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Overview

Renal cell carcinoma: molecular pathways and targeted therapy

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Abbreviations:

RCC = renal cell carcinoma
VHL = von Hipple-Lindau
MET = mesenchymal-epithelial transition
HGF = hepatocyte growth factor
FH = fumarate hydratase
SDH = succinate dehydrogenase
FLCN = folliculin
TSC = tuberous sclerosis complex
VEGF = vascular endothelial growth factor
VEGFR = vascular endothelial growth factor receptor
mTOR = mammalian target of rapamycin
HIF = hypoxia inducible factor
HRE = hypoxia-response elements
TGF = transforming growth facor
PDGF = platelet derived growth factor
PDGFR = platelet derived growth factor receptor
GLUT1 = glucose transporter
PHD = prolyl hydroxylase
TKI = tyrosine kinase inhibitor
KIT = steam cell factor
AMPK = mitogen-activated protein kinase
BHD = Birth-Hogg-Dube
IGFR = insulin-like growth factor receptor
CTLA4 = cytotoxic T-lymphocyte-associated antigen 4
PD-1 = programmed cell death 1 receptor
PD-L1 = programmed cell death ligand 1

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Abstract

Renal cell carcinoma (RCC) is not a single disease. A number of different types of cancer occur in the kidney and each is caused by different genes with different histology and clinical course. Studies of hereditary kidney cancer sydromes have led to identification of the main kidney cancer genes: VHL, MET, FH, SDH, FLCN, TSC1 and TSC2. Mutations in each of these genes lead to dysregulation of at least one metabolic pathway that is mediated by oxygen, iron, energy and nutrient sensing suggesting that renal cell cancer is a disease of dysregulated cellular metabolism. A more improved understanding of molecular pathways has led to development of targeted therapies. Targeted agents against VEGF, VEGFR and mTOR continue to play a crucial role in the management of metastatic RCC. However, complete response is extremely rare, resistance in tumor cells develops frequently and adverse effects of therapy are not unusual finding. For that reason further genetic and epigenetic changes, metabolic aberrations as well as immune response are beeing investigated in numerous studies to find new targets for more personalized therapy.

INTRODUCTION

Kidney cancer acounts for 2-3% of all malignant diseases in adults. The most common forms of kidney cancer (>90%) are various forms of renal cell carcinoma (RCC) originating from different parts of nephron. RCC is the seventh most common cancer in men and the ninth most common in women. Incidence worldwide is about 209 000 new cases per year with 102 000 deaths per year and has increased over the last several years *(1, 2)*. Between 20% and 30% of patients with RCC have metastatic disease at the time of diagnosis, and another 30% subsequently develops metastasis after resection. The overal 5-year survival rate of RCC is in the range of 50-60%, whereas in metastatic disease long-term survival dramatically decreases and does not exceed 10% *(3, 4)*.

RCC is not a single disease. A number of different types of cancer occur in the kidney and each is caused by different genes with different histology and clinical course that respond differently to therapy. Historically, main histologic subtypes of RCC in the Heidelberg classification are: clear cell RCC (60-80%), papillary RCC (10-15%), chromophobe RCC (5%), oncocytoma (5%), collecting duct carcinoma (1%). Recently, Vancouver classification added some new subtypes to this list: translocation-linked, mucinous tubular, spindle-type RCC and tubulocystic carcinoma (all composing <1% of cases) *(5, 6)*.

