Metabolism and Oxidative Stress Response Pathways in Kidney Cancer: A Tale of Chance and Necessity

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OVERVIEW

Over 270,000 patients are affected with kidney cancer worldwide and 120,000 died from this disease in 2014. Over the last few decades, important progress has been made in our understanding of the genetic and molecular mechanisms underlying the growth of these tumors, which has led to improvement in patient care. Some of the most significant recent advances came from the increasing number of large datasets generated by bioinformatics (genomics, proteomics, etc.) and their integration to characterize the genetic and molecular factors responsible for kidney tumor development and survival. Interestingly, deregulated metabolism and oxidative stress pathways are commonly found in advanced-stage kidney tumors and are important factors to consider and potentially target when developing therapeutic approaches.

Patients with small and localized kidney tumors (known as renal cell carcinoma; RCC) who are treated with surgical resection or surveillance have a very high 5- and 10-year survival rate (over 95%). However, less than 15% of patients with advanced disease (T4 or M1) will survive longer than 5 years.1,2 RCC is not a single disease; it is made up of a number of different types of cancer that occur in the kidney. Each type is characterized by different histology, a distinctive clinical course, disparate genetic changes, and requires distinct clinical approaches. The most common type of RCC is clear cell renal cell carcinoma (ccRCC). ccRCC represents 75% of all RCC and is characterized in 90% of cases by mutation of the von Hippel-Lindau (VHL) gene.3,4 The second most common type of RCC is papillary RCC, representing approximately 15% of cases. Papillary tumors can be subcategorized into two distinct histologic subtypes: papillary type I and papillary type II. Papillary type I includes tumors with MET mutations and chromosome-7 amplification. Papillary type II, a heterogeneous group of tumors, includes tumors with tricarboxylic acid cycle (TCA) enzyme mutations, such as fumarate hydratase (FH), leading to a highly aerobic glycolytic phenotype and upregulation of the Nrf2-antioxidant response pathway. There are few therapeutic strategies specifically tailored to papillary tumors, and agents commonly used in ccRCC have only limited effectiveness in advanced papillary RCC.

It is known that dysregulation of cellular energetics and a metabolic shift to aerobic glycolysis is a hallmark of many types of cancer.5-7 Initially described by Otto Warburg in 1924, cancers characterized by aerobic glycolysis undergo a metabolic shift to increased glycolysis despite the presence of oxygen and functioning mitochondria. Currently recognized as one of the “12 hallmarks of cancer,”6 dysregulation of cellular energetic needs is supported by multiple genetic and molecular events. Some examples include the activation of the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway or the VHL/hypoxia-inducible factor (HIF) pathway, which would affect different aspects of cellular metabolism and also the activation of “coping” pathways, such as the Nrf2 pathway, which allows cells to survive the oxidative stress linked with an aberrant metabolism.8 Regardless of whether metabolic alterations of tumor cells are an initiating event leading to tumorigenesis or are an adaptation associated with an aggressive phenotype, identifying the metabolic events associated with RCC survival is an important step toward the development of effective forms of therapy for this group of tumors.

