour type occurs in a sporadic or in a hereditary form. This has led to great efforts in cytogenetic and molecular genetic research, which has brought deep insights and correlations of phenotypes and genotypes in RCC. Table 2 presents an overview of the hereditary renal tumour syndromes and their morphologic and clinical manifestations. With respect to these findings, the cytogenetic background of renal carcinogenesis became more important as questions of an adenoma/carcinoma sequence and initiation steps and tumour progression were analysed. It should be noted that this research is still ongoing and is completed by new molecular genetic results. As we distinguish a couple of different RCCs morphologically (Table 1) the corresponding genetic model is still fragmentary and limited (Table 3) [5]. Based on that genetic background, there are some chromosomal markers associated with prognosis. In clear-cell RCC loss of chromosome 9p and 10q as far as 14q results in a poor prognosis. Looking at papillary carcinomas type 2 mutations of the fumarate hydratase gene on chromosome 1q42-43 and loss of heterozygosity (LOH) 9p13 account for a poor prognosis also. Tremendous loss of chromosomes (monosomies) indicates progression from oncocytic adenoma/hybrid tumour to chromophobe RCC (cRCC).

Another result of the intensive morphologic and genetic research of recent years was the characterisation of the antigen spectrum of the different tumour types mentioned above, which has led to immunohistologic profiles for differential diagnosis. For example α-methyl acyl-coenzyme A racemase (AMCAR) expression is typical for papillary RCCs, TFE3 expression is typical for translocation carcinomas, CD117 (c-kit) expression is typical for chromophobe carcinomas, and RON is typical for oncocytic adenomas and chromophobe RCCs.

Based on the knowledge of these different cytogenetic pathways, there was an intensive research for molecular changes that were associated with these chromosomal aberrations. This finally led to some important tumour suppressor genes such as the von Hippel-Lindau gene (VHL) in clear-cell RCC, among others, and oncogenes that are involved in cell cycle regulation and differentiation [6,7]. The following discussion will focus on this question.

2. Understanding the function of the VHL protein in renal cancer: prerequisite for the novel therapeutic interventions

Between 24% and 45% of patients with VHL develop clear-cell RCC. Inactivating germline mutations of the VHL gene represents the genetic hallmark of this syndrome and have been demonstrated in almost all patients with VHL. Sporadic clear-cell RCC (the most
frequent subtype of sporadic renal cancer [8]) is characterised by inactivation of the VHL gene by deletion, mutation, or promoter hypermethylation in about 70% of the tumours.

The functions of the VHL protein (pVHL) have been extensively studied in the last 15 yr. The pVHL is implicated in cell cycle control and gene regulation and requires transcription-dependent nuclear–cytoplasmic trafficking for its function. There are two biologically active VHL protein isoforms: pVHL(30) and pVHL(19). The distribution of pVHL isoforms varies in the nuclear and cytoplasmic compartments of renal tumours and alteration of subcellular pVHL trafficking is of potential relevance for the biologic behaviour of clear-cell RCC [9].

The pVHL functions as a recognition subunit in an E3 ubiquitin protein ligase complex, targeting the hypoxia-inducible transcription factor Α (HIF-α) for ubiquitin-mediated degradation in the presence of oxygen [10]. Under normoxic conditions, hypoxia-inducible factor 1 (HIF-1) is hydroxylated (-OH) on two conserved proline residues by a family of prolyl hydroxylases at its oxygen-dependent degradation domain. This hydroxylation provides a substrate-recognition site for the VHL E3 ubiquitin ligase complex, which contains elongins C and B, cullin-2 (CUL2) and RBX1. Polyubiquitinylation of HIF1- by the VHL complex leads to its proteasomal degradation by the 26S proteasome. Hypoxic conditions block hydroxylation, allowing HIF-1 subunits to accumulate and activate transcription of hypoxia-responsive genes [11]. VHL inactivation, as occurs in renal cells from patients with a germline VHL mutation and loss of the wild-type allele, mimics the hypoxic response by preventing degradation of HIF-1 subunits. Loss of VHL function causes accumulation of HIF-1 subunits in the cytoplasm and their translocation to the nucleus. HIF-1 dimerises with HIF-1 and is coactivated by CBP/p300. HIF binds to hypoxia response elements (HREs) in gene promoters, thereby activating transcription of genes up-regulated in clear-cell renal tumours, including