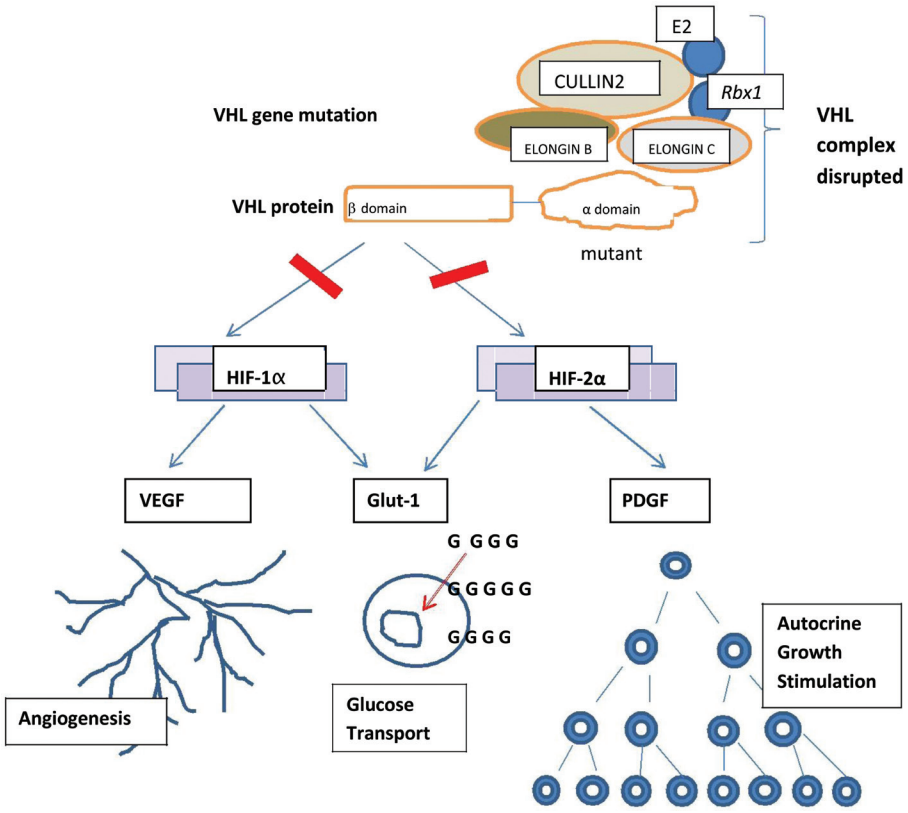

Figure 1. *The VHL complex targets HIF-1α and HIF-2α for degradation.*

Although hereditary RCC represents <5% of cases, it played a pivotal role in understanding and characterising the molecular pathways involved in sporadic RCC. Studies of hereditary kidney cancer sydromes have led to identification of the main kidney cancer genes: VHL, MET, FH, SDH, FLCN, TSC1 and TSC2. Mutations in each of these genes lead to dysregulation of at least one metabolic pathway that is mediated by oxygen, iron, energy and nutrient sensing suggesting that RCC is a disease of dysregulated cellular metabolism *(7)*.

Historically, therapeutic options for RCC were limited due to the lack of sensitivity to both chemo- and radiotherapy and low efficiency as well as high toxicity of interleukin-2 or interferon-alpha immunotherapy. In recent years we witnessed a profound revolution in RCC treatment. An improved understanding of RCC pathogenesis and cancer cell biology, identification of relevant molecular targets such as pro-angiogenic VEGF and mTOR pathways has led to development of biology-driven therapies. In the last 10 years seven targeted agents have been approved for metastatic RCC including agents that are directed against VEGF (sorafenib, sunitinib, bevacizumab, pazopanib, axitinib) and mTOR (temsirolimus and everolimus). Sequencing of these new targeted therapies improved overal survival of metastatic clear cell RCC from one to over 3 years. However, tumor cells are not target strictely by these new agents, their target are mostly endothelial cells, i. e. VEGF receptor. Also, tumor cells become resistant to targeted agents. So targeted therapy failed to cure metastatic RCC patients and came with a number of side effects and there is a greate need for novel therapeutic approaches *(8)*.

VON HIPPLE-LINDAU GENE PATHWAY

Hereditary von Hipple-Lindau (VHL) disease is associated with mutations of VHL tumor suppressor gene on the short arm of chromosome 3 and predisposes to the development of clear cell RCC and other tumors such as hemangioblastomas of the central nervous system, retinal agiomas, pheochromocytomas. Somatic inactivation of the VHL gene were also found in 60-80% of sporadic clear cell RCC. VHL gene encodes VHL protein which forms a complex with elongin B, elongin C and cullin 2 known as the E3 ubiquitin-ligase complex which, under normoxic cell situation, brings hypoxia inducible factor (HIF) to proteasomal degradation. Hypoxia-inducible factor (HIF) is oxygen-sensitive transcription factor that consists of HIF-α and HIF-β subunit which bind to hypoxia-response elements (HRE) in gene promotors to regulate the expression of genes that are involved in energy metabolism, angiogenesis, erythropoiesis, iron metabolism, cell proliferation and apoptosis. HIF mediates transcription of a number of downstream genes important

in carcinogenesis such as transforming growth factor alpha (TGFα), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and glucose transporter (GLUT1). During normoxia, HIF-α is hydroxylated by HIF prolyl hydroxylase (PHD) which requires oxygen, 2-oxoglutarate, ascorbate and iron as cofactors. Hydroxylated HIF-α then binds to VHL protein and is degraded by proteosome. Under hypoxic conditions and in situations of VHL protein deficiency, PHD cannot hydroxylate HIF-α which then cannot be dergraded by proteosoma what leads to HIF over-accumulation. There are two HIFs: HIF1-α and HIF2-α. Studies have indicated that HIF2-α is more important for carcinogenesis of clear cell renal cancer. The over-accumulation of HIF leads to overproduction of proangiogenic factors and tumor proliferation, (Figure 1), *(9–16).*

