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Application of Proteomic and Metabolomic Technologies in Renal Cell Carcinoma and Research Progress of Related Biomarkers

Liyuan Zhang, Junyan Li, Youfu Pan*

Department of Medical Genetics, Zunyi Medical University, Zunyi 563000, Guizhou, China

**Author to whom correspondence should be addressed.*

Abstract: Renal cell carcinoma (RCC), which accounts for about 90 percent of kidney cancers, has a distinct metabolic reprogramming profile characterized by increased aerobic glycolysis (Warburg effect), abnormal accumulation of lipids, and impaired mitochondrial function. Recent advances in high-throughput proteomic and metabolomic technologies have revolutionized our understanding of the pathophysiology of RCC, allowing for the systematic identification of disease-specific molecular signatures, elucidation of drug resistance mechanisms, and possible targets for intervention. The review focuses on the use of proteomic and metabolomic technologies in renal cell carcinoma and the research progress on related biomarkers, and is expected to provide useful information for the early detection and treatment of RCC.

Keywords: Renal cell carcinoma; Proteomics; Metabolomics; Early diagnosis; Biomarkers

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1. Introduction

Kidney cancer is one of the most common and fatal urological diseases in the world, with 430,000 new cases diagnosed each year by 2020. Histologically, clear cell renal cell carcinoma (ccRCC) accounts for approximately 75% of renal cell carcinoma (RCC), which is the overwhelming majority (90%) of kidney cancers [1]. More than 30% of patients with ccRCC have metastasis at the time of initial diagnosis and 40% of patients undergoing surgical resection eventually relapse [2].

Extensive studies have shown that RCC is a cellular metabolic disease. Metabolic alterations include an increase in aerobic glycolysis, phosphorylation by the pentose phosphate pathway, synthesis of fatty acids, metabolism of glutamine and glutathione, and a decrease in the tricarboxylic acid (TCA) cycle, as well as the oxidative oxidation and phosphorylation of fatty acids (Oxphos) [3]. Cancer metabolites have a major influence on chromatin remodeling and epigenetic dysregulation, which may result in characteristic hypermethylation, EMT

phenotype switching, and pseudohypoxic features [4].

Currently, the main treatment for metastatic RCC is targeted therapy and immunotherapy, but these are effective only in some patients and are prone to drug resistance [5]. As the hypoxia-inducible factor (*HIF*) pathway is critical in the pathophysiology of the ccRCC, *HIF* and downstream targets such as *VEGFA* have always been highly anticipated as drug targets [6]. However, researchers have a great interest in seeking novel biomarkers or druggable targets. In recent years, high-throughput proteomics and metabolomics technologies have been used to screen for biomarkers of various tumors, including RCC. The discovery of specific markers could provide new strategies for clinical diagnosis, treatment, and prediction of disease. This review focuses on metabolic reprogramming and the use of proteomic and metabolomic approaches, as well as the research progress on related biomarkers in renal cell carcinoma, to provide new insights for translational research in renal cell carcinoma.

2. Metabolic signature of renal cell carcinoma

RCC is a known metabolic disease that involves a range of metabolic disorders. Among the many metabolic pathways, the Warburg effect, increased fat deposition, and mitochondrial dysfunction are typical of the abnormal metabolic pathways.

2.1. Warburg effect in RCC

The Warburg effect is a major metabolic characteristic of tumor cells, meaning that even in the presence of abundant oxygen, the tumor cells still obtain their energy mainly by glycolysis. In RCC, the Warburg effect, i.e. the aerobic glycolysis rather than oxidative phosphorylation, is favored in cellular energy metabolism.

Inactivation of the *VHL* gene is the primary genetic cause of most RCCs and also contributes to the Warburg effect. Deficiency of *VHL* can stabilize *HIF* and thereby promote angiogenesis and tumor growth [7]. *HIF* can reduce the glucose and glutamine oxidation by altering the TCA cycle and may regulate metabolism by altering the transcription of key genes in the glycolysis pathway, such as *FKBP10* and *TET2* [8–11].

The opposite reaction to glycolysis is gluconeogenesis, which is the process by which organisms convert various non-sugar substances to glucose or glycogen. Fructose-1,6-bisphosphatase (FBP) is a rate-limiting enzyme in gluconeogenesis and is subdivided into two subtypes, *FBP1* and *FBP2*. Li and colleagues have shown that *FBP1* can interact directly with *HIF*, thereby inhibiting the transcriptional activity of both *HIF1α* and *HIF2α* and also inhibiting glycolysis [12]. Moreover, *HIF1α* is shown to be primarily involved in glycolysis, while *HIF2α* is mainly involved in the regulation of genes involved in lipoprotein metabolism, ribosome biogenesis [13].

PBRM1 is the second most frequently mutated gene in ccRCC with a mutation rate of 38.1% [14]. Recently, we have demonstrated that when *PBRM1* is knocked down, the levels of some major metabolic enzymes involved in the glycolytic pathway are increased, including enolase 1 (ENO1, pyridostigmine), pyruvate kinase (PKI), and lactate dehydrogenase A (LDHA) in ccRCC cells [15].

In addition to the major metabolic enzymes involved in the glycolytic pathway, other proteins have been shown to play a role in the development of RCC by influencing the glycolytic pathway. These include *PFKFB*3, *ATAD2*, *TRIM21*, *SIRT3*, *METTL14*, among others [16–19].

