Abstract. Several genes play essential roles in human development and alteration of their expression or regulation leads to various pathologies. This review examines the literature on the expression and the roles of neurogenic locus notch homolog protein (NOTCH), sonic Hedgehog (SHH) and wingless-type (WNT) pathways, as well as the nephrogenic transcription factors Wilms' tumor 1 (WT1), paired box 2 (PAX2) and homeobox protein lim-1 (LIM1) in clear cell renal cell carcinoma. Besides being re-expressed in human tumors, the inhibition of these factors has strong antitumor activity both in vitro and in vivo. Interestingly, these pathways are also part of the molecular network involved in the development of organs including nephrogenesis. The identification of developmental pathways involved in clear cell renal cell carcinoma growth places an additional piece into the molecular puzzle of cancer mechanisms. Moreover, the evaluation of these molecules could pave the way to innovative and safe therapies for this refractory disease. Valuable prognostic markers might also be identified through these studies. Finally, the proof of concept in other types of cancer is reviewed.

Renal cell carcinoma (RCC) is the most lethal urological tumor and the sixth leading cause of cancer deaths in Western countries. Each year, approximately 270,000 patients are diagnosed with this malignancy, resulting in an estimated of 110,000 deaths, and its incidence is steadily increasing (1-3). Eighty percent of RCCs are clear cell renal cell carcinoma (CCC) and are thought to originate from the renal proximal tubule. RCC is resistant to radio-, hormono-, and chemotherapy, and immunotherapy is effective in only 15% of selected patients (1-3).

The best known oncogenic signal in human CCC is constituted by the von Hippel-Lindau (VHL) tumor suppressor gene and hypoxia-inducible factors (HIFs). Inherited and sporadic forms of CCC are associated with inactivation of the VHL gene (4). In hypoxic conditions or with VHL gene defects, as is the case in 60% of CCCs, HIF-α is stabilized, allowing for the expression of a large panel of target genes involved in growth, motility, metabolism and angiogenesis, such as vascular endothelium growth factor (VEGF), tumor growth factors (TGFs), parathyroid hormone-related protein (PTHrP), and glucose transporters, all shown to contribute to CCC (5, 6).

Recent advances in our understanding of the HIF molecular network have led to several novel targeted therapies. Drugs that modulate the downstream targets of the HIF pathway, including sunitinib, sorafenib, temsirolimus, everolimus, axitinib and bevacizumab, have proven beneficial in treating CCC. However, drug-induced resistance observed after a few months of treatment with these compounds and the lack of validated biomarkers restricts our ability to tailor specific drugs to patients and this might be considered as the most important barrier to a better clinical response (4, 7, 8). Thus, treating RCC represents a challenge that pushes the scientific community to be more effective in discovering new therapeutic targets against this refractory disease.

A Novel Perspective to Explore

There are three questions that remain in the field of biological and medical sciences: (i) How is it possible that one cell or the recruitment of a few of them may lead to the development of a functional organ? (ii) How is it possible that one cell or few cells starting to act differently may lead to the development of pathologies? The third question arises directly from the first
two: (iii) What are the molecular and cellular mechanisms involved in these profound modifications? Recent findings in oncology point to a new perspective for exploration, by combining the first two questions with the third: Are the developmental pathways involved in the genesis and the growth of an organ responsible for the development and the growth of a tumor, as might be the case for human CCC?

As the formation of the kidney is very well-documented in literature, it is not surprising that this organ serves as an example to uncover the links between organogenesis and pathologies from a mechanistic point of view. Additional oncogenic events are required for CCC formation, and this concept has been clearly proven recently by molecular and genetic approaches (9-11). The emerging idea is that tumors hijack signaling pathways involved in normal development for their own growth. Two categories of actors have been revealed so far: the first includes transcription factors and the second is represented by complex signaling pathways. Regarding the kidney, this is at least the case for paired box 2 (PAX2), homeobox protein lim-1 (LIM1) and Wilms’ tumor 1 (WT1) transcription factors, and the Sonic Hedgehog (SHH), neurogenic locus notch homolog protein (NOTCH) and wingless-type (WNT) signaling pathways. Keeping in mind that it is not only the presence but also the level of expression/activation of the proteins that allow them to regulate mechanisms, here we aim to review their role in kidney tumorigenesis in the light of their involvement in normal developmental processes (Figure 1 and Table 1).