CLEAR CELL RENAL CELL CARCINOMA TUMORS Targeting the VHL/HIF Pathway

Current therapies for patients affected with advanced ccRCC mostly target the VHL/HIF pathway. The VHL protein binds other proteins, such as elongin B, elongin C, and CUL2, to target the HIF for ubiquitin-mediated degradation. This is an oxygen-sensing mechanism.9-11 The VHL complex targets hydroxylated proteins, such as the HIF1-alpha and HIF2-alpha, for proteasomal degradation. In normoxic conditions, HIF prolyl hydroxylase hydroxylates two proline res-
idues on HIF-alpha. Under hypoxia, HIF is not hydroxylated, is not recognized by the VHL complex, and is stabilized. HIF1-alpha and HIF2-alpha are transcription factors that have downstream targets that are important for cell survival under hypoxic conditions and that regulate multiple biologic functions such as angiogenesis, energy metabolism, cell proliferation, erythropoiesis, and iron metabolism. Transforming growth factor alpha, vascular endothelial growth factor and its receptor, as well as the glucose transporters GLUT1 and GLUT4, and lactate dehydrogenase A (LDHA) are among HIF’s downstream transcriptional targets. Since the identification of VHL gene mutations in ccRCC and elucidation of the role played by VHL in regulating the HIF pathway, numerous therapeutic approaches targeting this pathway have been developed.10,12 However, although response rates can be substantial (in some cases as high as 40%), most disease eventually become resistant to therapy and progress. It has been shown in preclinical studies that HIF2-alpha is a critical pathway in VHL-deficient tumors.13-15 Several strategies to inhibit HIF2-alpha have shown promising results in vitro in VHL-deficient tumor cell lines, and further studies are being conducted to facilitate evaluating this strategy in the clinic.16-18 To improve the current clinical outcomes for patients with advanced ccRCC, development of more selective and potent agents targeting the vasculature might be necessary as well as a better understanding of how and why tumor cells are becoming resistant.

UNDERSTANDING THE METABOLIC ADAPTATION OF CLEAR CELL RENAL CELL CARCINOMA TUMORS

Deciphering the molecular basis of ccRCC is critical for the development of effective targeted therapies. In report on ccRCC by The Cancer Genome Atlas, 19 substantially mutated genes were identified, including VHL, chromatin remodeling genes (PBRM1, SETD2, and BAP1), genes of the PI3K/Akt pathway (PTEN, PI3KCA), the mTOR pathway (MTOR), and P53. By integrating genomic, proteomic, and transcriptomic data, this study reported findings in high-grade, high-stage ccRCC that were consistent with a metabolic shift toward aerobic glycolysis and decreased oxidative phosphorylation. These data provide the basis for the development of additional therapeutic approaches in patients with advanced ccRCC.

Over the last few decades, signaling pathways, such as PI3k/Akt/mTOR, AMPK, or PKC-theta, have been shown to be promising therapeutic targets for ccRCC in clinical or preclinical studies.19-22 Many of these signaling pathways are implicated directly or indirectly in regulating cell metabolism (Fig. 1). For example, two agents targeting mTOR, a key node in cell metabolism, have been approved for the treatment of ccRCC (everolimus and temsirolimus).23 mTOR is a serine/threonine protein kinase that interacts with several proteins to form two distinct mTOR complexes (mTORC1 and mTORC2) that have different metabolic and biologic functions, different regulators, but share the same catalytic mTOR subunit (for review see Laplante M and Sabatini DM).24 mTORC1 stimulates glucose metabolism and fatty acid synthesis via regulation of HIF1-alpha and SREBP1/2 activation respectively, while inhibiting autophagy and supporting macromolecule biosynthesis. mTORC2 activates Akt and supports cell survival and cytoskeleton dynamics.24 It is thought that resistance to mTOR inhibitors such as rapamycin is at least partly due to the fact that rapamycin only inhibits mTORC1, and mTORC2 is able to overcome this inhibition by phosphorylating and activating Akt (and glucose uptake). Thus, novel mTOR inhibitors targeting the catalytic subunit (i.e., dual TORC1/2 inhibitors) as well as approaches combining PI3K/Akt inhibitors with mTORC1 inhibitors and dual PI3K/mTOR inhibitors are being developed. The PI3K/Akt signaling pathway is involved in the insulin response pathway and promotes glucose uptake via regulation of GLUT1/4.25 Identified about 9 years ago as aberrantly activated in ccRCC and a potent preclinical therapeutic target,19,26 it was shown to be associated with ccRCC tumor progression, partially because of its role in supporting glycolysis.26 Several agents targeting the PI3K/Akt pathway are currently in development for ccRCC. Studies are underway to determine if targeting glucose uptake combined with inhibition of glutamine metabolism might be therapeutically beneficial in ccRCC. Since both glucose metabolism and reductive glutamine metabolism have been shown to be upregulated. Since glutamine uptake also promotes lipid biosynthesis via reductive carboxylation, thus both glutamine uptake and lipids biosynthesis might be potential therapeutic targets. Inhibition of fatty acid synthase has been shown to have a potent effect in vitro in ccRCC cell lines; however, more work will be needed before this is translated into clinical trials. Preclinical studies have shown that cancer cells shift their metabolism to glutamine metabolism to support anabolism and growth.27 In VHL-deficient tumor cells, HIF2-alpha expression is sufficient to induce reductive carboxylation because of reduced intracellular citrate levels.28-31

