Metabolomics and Metabolic Reprogramming in Kidney Cancer

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Summary: Kidney cancer, or renal cell carcinoma (RCC), is a disease of increasing incidence that commonly is seen in the general practice of nephrology. Despite this state of affairs, this fascinating and highly morbid disease frequently is under-represented, or even absent, from the curriculum of nephrologists in training and generally is underemphasized in national nephrology meetings, both scientific as well as clinical. Although classic concepts in cancer research in general had led to the concept that cancer is a disease resulting from mutations in the control of growth-regulating pathways, reinforced by the discovery of oncogenes, more contemporary research, particularly in kidney cancer, has uncovered changes in metabolic pathways mediated by those same genes that control tumor energetics and biosynthesis. This adaptation of classic biochemical pathways to the tumor's advantage has been labeled *metabolic reprogramming*. For example, in the case of kidney cancer there exists a near-universal presence of von Hippel–Lindau tumor suppressor (pVHL) inactivation in the most common form, clear cell RCC (ccRCC), leading to activation of hypoxia-relevant and other metabolic pathways. Studies of this and other pathways in clear cell RCC (ccRCC) have been particularly revealing, leading to the concept that ccRCC can itself be considered a metabolic disease. For this reason, the relatively new method of metabolomics has become a useful technique in the study of ccRCC to tease out those pathways that have been reprogrammed by the tumor to its maximum survival advantage. Furthermore, identification of the nodes of such pathways can lead to novel areas for drug intervention in a disease for which such targets are seriously lacking. Further research and dissemination of these concepts, likely using omics techniques, will lead to clinical trials of therapeutics specifically targeted to tumor metabolism, rather than those generally toxic to all proliferating cells. Such novel agents are highly likely to be more effective than existing drugs and to have far fewer adverse effects. This review provides a general overview of the technique of metabolomics and then discusses how it and other omics techniques have been used to further our understanding of the basic biology of kidney cancer as well as to identify new therapeutic approaches.

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With the discovery of clonal proliferation and subsequently of oncogenes, cancer had long been considered a disease of uncontrolled cell proliferation mediated by such phenomena as constitutively activated growth factor receptors. However, long after the initially puzzling observation of enhanced glycolysis—an energetically inefficient process—occurring in aggressive tumors despite normoxia,[1] a steady stream of more recent discoveries has linked cancers with a variety of metabolic changes that are only indirectly related to the more obvious phenotype of cell proliferation. Many of these metabolic derangements, especially in cancer of the kidney,[2,3] now have been linked directly to oncogenic mutations (Table 1).[4–7] This research has led to the concept that the evolution of altered metabolism, so-called metabolic reprogramming, is associated with a kidney cancer that is most likely to thrive within the body. When evaluated critically, such reprogramming makes obvious sense because to enable uncontrolled proliferation of cancer cells, a tumor has basic needs for augmented levels of cellular building components, such as DNA, nucleotides, and membrane constituents, as well as high levels of molecules that regulate the enhanced tumor energetics.

With the recent advances in high-throughput technologies, the pace of parallel advances in the omics fields that are relevant to clinical medicine, including nephrology, has accelerated markedly. If used cautiously and with full knowledge of their inherent limitations, these techniques have the potential to alter how we diagnose and treat human diseases, and their use in nephrology, although lagging compared with other fields, has now begun to bear fruit, most notably

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Table 1. Selected Oncogenes and Tumor-Suppressor Genes That Regulate Metabolism

Gene	Metabolic Pathway	Relevance to RCC	References
PTEN	Inhibition of glycolysis through inactivation of Akt	2.6% of patients have biallelic loss and 16.6% of patients have monoallelic loss of *PTEN* Loss of *PTEN* is associated with high stage and grade of RCC	49,50
TSC1/2	Deficiency leads to the Warburg effect and glutamine addiction through activation of mTOR	Mutation is a risk factor for RCC	51,52
AKT	Up-regulation of glycolysis through activation of enzymes including HK	Akt mutation is rare in RCC; activation is through PTEN loss Akt inhibitors are being tested in clinical trials for RCC	53–56
VHL	Inhibition of the Warburg effect through deactivation of HIF	Loss-of-function mutation found in >90% of patients	57,58
p53	Down-regulation of glycolysis by deactivation of GLUT1/4 and up-regulation of TIGAR Up-regulation of glutamine metabolism via increased transcription of GLS2	Mutation is rare in RCC	59–62
LKB1	Up-regulation of glycolysis and β-oxidation and down-regulation of lipid synthesis through activation of AMPK	Activity compromised in vitro, in vivo, and in patients	63–65
Myc	Up-regulation of the Warburg effect through activation of HK, LDH-A, and PDK1 Up-regulation of glutamine metabolism through GLS1 activation Up-regulation of lipid synthesis through activation of FAS and SCD1	Often mutated and overexpressed in RCC Activated by HIF-2α Overexpression induces RCC in mice	66–70

Abbreviations: mTOR, mammalian target of rapamycin; HK, hexokinase; HIF, hypoxia-inducible factor; GLUT, glucose transporter; TIGAR, p53-inducible glycolysis and apoptosis regulator; GLS, glutaminase; APMK, AMP-activated protein kinase; LDH-A, lactate dehydrogenase A; PDK1, pyruvate dehydrogenase kinase 1; FAS, fatty acid synthase; SCD1, Stearoyl-CoA desaturase 1.
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in kidney cancer. The advent of metabolomics, in which all of the metabolites in a given tissue or biofluid are examined (with the caveat that some metabolites will be below the detection limit of the instrumentation used and others will not be readily chemically identified), is one of the latest advances in the field of omics. Although not necessarily superior to the other, older omics technologies, metabolomics does have several advantages in that there are comparatively fewer metabolites compared with genes, transcripts, and proteins, which means that there are less data to manipulate. In addition, changes in metabolites are the most accurate predictors of the signature of the actual processes that are occurring within the body, rather than changes in compounds (such as untranscribed DNA or proteins modified before or after translation) that might be superfluous to these processes.[8] In light of this advantage and the fact that metabolomics analysis can be performed readily on biofluids such as blood and urine as well as tissue, which is harder to obtain, it is becoming increasingly clear that metabolomics has great potential for biomarker discovery.[9] However, serious limitations to this technique exist, mostly connected to consistency of sample collection, potential confounding effects, and limitations in detection of low-abundance or unidentifiable metabolites.[9]

Metabolomics, in its nontargeted, hypothesis-generating, and thus most useful form, is defined as the nontargeted measurement of all metabolites produced by the body. Now recognized as an established omics,[10] metabolomics has shown utility in biomarker discovery and, more successfully (at least in our hands), in the identification of new therapeutic targets and paradigms. The utility of metabolomics, perhaps more so than other established omics, lies in the fact that the metabolites measured in tissue or biofluids represent actual biochemical processes occurring in the body, such that when such processes are altered in disease states, the set of metabolites measured generated will change accordingly. This may not be the case in other omics techniques because the quantitation of genes and proteins may not correlate with their activities. Indeed, combining metabolomics with proteomics[11] or with transcriptomics[12] can greatly increase its utility in helping to understand renal cell carcinoma (RCC) biology.

METABOLIC REPROGRAMMING IS TO THE CANCER'S ADVANTAGE

Many classic metabolic pathways are increased, decreased, or bypassed entirely in cancer cells; kidney