Table 2 – Hereditary renal cell tumours

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Chromosome</th>
<th>Gene</th>
<th>Protein</th>
<th>Tumour type</th>
<th>Extrarenal manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dermis</td>
</tr>
<tr>
<td>von Hippel-Lindau</td>
<td>3p25</td>
<td>VHL</td>
<td>pVHL</td>
<td>Multiple, bilateral clear-cell RCC, renal cysts</td>
<td>—</td>
</tr>
<tr>
<td>Hereditary papillary RCC</td>
<td>7p31</td>
<td>c-MET</td>
<td>HGF-R</td>
<td>Multiple, bilateral papillary RCC (type 1)</td>
<td>—</td>
</tr>
<tr>
<td>HLRC</td>
<td>1q42</td>
<td>FH</td>
<td>FH</td>
<td>Papillary RCC (non-type 1)</td>
<td>—</td>
</tr>
<tr>
<td>Familial papillary thyroid carcinoma</td>
<td>1q21</td>
<td>?</td>
<td>?</td>
<td>Papillary RCC, oncocytomas</td>
<td>—</td>
</tr>
<tr>
<td>Hyperpara-thyroidism, jaw tumour (HP-JT)</td>
<td>1q25</td>
<td>HRPT2</td>
<td></td>
<td>Epithelial-stromal mixed tumours, papillary RCC</td>
<td>—</td>
</tr>
<tr>
<td>Birt-Hogg-Dubé</td>
<td>17p11</td>
<td>BHD</td>
<td>Folliculin</td>
<td>Multiple chromophobe RCC, onco-cytic adenoma, papillary RCC</td>
<td>Facial fibrofolli-culoma</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>9p34</td>
<td>TSC 1</td>
<td>Hamartin</td>
<td>Multiple, bilateral angiomyolipomas, lymphangioleio-myomatosis; rare clear-cell RCC</td>
<td>Angio-fibroma; peau chagrin; subungual fibroma</td>
</tr>
<tr>
<td>16p13</td>
<td>TSC 2</td>
<td>Tuberin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constitutional translocation chromosome 3</td>
<td>3p13-14</td>
<td>?</td>
<td>?</td>
<td>Multiple, bilateral Clear-cell RCC</td>
<td>—</td>
</tr>
</tbody>
</table>

VHL = von Hippel-Lindau; RCC = renal cell carcinoma; CNS = central nervous system; HLRC = hereditary leiomyomatosis renal cell carcinoma.
vascular endothelial growth factor (VEGF), erythropoietin, and platelet-derived growth factor (PDGF) [12]. Fig. 1 provides a simplified scheme of these events, which form the basis for therapeutic intervention. Clear-cell RCC is characterised by rich neovascularisation and an often prominent vascular network around tumour cells, suggesting the elaboration of tumour angiogenesis factors by neoplastic cells. It has been demonstrated that strong vascularisation in clear-cell RCC is correlated with VEGF expression, suggesting that this growth factor plays a role in the vascular biology of clear-cell RCC tumours as a consequence of VHL inactivation [13]. VEGF stimulates endothelial cell proliferation in vitro and has also an angiogenic activity in vivo.

Recently, it was shown that loss of VHL function results in strongly enhanced transcription of HIF-a-inducible genes, especially in up-regulation of CXCR4 [14]. Therefore, the VHL tumour suppressor gene product is one of the major regulators of CXCR4 expression and increased CXCR4 expression levels are most likely a consequence of impaired VHL function in clear-cell cRCC. Strong CXCR4 expression is associated with poor prognosis of RCC. Thus, by expressing CXCR4, tumours obviously acquire properties that enable them to invade tissue barriers, migrate to secondary organs, and form metastases. Although CXCR4/CXCL12 expression patterns may explain selection of specific organs for the formation of metastasis, the exact molecular mechanisms by which the CXCR4/CXCL12 axis promotes tumour invasion are still unclear.

Other pVHL functions include fibronectin matrix assembly, p53 stabilisation, and transactivation [15,16]. In addition, pVHL has the ability to bind and stabilise microtubules by protecting them from depolymerisation, which is a prerequisite for cilia formation [17]. In fact, two previous in vitro studies showed that by re-expressing pVHL in VHL null clear-cell RCC cell lines, pVHL regulates the formation of primary cilia [18,19]. These observations strongly suggest that loss of VHL function in renal epithelial cells leads to degeneration of primary cilia, which represents a critical step towards cyst formation and clear-cell RCC development in patients with VHL. Interestingly, renal cysts are also present in about 60% of individuals suffering from the VHL disease. One might hypothesise that cyst formation is one of the first visible renal alterations in VHL-caused tumour formation.

