The panoramic view of clear cell renal cell carcinoma metabolism: values of integrated global cancer metabolomics

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Kidney is a highly metabolic organ that filters approximately 180 liters of plasma every day through glomeruli in adult human, reabsorbs most of filtered small molecule nutrients such as amino acid, glucose, fatty acid through renal tubules, consumes a large amount of energy through oxidizing free fatty acid, lactate and ketoacid, and produces glucose through gluconeogenesis during a fight-or-flight situation. Clear cell renal cell carcinoma (ccRCC) is the most common (~75%), lethal subtype of kidney cancer, and originates from the renal tubule epithelium (1). Morphologically, ccRCC cells are lipid- and glycogen- laden, implicating altered fatty acid and glucose metabolism in the development of ccRCC. However, the casual relationship between these unique metabolic derangements and the pathobiology of ccRCC is unknown. Furthermore, the global metabolic shifts during the pathogenesis and the progression of ccRCC remain to be determined.

Changes in cellular metabolism contribute to the development and progression of tumors, and can render tumors vulnerable to interventions. Tumors reorganize their metabolism to produce sufficient energy and biosynthetic building blocks, such as nucleotides, lipids, and amino acids, for malignant cellular proliferation (2). Hence, a better understanding of caner metabolism presents opportunities for cancer diagnostics, prognostics, and therapeutics. However, studies of human cancer metabolism remain limited due to technical challenges of detecting and quantifying small molecules, the highly interconnected nature of metabolic pathways, the lack of designated tools to analyze and integrate metabolomics with other omics data, and the difficulty in acquiring a large set of high quality fresh frozen tumor samples.

The Cancer Genome Atlas (TCGA) KIRC working group reported the most comprehensive omic study on a large ccRCC cohort (n=470) (1). It encompasses genomics, transcriptomics, epigenomics, and proteomics, and offers invaluable biological insights among which several outstanding metabolic derangements were noted. In that study, worse patient survival was shown to correlate with upregulation of pentose phosphate pathway and fatty acid synthesis pathway genes, and downregulation of TCA cycle genes (1). To further investigate kidney cancer metabolism, we performed global metabolomic profiling on 138 well-annotated, matched ccRCC/normal tissue pairs (3).

To bridge the gap between TCGA KIRC transcriptomics and our MSK ccRCC global metabolomics, an analytic pipeline and a visualization tool ("metabolograms") (http://sanderlab.org/kidneyMetabProject/) were developed, which enabled us to assemble an integrated metabolic atlas of ccRCC at the pathway level. Surprisingly, a marked discordance between transcriptomics and metabolomics was detected in ccRCC, demonstrating the value of incorporating metabolomics into the current standard multi-omic platform for a better understanding of cancer

metabolism (3). Moreover, data generated from global ccRCC metabolomics not only helped validate prior cell-based discoveries and small cohort studies but also provided novel metabolic insights that could have prognostic and therapeutic impacts (3).

ccRCC is characterized by a biallelic loss of the Von Hippel-Lindau (*VHL*) tumor suppressor gene which encodes an E3 ubiquitin ligase that degrades hypoxia inducible factors (HIFs) (4). In low oxygen condition such as ischemia, HIFs are stabilized to activate target genes that promote angiogenesis to expedite the repair of ischemic injury, impede oxidative phosphorylation to cope with low oxygen supply, and turn on glycolysis to meet urgent energy demand (5). In ccRCC, the pathological inactivation of *VHL* in turn leads to the aberrant accumulation of HIFα despite an adequate oxygen supply. Hence, ccRCC manifests with a "pseudohypoxia" metabolic state. With this information in mind, we focus on a few key metabolic findings to illustrate the value of global cancer metabolomics.

When comparing tumors to normal as a whole, a few findings were notable from our study (3). First, at the molecule-by-molecule level, intermediate products of glycogen biosynthesis such as maltose, maltotriose, and maltotetraose were highly abundant and most distinguishable in ccRCC than normal renal cortex, reaffirming the known high glycogen content in ccRCC. Second, most of the amino acid pathways were downregulated, consistent with the de-differentiation of renal tubules that normally reabsorb all filtered amino acids. Third, the upper half of glycolytic pathway was upregulated in favor of the pentose phosphate pathway whereas the lower half was downregulated, which was also demonstrated by Lucarelli *et al*. (6) and could reflect the intrinsic gluconeogenic capacity that needs modulation, e.g., through downregulating fructose 1,6 bisphosphatase (FBP) (7). Fourth, Krebs cycle intermediates displayed a dichotomized, asynchronous pattern, i.e., citrate, cis-aconitate and succinate were up whereas fumarate and malate were down. This seemingly paradoxical phenomenon likely reflects the global inhibition of the Krebs cycle due to HIF overabundance and the increased use of glutamine as carbon source for citrate production that had been shown in cell-based studies (8-10). Altogether, it results in the over accumulation of fatty acid in the cytosol. Hence, the clear cytosolic appearance due to high glycogen and lipid observed in ccRCC likely reflects the presence of uncontrolled hyperactive HIFs.

When we compared metabolic changes stratified based on high (III–IV) versus low (I–II) clinical stages (3), three interesting metabolic features worth further discussions were found. First, the more aggressive ccRCC tumors contained more dipeptides, likely reflecting an increased lysosome activity. Second, there was a redirection of glutamine metabolism towards glutathione biosynthesis, likely reflecting the increased oxidative stress in aggressive ccRCC. Third, there was an increased usage of the one-carbon metabolic network in ccRCC based on elevated levels of serine, homocysteine, methionine, S-adenosyl methionine (SAM), and S-adenosyl homocysteine (SAH), which is consistent with an increased feed of one methyl group from serine into the tetrahydrofolate (THF) cycle for nucleotide synthesis.

There are ~42,000 small molecular weight metabolites being registered in the human metabolome database thus far and with the advancement of technology, this number expects to grow quickly. Although our platform focused on 577 named metabolites, our study generates the most comprehensive metabolomics dataset on a single cancer type and enables integration of metabolomics with sequencing data, and demonstrates the value of large-scale tumor metabolomics. Our results highlight the massive re-organization of cellular metabolism as ccRCC tumors progress and acquire more aggressive features, which could be shared with other human cancer types. Furthermore, our data also provide pathologic insights that could have prognostic and/or therapeutic values. Lastly, the analytic tools and the datasets of our ccRCC metabolomics along with clinical annotations are made available through an interactive public data portal for cancer research community to explore.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013;499:43-9.

2. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029-33.

3. Hakimi AA, Reznik E, Lee CH, et al. An Integrated Metabolic Atlas of Clear Cell Renal Cell Carcinoma. Cancer Cell 2016;29:104-16.

4. Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 2010;7:277-85.

5. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest 2013;123:3664-71.

6. Lucarelli G, Galleggiante V, Rutigliano M, et al. Metabolomic profile of glycolysis and the pentose phosphate pathway identifies the central role of glucose-6-phosphate dehydrogenase in clear cell-renal cell carcinoma. Oncotarget 2015;6:13371-86.

7. Li B, Qiu B, Lee DS, Walton ZE, et al. Fructose-1,6-bisphosphatase opposes renal carcinoma progression. Nature 2014;513:251-5.

8. Mullen AR, Wheaton WW, Jin ES, et al. Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature 2011;481:385-8.

9. Wise DR, Ward PS, Shay JE, et al. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci U S A 2011;108:19611-6.

10. Metallo CM, Gameiro PA, Bell EL, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature 2011;481:380-4.