A number of targeted agents currently approved to treat clear cell RCC target downstream components of the HIF pathway including VEGF as well as VEGF and PDGF receptors or act as inhibitors of mTORC1. The response to these agents observed in patients with metastatic clear cell RCC is proof of principle that tumor regression can be induced by targeting VHL/HIF pathway. Current first line therapies for metastatic clear cell renal carcinoma that target VEGF are sunitinib, pazopanib, bevacizumab with interferon-alpha and the one that targets mTOR is temsirolimus for poor risk patients. **Sunitinib** is a small molecule multiple tyrosine kinase inhibitor (TKI) that inhibits VEGFR1 and VEGFR2, PDGFR-α and PDGFR-β, steam cell factor receptor (KIT), glial cell-line derived neurotrophic factor receptor (RET), receptor of macrophage-colony stimulating factor (CSF-1R), FMS-like tyrosine kinase-3 (FLT3). **Pazopanib** is another TKI that also targets VEGFR1-2-3 and PDGFR-α/β, KIT and fibroblast growth factor receptor (FGFR), and other, interleukin-2 receptor (ltk), leukocyte-specific protein TK (Lck), transmembrane glycoprotein receptor TK (c-Fms). **Bevacizumab** is recombinanat humanized monoclonal antibody that binds proangiogenic factor VEGF-A and prevents interaction of VEGF and its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells. **Temsirolimus** is mTOR inhibitor that binds to FK506-binding protein and inhibit mTORC1 but not mTORC2. Current second-line therapies for clear cell RCC are axitinib, everolimus and sorafenib. **Axitinib** is TKI that targets VEGFR1-2-3, and it inhibits less PDGFR and KIT. **Everolimus** is mTORC1 inhibitor. **Sorafenib** is TKI that targets VEGFR2-3, PDGFR-β, KIT, FLT-3, BRAF and CRAF.

MESENCHYMAL-EPITHELIAL TRANSITION GENE PATHWAY

Hereditary papillary RCC type 1 is associated with gain-of-function mutations of mesenchymal-epithelial transition (MET) proto-oncogene located at chromosome 7q31 which encodes thyrosine kinase membrane receptor and its ligand the hepatocyte growth factor (HGF). Interestingly, sporadic papillary RRCs show more trisomy 7 than MET mutations. Gain-of-function mutations of MET gene result in consecutive activation of the thyrosine kinase receptor and downstream signaling cascade such as posphatidylinositol 3-kinase (PI3K)-Akt-mTOR signaling pathway and LKB1-AMPK-mTOR pathway. Activation of these pathways leads to increased cell surface expression of nutrients transporters increasing uptake of amino acids, glucose and other nutrients what causes cell proliferation *(17, 18).* Multikinase inhibitors (i. e. foretinib) and anti-HGF antibody are beeing investigated for the future treatment of papillary RCC, (Figure 2), *(19).* Observation that MET and VHL signaling pathways intersect via VHL protein mediated regulation of HIF function is of particular interest for renal carcinogenesis.HIF over-expression results in transcriptional upregulation and therefore promotion of the transforming potential of MET receptor. This crosstalk between VHL and MET pathways may explain why clear cell and papillary histologies often coexist in the same tumor *(20, 21).*

FUMARATE HYDRATASE AND SUCCINATE DEHYDROGENASE GENE PATHWAY

Mutations in the another tumor supressor gene, fumarate hydratase gene (FH) located at cromosome 1q42, are found in patients with hereditary leiomyomatosis RCC who develop cutaneous leiomyomas and leiomyomas of the uterus and minority of them develop papillary type 2 RCC. Fumarate hydratase is an important enzime of the mitochondrial tricarboxylic acid cycle or Krebs cycle which coverts nutrients to energy in aerobic cells. FH mutations leads to fumarate accumulation and upregulation of HIF via inhibition of HIF prolyl hydroxylase (PHD) and inhibition of HIF degradation, (Figure 2), *(22).* The impaired oxidative phosphorylation in FH-deficient renal cancer results in a nearly total dependence on glycolysis, anaerobic glucose metabolism for energy production while downstream HIF genes such as VEGF and GLUT1 increase vasculature and glucose transport. Targeting angiogenesis in a cancer whose metabolism is completely dependent on glucose transport could provide a more effective approach to therapy *(23).*