**Developmental Pathways and Markers during Nephrogenesis**

The study of the development of the mammalian kidney has helped elucidate the general concepts of mesenchymal-epithelial interactions, epithelial cell polarization and branching morphogenesis (12-15). The early molecular events occurring in the developing kidney are becoming increasingly clear through the use of genetically engineered mouse models, experimentations on frog and chick embryos, as well as through the identification of human genes responsible for renal disease.

Early patterning of the kidney region depends on interactions between PAX/eye absent homolog (EYA)/sine oculis homeobox homolog (SIX) transcription factors, with essential roles for LIM1 and odd-skipped related 1 (ODD1) transcription factors (15-18). The proto-oncogene c-Ret (RET)/ glial-derived neurotrophic factor (GDNF) (receptor/ligand) pathway, which is subject to ‘on and off’ regulations by a broad range of developmental factors and signaling pathways, controls bud outgrowth and branching morphogenesis (15, 19). One of the most important factors during nephrogenesis is *LIM1* (also known as LHX1). This gene was described for the first time in *Xenopus*. It belongs to the class LIM-hd in the homeobox gene family (20). LIM1 transcription factor is expressed throughout the urinary tract during development (16). Although most *Lim1*−/− mice embryos died at the embryonic stage E10 because of a disability in the forming placenta, the few surviving newborns had no kidney (21).

An important actor of nephrogenesis for the regulation of mesenchymal-to-epithelial transition and the development and maturation of podocytes is the zinc finger protein WT1, also identified as a tumor suppressor protein. While the protein is barely detectable in newborns, the pattern of expression during the early steps of the development of the organs and its interactions with the WNT proteins and PAX2 suggests a crucial role for this protein during kidney development. In accordance with this statement, it is important to notice that *Wt1*-null mutant mice fail to develop kidneys (22, 23).

It has been proven that WNT proteins are involved in the induction of the kidney mesenchyme. Patterning along the proximal-distal axis, as the nephron develops, mostly involves NOTCH signaling along with additional markers (24). This is particularly interesting since CCC develops from proximal tubular epithelial cells, as mentioned above. Interestingly, LIM1 transcription factor controls the activity of the NOTCH signaling pathway during tubule patterning.

In addition to the NOTCH signaling pathway, which is critical for cell–cell communication and the regulation of a broad spectrum of cell fate specifications (25), the involvement of the phosphoinositide-3-kinase (PI3K)/protein kinase B alpha (AKT), nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB), mitogen-activated protein kinase (MAPK), bone morphogenetic proteins (BMPs) and Jun kinase pathways in the different steps of kidney development has been well-documented (26-28). The SHH-glioma-associated oncogene homolog (GLI) signaling pathway, discovered in 1993, carries-out essential roles in cell differentiation and proliferation during embryogenesis (29). The SHH-GLI pathway mainly controls the expression of three classes of genes in normal kidney development: the genes responsible for the spatial organization and the segmentation *PAX2* and sal-like protein 1 (*SALL1*); the modulators of the cell cycle (cyclin D1 and *N-MYC*); and the effectors of the SHH pathway itself: GLI1 and GLI2. It is noteworthy that the SHH-GLI pathway plays an orchestral role in the activation of several signaling pathways (29) mentioned above.

With the advance of various expression arrays, nephrogenesis is becoming one of the best characterized organ systems in developmental biology, despite the kidney’s architectural complexity.

**PAX2 and the Proof of Concept**

Human CCC remains a challenge for clinicians and scientists working in this field. Indeed, as presented above, CCC is not only characterized by a high intrinsic resistance but also by
therapy-induced resistance; the mechanisms accounting for such characteristics have not received much attention yet, and remain largely unknown. The developmental signaling pathways/markers described above may constitute the keys to uncovering the mechanisms of tumor resistance, and thus lead to innovative and efficient therapies for human CCC, either alone or in combination.