The elucidation of the different pVHL functions is the basis for understanding the novel therapeutic strategies for patients with RCC [20]. Targeting the VHL pathway for therapeutic intervention can theoretically occur at many sites. VHL protein function could be replaced, restoring binding to HIF-1 and allowing its proteasomal degradation. Further, the activity of HIF-1 could be a target for inhibition. Finally, molecules up-regulated by HIF-1 (eg, CXCR4) also provide specific targets for potential downstream inhibition of the VHL pathway.

3. Expression profiling of RCC

Array-based expression profiling, introduced in the late 1990s, is a powerful technique to characterise the transcriptome of tumours. It allows for a molecular classification of tissues and tumours and is an excellent research tool to identify differentially expressed genes that might play a role in tumour biology or represent candidate diagnostic markers or therapy targets. The first gene expression profiling study in RCC was published in 2001 by Young et al [21]. They analysed a total of seven samples consisting of clear-cell RCC, cRCC, and oncocytoma with matching normal renal tissue and found distinct differences in gene expression among these entities. Clear-cell RCC could be distinguished from chromophobe RCC/oncocytoma by its expression of vimentin, whereas
galectin-3 expression was more prevalent in oncocytoma/cRCC. In the same year Takahashi et al published the first correlation of RCC expression profiles with clinical follow-up data and could define a group of transcripts that predicted patient risk of progression more accurately than conventional tumour staging in a small cohort \((n = 29)\) [22]. Candidate diagnostic markers were identified by the same group in a larger study \((n = 70)\): glutathione S-transferase was highly expressed in clear-cell RCC, AMCAR was typical of papillary RCC and carbonic anhydrase II was found in cRCC [23]. The studies of Vasselli et al (58 tumours) and Jones et al (49 RCCs) focused on a cluster- or signature-based prediction of patient prognosis [24,25]. Importantly, both found prognostic groups of genes that await further confirmation, ideally in prospective studies.

In an array-based profiling study of RCC conducted at the Charité (Berlin), we also found a variety of differentially expressed genes, which are currently being validated. An example is the overexpression of stanniocalcin 2 (STC2), which was prognostic of shorter patient survival (manuscript in preparation).

### 4. Molecular targets in RCC

Conventional cytotoxic chemotherapy or antihormonal therapy is largely ineffective in metastatic RCC [26]. In selected patients cytokine therapy has been the only effective option so far but was limited by an inconvenient toxicity profile [27]. Our refined understanding of the molecular pathways involved in renal carcinogenesis has provided a rationale for novel therapeutics that specifically target molecules of aberrantly activated pathways.

The VHL tumour suppressor gene is epigenetically silenced or mutated in the majority of clear-cell RCCs. As described, the decreased pVHL expression leads to a stabilisation of the HIF-\(\alpha\)

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**Table 3 – Cytogenetic model of epithelial renal cell tumours**

<table>
<thead>
<tr>
<th>Phenotypic Association</th>
<th>Initiation</th>
<th>Promotion</th>
<th>Adenoma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>(proximal tubule system)</td>
<td>VHL-Mutation</td>
<td>-3p, -2p13</td>
<td>Clear-cell nphrogenic papillary tumour</td>
<td>Clear-cell translocation tumour</td>
</tr>
<tr>
<td>?</td>
<td>c-Met-Mutation</td>
<td>-7, +17, -Y.</td>
<td>tub. muc. tumour</td>
<td>tub. muc. tumour</td>
</tr>
<tr>
<td>Intercalated Cell collecting duct system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principal Cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4 – Selected novel therapeutic agents for metastatic renal cell carcinoma**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Class</th>
<th>Target molecule</th>
<th>Clinical trial phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab</td>
<td>Monoclonal antibody</td>
<td>EGFR</td>
<td>2</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>Monoclonal antibody</td>
<td>EGFR</td>
<td>2</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Monoclonal antibody</td>
<td>VEGF</td>
<td>2, 3</td>
</tr>
<tr>
<td>G250</td>
<td>Monoclonal antibody</td>
<td>CAIX</td>
<td>2</td>
</tr>
<tr>
<td>Sorafenib (RAY 43-9006)</td>
<td>Small molecule</td>
<td>VEGFR 2,3, PDGFR, Raf-1, fli-3</td>
<td>2, 3</td>
</tr>
<tr>
<td>Sunitinib (SU11248)</td>
<td>Small molecule</td>
<td>VEGFR 1-3, PDGFR, c-kit, fli-3</td>
<td>2, 3</td>
</tr>
<tr>
<td>Valatanib (PTK787/ZK)</td>
<td>Small molecule</td>
<td>VEGFR 1-3, (PDGFR, c-kit)</td>
<td>1</td>
</tr>
<tr>
<td>Temsirolimus (CCI-779)</td>
<td>Small molecule</td>
<td>mTOR</td>
<td>2, 3</td>
</tr>
<tr>
<td>Lapatinib (GW572016)</td>
<td>Small molecule</td>
<td>EGFR/ErbB-2</td>
<td>2, 3</td>
</tr>
</tbody>
</table>

