Editorial

# Multi-omics approach reveals the secrets of metabolism of clear cell—renal cell carcinoma

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Renal cell carcinoma (RCC) accounts for 2–3% of all adult malignant tumors, having the highest incidence in Western countries. It is estimated that in 2016, about 62,700 new cases will be diagnosed (3.7% of all new cancer cases) in the United States, and nearly 14,240 patients will die of this cancer (2.4% of all cancer deaths) (1). Patients with low stage, localized disease have an excellent prognosis after surgical treatment, but a significant percentage of subjects still present with a surgically unresectable tumor or will subsequently develop metastatic disease (2,3). Recent studies have provided novel insights into the molecular mechanisms involved in the RCC pathogenesis, leading to the identification of potential biomarkers for early diagnosis, risk assessment, and outcome prediction. Many molecular markers such as C-reactive protein, CA15-3, αKlotho and some metabolic enzymes have been investigated, although none of these molecules has improved the predictive accuracy of current prognostic systems and their use is not recommended in clinical practice (4-8). The recent introduction of large-scale methods to purify, identify, and characterize DNA, RNA, proteins, metabolites and other molecules, has led to an in-depth exploration of the molecular bases underlying the development of urological cancer, together with the identification of new biomarkers and potential therapeutic targets (9,10). In this scenario, analysis of cancer cell metabolism has shown that tumor cells exhibit an atypical reprogramming of the energy metabolism that serves to promote cell growth and proliferation (11). RCC is fundamentally a metabolic disease (12) and many studies have suggested that in some diseases characterized by an altered metabolism there is an increased risk to develop this tumor (13-15). Moreover, a bird's eye view of the genetic alterations involved in the RCC pathogenesis shows that many genes play an important role in controlling cell metabolism (16).

The recent comprehensive molecular characterization of clear cell RCC by the cancer genome atlas (TCGA) research network has confirmed that oncogenic metabolism and epigenetic reprogramming are two main features of RCC (17). In particular, an inverse correlation has been shown between patients' survival and the activation of a metabolic shift characterized by a Warburg effect-like state (i.e., the shift to aerobic glycolysis with lactate production), an increased dependence on the pentose phosphate pathway (PPP), a reduced Krebs cycle activity, increased glutaminolysis and fatty acid production (17). A later study explored the specific role of glycolysis and PPP in sustaining RCC cell proliferation, the maintenance of NADPH levels, production of reactive oxygen species (ROS) and in reducing chemotherapy-induced cytotoxicity (7). Clear cell RCC was characterized by high levels of glucose and other sugars, in association with an increase in upstream glycolytic intermediates (glucose 6-phosphate and fructose 6-phosphate), a reduction in downstream intermediates (3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate), and an increased lactate production. Other important findings were the higher expression in neoplastic tissue of two cancer-specific isoenzymes, namely Pyruvate kinase isoform M2 (PKM2) and L-lactate dehydrogenase isoform 5 (LDH5). These metabolic alterations, in association with high levels of PPP enzymes (Glucose-6-phosphate dehydrogenase-G6PDH and Transketolase-TKT) and metabolic intermediates (sedoheptulose 7-phosphate, ribose 5-phosphate, and ribulose

5-phosphate/xylulose 5-phosphate), suggested a rerouting of the sugar metabolism toward the PPP, with the aim of promoting both anabolic reactions and redox homeostasis in RCC (7). Ribose-5-phosphate is a sugar used in the synthesis of nucleotides, so the increased flux of metabolites through the PPP provides an advantage for cell growth and survival. Since G6PDH is the rate-limiting enzyme of the PPP, its inhibition causes a significant decrease in cancer cell growth, confirming the importance of this pathway in RCC. Another important byproduct of PPP is NADPH, a reducing compound that is used for biosynthetic reactions and to control the redox state. Cancer cells pre-treatment with a G6PDH inhibitor induces a significant reduction in NADPH levels and an increased production of ROS in renal cancer cells, suggesting that G6PDH, and hence the PPP, have a fundamental role in maintaining redox homeostasis in RCC (7).

These findings were recently confirmed by Hakimi *et al*. that showed how clear cell RCC is characterized by a reprogramming of central carbon metabolism, one-carbon metabolism, and the antioxidant response (18). In particular, consistent with previous studies (7,17), these authors showed increased levels of metabolites in upper glycolysis, and a reduction of metabolic derivatives in lower glycolysis, downstream of fructose 6-phosphate. This different abundance in metabolites production between upper and lower glycolysis suggests that the metabolic flux through this pathway may be differentially partitioned. In fact, while the sugars produced in the upper part of glycolysis are diverted to the PPP, the triose phosphates generated in the lower part are rerouted towards the Krebs cycle or one-carbon metabolism. In accordance with these results, analysis of tricarboxylic acid (Krebs) cycle metabolites (reduced levels of malate and fumarate, and accumulation of succinate) suggests that in RCC the mitochondrial bioenergetics and oxidative phosphorylation processes are impaired. It has recently been demonstrated that NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4-like 2 (NDUFA4L2) is a HIF-1 target gene that encodes for a component of the electron transport chain (ETC) Complex I (19). In particular, Complex I catalyzes the first step of mitochondrial respiratory chain reactions, and it couples the Krebs cycle to oxidative phosphorylation. Tello *et al*. have shown that hypoxia-induced NDUFA4L2 attenuates mitochondrial oxygen consumption through inhibiting Complex I activity, and reduces intracellular ROS production under low-oxygen conditions (19). The most surprising fact is that NDUFA4L2 is one of the most highly expressed genes in clear cell RCC, while its knockdown impairs cell proliferation, alters metabolic pathways and induces autophagy in renal cancer cells (20). Taken together, these findings outline a clear cell RCC metabolic signature characterized by an anaerobic switch that favors the upper part of glycolysis and the rerouting of the sugar metabolism toward the PPP, and attenuates the mitochondrial activity though the overexpression of NDUFA4L2.

Another important advance highlighted by the Hakimi study (18) was the development of a metabologram, a web-based application that integrates transcriptomic and metabolomics data (http://sanderlab.org/kidneyMetabProject), with the aim of providing a rapid evaluation of metabolic changes in RCC. This visual tool explores the metabolic pathways using both gene expression (derived from TCGA database) and metabolite abundance data (derived from MSK Institute). Using metabolograms, it is possible to simultaneously evaluate changes in gene expression and metabolite abundance between tumor and normal tissues, as well as between different tumor stages. In addition, this web platform makes it possible to explore the association between the abundance of particular metabolic intermediates and 24 clinical parameters such as gender, age, the BMI, pathological characteristics, the onset of recurrence or metastasis.

In conclusion, the field of cancer metabolomics is evolving very rapidly. Recent amazing discoveries have cast new light on the molecular regulation of cancer cells metabolism, and novel biochemical pathways are now under investigation. In the next years we may expect a progressive integration of data derived from different platforms and other omics approaches and a more extensive use of the systems biology approach to identify a panel of molecular factors as biomarkers for diagnosis, prognosis, and as potential targets for specific therapies.

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