Succinate dehydrogenase (SDH) is another Krebs cycle enzyme gene. Germline mutations in three out of four SDH genes (SDHB, SDHC, SDHD) have been associated with familial paraganglioma and familial pheochromocytoma. SDHB mutations predispose to renal cancer with or without pheochromocytoma. Inactivation of SDH increases levels of succinate which has been shown to inhibit HIF degradation like FH inactivation. Best therapeutic targets for these tumors are also tumor angiogenesis and glucose transport, (Figure 2), *(7, 24).*

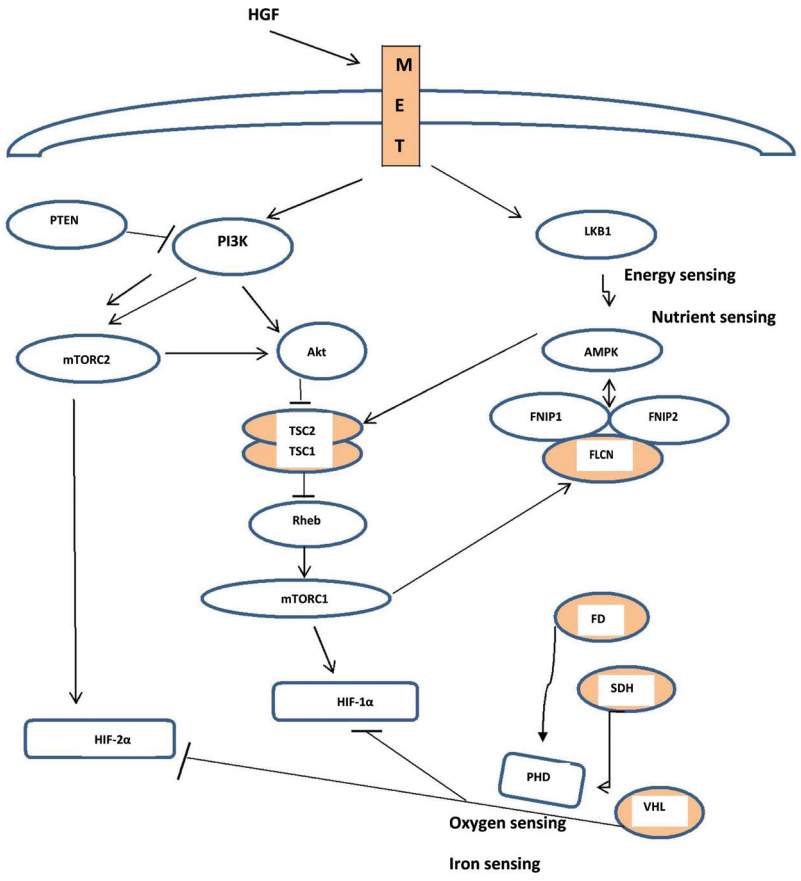

Figure 2. *The genes known to cause renal cancer and signaling pathways.*

FOLLICULIN GENE PATHWAY

Hereditary syndrome Birt-Hogg-Dube (BHD) is characterised by loss-of-function mutations in the folliculin gene (FLCN) at chromosome 17p11.2. Patients with this syndrome develop fibrofolliculomas of the face and neck, spontaneous pneumothorax, lung cysts and RCCs. Renal tumors of BHD show variable histology like chromophobe RCC which is most frequent, oncocytic-chromophobe hybrid, clear cell RCC and oncocytoma. The funtion of protein folliculin is not completely known but loss of function of FLCN gene is associated with mTORC1 activity. Namely, folliculin forms a complex with folliculin interacting proteins 1 and 2 (FNIP1, FNIP2), which binds the gamma-subunit of AMPK and as a consequens inhibits mTOR pathway activity, (Figure 2). These results suggest that mTOR inhibitors might be potential therapeutic agents against BHD associated renal cancer *(25–27).*

TUBEROUS SCLEROSIS COMPLEX GENE PATHWAY

Tuberous sclerosis is hereditary syndrome characterised by mutations of TSC1 or TSC2 gene located at chromosome 9p34 and 16p13 and multiple hamartomatous lesions of the skin, retina, brain, lungs and kidney. Renal tumors include benign angiomyolipomas, cysts, oncocytoma, rare clear cell RCC. TSC1 and TSC2 genes are also involved in the AMPK/mTOR pathway. TSC1 encodes hamartin and TSC2 encodes tuberin which form a heterodimer that inhibits Rheb, a Ras-family GTPase that activates mTORC1. Lack of TSC1/2 inhibition would presumably also result in HIF accumulation through increased HIF mRNA translation by activated mTORC1, (Figure 2). Sirolimus, mTORC1 inhibitor has been shown to cause regression of renal angiomyolipomas in patients with tuberous sclerosis complex *(28, 29).*