Following the findings that PAX2 is re-expressed in nearly 80% of CCCs (30, 31), the best example of such a concept has been described by Hueber and colleagues (32). Indeed, in
this study, PAX2 was shown to confer resistance to cisplatin-induced apoptosis in non-transformed kidney cells and fetal kidney explants, and its overexpression in CCC cells also contributed to cisplatin resistance. Thus, from a therapeutic point of view, a strategy of inactivating PAX2 expression/activity might enhance the efficacy of cytotoxic drugs against CCC. To explain the antitumor activity induced by the targeting of PAX2, several targets have been unveiled, such as metalloproteases (disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), phosphatase and tensin homolog (PTEN) and the PI3K-AKT pathway (33, 34). It should be stressed-out that the regulation of PAX2 expression in CCC is at least under the control of the VHL/HIF system and hypoxia, although no induction was obtained despite the presence of six hypoxia response element (HRE) motifs in the promoter of PAX2 (35, 36). In addition, the PAX2 repressor WT1 and hypo/hypermethylation were not important for transcriptional regulation of PAX2. Nevertheless, this latter study suggested that VHL loss and hypoxia might be part of the cellular machinery leading to the re-expression/reactivation of developmental markers and clearly of developmental signaling pathways through effects, for example, on growth factor receptors. In addition, further investigations on PAX2 concluded that this actor of the kidney development described recently as an oncogene, is a very useful histological marker to differentiate between various forms or stages of renal cancer (37-43).

**Table I. Regulatory molecules involved in nephrogenesis and in RCC and their cellular effects during the processes.**

<table>
<thead>
<tr>
<th>Regulatory molecule</th>
<th>Roles in kidney development</th>
<th>Roles in carcinogenesis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX2</td>
<td>Epithelial differentiation of the metanephric mesenchyme; cell proliferation; cell adhesion</td>
<td>Involvement in RCC resistance to chemotherapy</td>
<td>Reidy and Rosenblum (15) Hueber et al. (32) Hueber et al. (36)</td>
</tr>
<tr>
<td>WT1</td>
<td>Epithelial differentiation of the metanephric mesenchyme; ureteric bud ingrowth in the metanephric blastema; repressor of myogenic cell fates; podocyte and epithelial differentiation</td>
<td>Promotes cell survival and cell proliferation</td>
<td>Kreiberg et al. (51) Md Zin et al. (23) Kawakami et al. (44)</td>
</tr>
<tr>
<td>LIM1</td>
<td>Growth of the ureteric bud; formation of the collecting system</td>
<td>Promotes cell proliferation; inhibits cell apoptosis; stimulates cell migration/invasion</td>
<td>Sitaram et al. (50) Reidy and Rosenblum (15) Kobayashi et al. (16) Varis et al. (58) Dormoy et al. (59)</td>
</tr>
<tr>
<td>SHH/Gli</td>
<td>Spatial organisation and segmentation; modulation of the cell cycle; control on the GLI effectors themselves</td>
<td>Promotes cell proliferation and cell survival</td>
<td>Gill and Rosenblum (29) Katoh et al. (55) Stanton and Peng (56) Dormoy et al. (57)</td>
</tr>
<tr>
<td>NOTCH</td>
<td>Cell-cell communication; regulation of apoptosis; regulation of proliferation; regulation of migration</td>
<td>Defect of control in cell fate in the absence of Notch signaling</td>
<td>Pulkinnen et al. (24) McCright et al. (25) Sun et al. (52) Aparicio et al. (53) Pulkinnen et al. (24) Kawakami et al. (44)</td>
</tr>
<tr>
<td>WNT</td>
<td>Cell proliferation; epithelial conversion of mesenchymal cells; inducer of tubulogenesis in the mesenchyme; epithelial differentiation of the renal mesenchyme</td>
<td>Promotes tumor growth and metastasis</td>
<td>Gill and Rosenblum (29) Katoh et al. (55) Stanton and Peng (56) Dormoy et al. (57)</td>
</tr>
</tbody>
</table>

PAX2: Paired box 2; WT1: Wilms’ tumor 1; LIM1: homeobox protein lim-1; NOTCH: neurogenic locus notch homolog protein; SHH: sonic hedgehog; GLI: glioma-associated oncogene homolog; WNT: wingless-type.