KEY POINTS

- Genetic and molecular mechanisms underlying renal cell carcinomas’ developments drive metabolic changes in tumor cells.
- Advanced clear cell RCC and hereditary leiomyomatosis and RCC tumors share a similar metabolic profile consisting of a shift to aerobic glycolysis, enhanced glutamine metabolism, and increased fatty acid synthesis.
- Improvement in outcomes for patients with clear cell RCC has been made over the last 10 years by strategically targeting the VHL/HIF pathway.
- Targeting metabolic changes supporting tumor cells’ survival might have a therapeutic value for clear cell RCC and papillary tumors.
- Imbalances in redox status are symptomatic of aggressive tumors and should be considered when developing a targeted therapeutic approach for RCC.
Glutamine starvation and glutaminase inhibitors were both lethal to VHL-deficient tumor cells in vitro and in preclinical animal models suggesting that glutamine metabolism may be a therapeutic target for VHL-deficient ccRCC, alone or combined with other approaches. The development of glutaminase inhibitors for ccRCC therapy is currently being evaluated in early phase clinical trials.

OXIDATIVE STRESS RESPONSE IN CLEAR CELL RENAL CELL CARCINOMA

The high-energetic needs of tumor cells, aerobic glycolysis, inhibition of autophagy, and rapid cell proliferation, all lead to an increased oxidative stress. Oxidative stress caused by unbalanced accumulation of reactive oxygen species (ROS), has been shown to promote tumor growth by inducing DNA damage and gene mutations or by activating signaling pathways promoting cell proliferation. However, how to therapeutically target or manage tumors’ oxidative stress response is still under study and it is unclear whether ROS scavengers or inducers might be beneficial. In ccRCC, both approaches have been evaluated in preclinical models. Agents affecting glutathione metabolism (glutaminase inhibitors), for example, or inducing endoplasmic reticulum stress (proteasome inhibitors and Hsp90 inhibitors) have been shown to be effective in preclinical models.

TYPE II PAPILLARY RENAL CELL CARCINOMA

Type II papillary RCC can occur in both a sporadic (nonhereditary) and inherited form. Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a hereditary cancer syndrome in which affected individuals are at risk for the development of cutaneous and uterine leiomyomas and an aggressive form of type II papillary RCC. The most prevalent gene mutation(s) responsible for sporadic papillary type II tumors are still unknown; however, studies have reported mutations of genes in the Nrf2 complex (Nrf2, KEAP1, CUL3).