MAMMALIAN TARGET OF RAPAMYCIN PATHWAY

MTOR is multifunctional serine-threonine kinase that plays a central role in the regulation of cell growth, proliferation, apoptosis and metabolism. MTOR forms multimolecular complexes with regulatory-associated protein of mTOR (rapTOR) to form mTORC1 and with rapamycine-insensitive companion (ricTOR) to form mTORC2. PIK3/Akt pathway activates mTOR by growth factors receptors such as epidermal growth factor

TABLE 1
Targeted therapies for the most common renal cell carcinomas *(7)*.

Histology	Gene/pathway	Drug
Clear cell	VHL	Anti-VEGF: sunitinib, sorafenib, bevacizumam, axitinib mTOR inhibitors: temsirolimus, everolimus
Papillary type 1	MET	Foretinib, anti-VEGF
Papillary type 2	FH, SDH	Anti-VEGF
Chromophobe Oncocytoma	FLCN, BHD	mTOR inhibitors
Angiomyolipoma	TSC1, TSC2	mTOR inhibitors

receptor (EGFR) or insulin-like growth factor receptor (IGFR). Also, mTOR pathway is affected by the other pathways of renal carcinoma tumorogenesis such as all these which affect HIF. The activated mTORC1 complex phosphorylates its downstream effectors, 4EBP1 and S6K1, which cause increased translation and protein biosynthesis, metabolism and cell proliferation as well as decreased autophagy *(30, 31)*. Temsirolimus and everolimus are mTOR inhibitors approved in RCC. Both inhibit mTORC1 complex, (Table 1).

NEW MOLECULAR PATHWAYS AND THERAPEUTIC STRATEGIES

Chromatin remodelling genes and epigenetic alterations

Althoug 80% of clear cell renal carcinomas have mutated VHL gene there are some tumors that do not have VHL gene mutations. Many efforts have been made to discover other genetic changes in clear cell RCC. There are genes involved in chromatin remodeling process near the VHL gene on the chromosome 3p. Chromatin remodelling refers to dynamic modification of chromatine architecture allowing selective access of transcription factors to the condensed DNA. The main genes are: polybromo 1 (PBRM1), genes encoding the SET domain containing 2 (SETD2) and BRCA-1 associated protein-1 (BAP1). PBRM1 was found to be mutated in 45% of clear cell RCC, STED2 in 10-15% of ccRCC as well as BAP1 mutations. Studies have shown that PBRM1 mutation alone carries better prognosis then BAP1, whereas patients with both mutations have the worst prognosis. However, the precise role of these new gene mutations is still unknown. These mutations show that epigentic processes are as important in carcinogenesis as genetic changes. Therefore, many studies of RCC investigate abberations in DNA methylation and microRNAs *(32, 33)*.

Immune-based therapeutic strategies

Lack of complete response and progression on targeted therapy has led to renewed interest in novel immunotherapy. The main targets are checkpoint receptors on immune effector cells such as CD8+ T lymphocytes that inhibit costimulatory signals of immune activation upon ligand binding, which result in peripheral tolerance and immunosupression to tumor cells. Agents that block these receptors can couse antitumor immune response. One of these new agents is ipilimumab, monoclonal antibody dirrected against cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) that has shown durable response in metastatic RCC. Another are agents that inhibit programmed cell death 1 receptor (PD-1) on T lymphocytes or agents that bind its ligands PD-L1 and PD-L2 which are expressed on the surface of tumor cells. The first investigated agent that targets PD-1 is nivolumab that shows promising response in PD-L1 positive tumors. There are many ongoing trials that evaluate inhibitors od PD-1 and PD-L1/2 alone or in combination with VEGF and mTOR inhibitors *(32)*.

CONCLUSION

Renal cell carcinoma is an example how better understanding of the molecular pathogenesis may help to improve outcome. Targeted agents against VEGF, VEGFR and mTOR continue to play a crucial role in management of metastatic RCC. However, complete response is extremely rare, resistance in tumor cells develops frequently and adverse effects of therapy are not unusual finding. For that reason further genetic and epigenetic changes, metabolic abberations as well as immune response are beeing investigated in numerous studies. A more improved understanding of dysregulated molecular pathways in RCC will help recognice the driving force of the disease and will also form the basis for the devolopment of novel targeted therapies and molecular biomarkers.

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