**CCC: From Kidney Development to Cancer**

While the example of PAX2 may seem isolated at first glance, there is numerous compelling evidence showing that proteins involved in developmental processes are re-expressed or deregulated after development, thus explaining at least partially, the physiopathology of a particular organ. Thus, an overview of literature concerning the WNT pathways reveals their implication in development (including kidney) and their role in tumor growth and metastasis (24, 44, 45). In brief, WNT antagonists induce apoptosis and inhibit proliferation in RCC (46-48).

It has been known for a long time that WT1 is re-expressed in CCC (49), but only recently has it been shown that WT1, through its multiple targets, may act as an oncogene in human CCC (50, 51). In particular, WT1 modifies telomerase activity to induce resistance of cancer cells to avoid programmed cell death.

Among the four NOTCH receptors responsible for NOTCH signaling, it has been shown very recently that CCC is characterized by a significant decrease of NOTCH 1 and NOTCH 4 receptor expression (52). This is important because
the proteins responsible for the loss of their expression might constitute a target to treat CCC. Of note, their evaluation as pathological markers is currently under investigation (53). It should be stressed-out that in another study, NOTCH 1 and the NOTCH ligand Jagged-1 were shown to be expressed at significantly higher levels in CCC than in normal human renal tissue, and that the growth of primary CCC cells was reduced upon inhibition of NOTCH signaling, both in vitro and in vivo in a xenografted nude mouse model (54). Although no clear explanation yet accounts for such an apparent discrepancy, it could be due to the difference in the experimental approach used to measure NOTCH and NOTCH ligand expressions, i.e. immunohistochemistry vs. western blotting.

In cancer, it has been shown that the blockade of the SHH-GLI signaling pathway induces death of cancer cells. In addition, recent evidence suggests that an SHH paracrine mechanism, mediating tumor mesenchymal interactions, may contribute to the metastatic capacity of cancer cells. Complete regression of tumor growth in xenografted nude mice treated with inhibitors of the SHH pathway has been reported (55). Targeting the SHH-GLI pathway has shown promising results in vivo for gastric, pancreatic, prostatic, breast cancer, and medulloblastoma (56). Recently, our laboratory demonstrated that this pathway is specifically re-expressed in human CCC and that its targeting might be particularly efficient against this disease, not only through inhibition of tumor growth but also by impeding tumor vascularization (57). As demonstrated during development, these studies also revealed that the SHH-GLI signaling pathway interacts with various oncogenic, but also with developmental pathways and tyrosine kinase receptors. In addition, LIM1 transcription factor acts as an oncogene in CCC and in chronic leukemia (59). Very recently, our group demonstrated that the LIM1 transcription factor acts as an oncogene in CCC and that it reproduces the main effects observed with the SHH-GLI pathway, including the regulation of various oncogenic pathways and tyrosine kinase receptors. In addition, LIM1 blockade induces substantial inhibition of cell movement, at the basis of metastatic invasion (59). Finally, it has been shown that deficiency in LIM1 contributes to the pathogenesis of nephroblastoma (60). Altogether, these data suggest that LIM1 regulation may represent a new target for refractory diseases of the kidney, such as CCC and nephroblastoma.

Targeting the Developmental Pathways to Trigger Anticancer Mechanisms

We demonstrated that players in the various steps of kidney development may later be re-expressed or de-regulated and contribute to cancerogenesis. In addition, these proteins may constitute an extremely useful strategy to target cancer cells efficiently and may define precious markers for the diagnosis of or staging of kidney cancer. Is it possible to use the same analysis for other types of cancer?