EGFR = endothelial growth factor receptor; VEGF = vascular endothelial growth factor; CAIX = carboxy anhydrase IX; VEGFR = vascular endothelial growth factor receptor; PDGFR = platelet-derived growth factor receptor; mTOR = mammalian target or rapamycin.
and consequently to the transcription of HIF-α target genes, many of which are involved in tumour-promoting processes such as proliferation, angiogenesis, and cell motility [28]. Targeting the transcription factor HIF directly is difficult, but a variety of agents have been identified that down-regulate HIF-α levels indirectly, for example, inhibitors targeting the mammalian target of rapamycin (mTOR) or heat shock protein 90 (HSP90) [29,30]. Another approach is to target HIF-α-regulated genes directly. HIF-α-responsive genes of major importance in tumour biology are VEGF, PDGF, transforming growth factor (TGF-α), epidermal growth factor receptor (EGFR, ErbB-1), and carbonic anhydrase IX (CAIX).

4.1. Target molecules

VEGF is a glycoprotein with several isoforms (VEGF-A–E) that plays an important role in tumour-associated angiogenesis. After binding to the extracellular domain of its receptor (VEGFR), downstream genes are expressed. VEGF is overexpressed in RCC and has prognostic properties [31]. It can be inhibited by various small molecules (see below) but also by the recombinant antibody bevacizumab (Avastin, Genentech).

PDGF is primarily expressed in platelets and has five isoforms (PDGF-A–E) that bind to three different receptors (PDGFR-α, PDGFR-β, and PDGFR-αβ). It functions in the regulation of cell growth and apoptosis. PDGFR-α correlates to higher Fuhrman grades and appears to be a prognostic marker in RCC [32].

TGF-α is a growth stimulatory protein overexpressed in RCC, which is also induced by hypoxia [33]. TGF-β is principally an inhibitory growth factor that is paradoxically overexpressed in various malignant tumours including RCC [33,34].

EGFR is overexpressed in the majority of RCC and is considered an important target in neoplasia for small molecules and monoclonal antibodies (e.g., panitumumab, Amgen; cetuximab, ImClone Systems) alike [35,36].

CAIX is a transmembrane enzyme involved in regulation of membrane ion channels and extracellular pH and is expressed in most normal tissues. CAIX is overexpressed in >90% of clear-cell RCC but is usually not found in normal tubulipus epithelium. Interestingly, in RCC it has been found as a negative marker of prognosis and is discussed as a predictive marker of interleukin 2 (IL-2) therapy response [37,38].

mTOR is a tyrosine kinase centrally involved in cell proliferation and apoptosis. Inhibiting mTOR by rapamycin or its analogues also has antiangiogenic effects, probably the result of an indirect VEGF down-regulation [39].

4.2. Other target genes not associated with HIF-α

KIT is a type III tyrosine kinase receptor that is overexpressed in cRCC and oncocyto ma, but rarely in clear-cell RCC [40].

Cyclooxygenase 2 (COX-2) is the inducible isoform of cyclooxygenases and is up-regulated in many malignant tumours including RCC. Possibly, COX-2 is a predictive marker for therapy response to COX inhibitors in RCC [41].

The matrix metalloproteinases (MMPs) are commonly up-regulated in malignant tumours and are associated with an invasive phenotype. In RCC they have been associated with a poor prognosis [42] and attempts to target MMPs therapeutically are underway [43].

5. New therapeutic options in RCC

Monoclonal antibodies targeting some of the aforementioned structures and a large variety of small molecules inhibiting tyrosine kinases have been developed and are currently being evaluated. Tyrosine kinases phosphorylate hydroxyl groups of tyrosine residues on signal transduction molecules, and thus activate multiple cellular pathways relevant to cell proliferation, motility, and angiogenesis [44]. Important pathways in RCC are the RAS/Raf/MEK/ERK signal transduction pathway and the already mentioned mTOR pathway [45,46]. Prominent examples of small molecules to interfere with their function are listed in Table 4. The clinical results of targeted therapy in RCC are subjects of a separate article in this supplement.

References