UNDERSTANDING THE METABOLIC ADAPTATION OF HEREDITARY LEIOMYOMATOSIS AND RENAL CELL CARCINOMATUMORS

In 2002 Tomlinson et al determined that FH, a gene encoding the TCA enzyme fumarate hydratase, was responsible for HLRCC. It is estimated that approximately 15% to 20% of patients bearing a FH germ-line mutation will develop kidney tumors. Loss of FH enzyme function leads to impaired oxidative phosphorylation, a metabolic shift to aerobic glycolysis, and accumulation of the metabolite fumarate. FH-deficient tumor cells are notably dependent on glucose for ATP production as well as on glutamine for citrate, glutathione, and lipid biosynthesis. Increased amounts of the metabolite fumarate accumulate because FH-deficiency drives multiple biologic processes including inhibition of prolyl hydroxylase and stabilization of HIF1-alpha, supporting both...
aerobic glycolysis (GLUT1/4, LDHA) as well as angiogenesis (e.g., vascular endothelial growth factor).\textsuperscript{39} Aerobic glycolysis in HLRCC-associated FH-deficient papillary RCC cells produces increased ATP, leading to downregulation of the energy-sensing master regulator, AMPK.\textsuperscript{22} Therapeutic strategies targeting aerobic glycolysis with silencing of LDHA or activators of AMPK have shown promising preclinical effects.\textsuperscript{22,41}

OXIDATIVE STRESS RESPONSE IN HEREDITARY LEIOMYOMATOSIS AND RENAL CELL CARCINOMA

FH-deficient tumor cells are characterized by oxidative stress due to elevated aerobic glycolysis and fumarate accumulation.\textsuperscript{42,43} Targeting this redox imbalance by further increasing ROS has shown promise in preclinical studies.\textsuperscript{44} In addition to its role on inducing ROS, fumarate accumulation is also critical in supporting the antioxidant response via the inhibitory succination of the Kelch-like ECH-associated protein 1 (KEAP1).\textsuperscript{45,46} KEAP1 is the endogenous Nrf2 inhibitor and promotes targeting Nrf2 for proteosomal degradation. Nrf2 is a transcription factor critical for the antioxidant response by transcriptionally activating genes with antioxidant response element sequences, including NQO1 and HMOX.\textsuperscript{47} The antioxidant signature of Nrf2 pathway activation has been found in both sporadic papillary type II tumors as well as HLRCC-associated kidney tumors, providing a potential therapeutic approach targeting this pathway.\textsuperscript{46}

In studies conducted to develop a therapeutic approach targeting the Nrf2 pathway in FH-deficient type II papillary RCC, we recently showed that the activity of tyrosine kinase ABL1 was critical for Nrf2 nuclear translocation opening a new perspective on how to target this pathway\textsuperscript{21} (Fig. 2). Based on current understanding of the mechanisms supporting HLRCC tumor cell survival with aerobic glycolysis needed to provide ATP and Nrf2 antioxidant response pathway needed to cope with this extreme metabolism, it is reasonable to speculate that targeting both pathways simultaneously might have a promising effect. A phase II trial for patients with advanced papillary type II tumors (NCT01130519) using bevacizumab to inhibit angiogenesis and erlotinib to inhibit EGFR and modulate glucose and lipid metabolism via regulation of the PI3K/Akt pathway is currently underway.\textsuperscript{48} In addition, we have also developed a therapeutic strategy targeting glycolysis and Nrf2 transcriptional activity via ABL1 inhibition.\textsuperscript{21} We showed that ABL1 supports glycolysis in an mTOR-dependent manner and promotes Nrf2 transcriptional activity by indirectly allowing its nuclear translocation. Promising results were found in in vivo experiments in HLRCC xenograft models using vandetanib (a potent ABL1 inhibitor) as a single agent and when combined with the AMPK-activator metformin. A clinical trial evaluating the effect of vandetanib and metformin in patients with sporadic as well as HLRCC-associated type II papillary RCC is under development.

CONCLUSION

Over the past two decades significant progress has been made in our understanding of the molecular mechanisms of RCC.
Ten years ago we had very limited options for the treatment of patients with advanced RCC. We currently have seven approved agents for patients with advanced RCC and have some potential approaches that could represent a new era of targeted therapies and precision medicine designed to target metabolic and stress response pathways critical to tumor survival. The development of new bioinformatic tools will certainly further improve our understanding of the metabolic basis of kidney cancer and will hopefully provide the basis for the development of effective forms of therapy for patients with this disease.

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References