To emphasize that our discussion not only concerns the renal system, we started with the example of PAX2 and it should be stressed-out that PAX2 and the Paired-box genes have been described as essential actors of cancer initiation and progression (61). PAX2 is overexpressed in prostatic cancer (62) and its inhibition triggers cell death of prostatic cancer cells (63, 64). In breast cancer, PAX2 confers a low invasive phenotype after estradiol activation (65) and modulates fate switching through progesterone modulation (66). It has been described that PAX2 regulates ADAM10 and its silencing reduced the migratory and invasive capacity of melanoma cells (67). In addition, the down-regulation of PAX2 abrogated chemoresistance of melanoma cells to cisplatin, indicating its role in survival and melanoma progression. Finally, PAX2 expression allows for distinction between various grades of ovarian carcinomas (68, 69).

Even if LIM1 has not been well-studied, it has been recently shown that this transcription factor is re-activated in nephroblastosomas and an overview of the literature indicates that LIM domain kinases play a critical role in tumor cell invasion (60, 70).

Overexpression of WT1 is characterized in leukemia and solid tumors, and several studies showed its role in cell growth and differentiation (71, 72). In addition, WT1 is used as a marker in acute and chronic leukemia, as well as for minimal residual disease detection and quantification in myeloid leukemia and myelodysplastic syndrome (71, 73, 74). Recently, WT1 immunotherapy has made progress and clinical trials of WT1 peptide vaccination for cancer patients were started (72, 75, 76).

Considering the NOTCH receptors, the WNT pathway and the SHH-GLI pathway, describing only few types of cancer where their implications have been shown would be extremely restrictive, as their role in the progression, survival or the metastasis of nearly every kind of cancer has been characterized. For an overview of the literature, we propose a selection of articles in the references (77-90).

While the number of clinical trials involving the proteins implicated in normal renal development described in this review is still limited, we may expect to see an augmentation in the near future, as the studies describing their relation with cancer are constantly increasing.
Conclusion

The data presented in this review suggest crucial roles for developmental markers and signaling pathways, not only in kidney tumorigenesis but also in intrinsic and therapy-induced CCC resistance. Focusing on the kidney, this review shows that similar molecular actors may orchestrate both the development of an organ and its pathological transformation (Figure 2). Thus, understanding in detail the development and the physiology of an organ may lead to a better understanding of the physiopathology related to this organ, and ultimately to the proposal of new therapeutic and diagnostic approaches for anticancer research. Finally, these studies revealed new options in targeting cancer cells, particularly in kidney cancer: several proteins playing a role during development can be re-expressed or regulated differently in this pathology to help its growth or its dissemination and perhaps to explain its resistance. A further step would be the analysis of these proteins in metastatic tumors, and this is particularly important because the main findings regarding urological oncology arise from studies on primary tumors. However, the follow-up of patients who have been treated is extremely delicate and makes obtaining metastatic samples difficult.

Although it clearly appears that CCC tumor cells hijack the signaling pathways involved in normal development for their own growth, many questions still need to be resolved. For example, are other nephrogenic markers such as SALL1 and ODD1, and additional developmental signaling pathways such as the BMP pathway, involved in kidney tumorigenesis? How is the molecular network responsible for tumorigenesis orchestrated? Could these nephrogenic pathways be exploited to restore or at least ameliorate the response to chemotherapy or other therapeutic approaches such as targeted anti-angiogenic therapies? Finally, does the molecular network act in the same way during nephrogenesis and in tumorigenesis? The answers to these questions require deep research, but should undoubtedly open new therapeutic, and probably prognostic, options for human CCC. After all, a meticulous analysis of the actors playing in kidney development and in tumorigenesis may lead to the discovery of new therapeutic targets for this refractory disease, and may also open some new areas of investigation for the treatment of other pathologies.

Finally, since an increasing amount of data on other cancer types has led to the same conclusion, it seems that this field of investigation on the involvement of developmental markers/signaling pathways in carcinogenesis should constitute a new and rich source of information on how cancer cells grow and resist conventional therapies.

Conflict of Interest

None declared.

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