The major mutated genes in RCC, such as *VHL* and *PBRM1*, as well as genes coding for key glycolytic enzymes, play a critical role in the Warburg effect and the development of RCC. Some may have potential predictive significance.

2.2. Increased fat deposition in RCC

Lipid droplets are the main sites for intracellular lipid storage and are markers for lipid deposition. Alterations in lipid metabolism can lead to increased lipid synthesis, abnormal lipid accumulation, and dysregulated lipid signaling, thereby promoting cell proliferation, invasion, and migration in ccRCC and other tumors [20,21].

HIF2α is related to lipoprotein metabolism and can regulate the expression of important lipogenic enzymes and transporters [22]. Being targets of *HIF2α*, the mediator complex subunit 15 (MED15) and transcription factor 7 analogue 2 (TCF7L2), both play a role in lipid accumulation and RCC progression or metastasis [23,24]. In addition, other enzymes such as glutathione peroxidase 8 (GPX8) can also contribute to the formation of lipids and thus to the development of tumors. GPX8 belongs to the glutathione peroxidase (GPX) family and can catalyze the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), protecting the biological membranes from damage by reactive oxygen species (ROS). RCC, depletion of GPX8 resulted in a significant reduction in lipid levels, fatty acid synthesis, and triglyceride esterification. Mechanistically, GPX8 regulates nicotinamide N-methyltransferase (NNMT) through IL6-STAT3 signaling and is independent of *VHL* [25].

Some others, such as *CPT2*, *MLYCD*, *GPR1*, *CMKLR1*, and *ACAT1*, among others, have the opposite effect. There is evidence showing that *CPT2* inhibits the proliferation, invasion, and migration of ccRCC cells mainly by inhibiting the ROS/PPARγ/NF-κB signaling pathway [26]. Malonyl-CoA decarboxylase (MLYCD) is an important regulator of fatty acid anabolism and is downregulated in ccRCC. When *MLYCD*-mediated fatty acid oxidation is inhibited, lipid droplet accumulation occurs, disrupting endoplasmic reticulum and mitochondrial homeostasis, increasing ROS levels, and inducing ferroptosis [27]. *GPR1* and *CMKLR1* are crucial for maintaining the balance of lipid metabolism. Adipose triglyceride lipase (ATGL), a key enzyme that initiates the hydrolysis of triglycerides to release fatty acids, can be inhibited by *GPR1* and *CMKLR1* to reduce lipogenic triglyceride lipase and increase lipid oxidation and ferroptosis. In addition, 2-(α-naphthol) trimethylammonium iodide (α-NETA) is a *CMKLR1* antagonist that can effectively inhibit the growth of ccRCC cells by regulating lipid metabolism and activating SREBP1 signaling [28]. Acetyl-coenzyme A acetyltransferase 1 (*ACAT1*) is the only enzyme in the cell that catalyzes the formation of cholesterol esters from free cholesterol and long-chain fatty acids, and plays an important role in maintaining cellular cholesterol homeostasis. In ccRCC tissues, *ACAT1* expression is downregulated. Overexpression of *ACAT1* can inhibit the progression of ccRCC through affecting the PPAR/CPT1 axis and AMPK signaling pathway [29].

2.3. Mitochondrial dysfunction in RCC

Mitochondria are double-membrane organelles that are ubiquitous in eukaryotic cells and provide cells with energy through oxidative phosphorylation. Mitochondrial dysfunction mainly refers to disorders of energy metabolism caused by damage to the mitochondrial membrane, inhibition of the respiratory chain, reduced activity of mitochondrial enzymes, and damage to mtDNA. When mitochondria are disrupted, they can release cytochrome c, produce mitochondrial reactive oxygen species and metabolites, thereby affecting the signaling cascade response of gene expression, cell proliferation, and differentiation [30].

While *HIF1α* promotes glycolysis, it can also weaken mitochondrial activity. *HIF1α*-dependent reduction in mitochondrial oxygen consumption increases the NADH/NAD^+ ratio and thereby inhibits the activity of the NADH-sensitive glycolytic enzyme GAPDH [31]. Other proteins, such as NADH dehydrogenase 1 alpha subcomplex 4-like 2 (NDUFA4L2), mitochondrial fusion protein 2 (MFN2), among others, also play a critical role in the pathophysiology of the mitochondria and RCC [32,33].

As a metabolic disease, ccRCC is characterized by the typical Warburg effect, increased fat deposition, and mitochondrial dysfunction. *VHL* inactivation or promoter hypermethylation occurs in ~90% of RCC. When *VHL* is inactivated, there may be upregulation of glycolysis, Wnt/**β**-catenin signaling, and mTORC1 signaling, as well as downregulation of fatty acid metabolism. Some dysregulated signaling pathways were also observed upon *PBRM1* depletion [15].

3. Application of proteomics in renal cell carcinoma

Proteomics is the study of the complete set of proteins that are produced in a cell or an entire organism, including amino acid sequences of proteins, protein abundance, protein modification, and interaction between proteins. Conventional proteomic research techniques can be divided into three categories: Untargeted proteomics, targeted proteomics, and modified proteomics. Here, we mainly introduce the related applications of the above three categories of proteomics technologies in RCC studies.

Untargeted proteomics can be used to identify and/or quantify the relative amount of many proteins [34]. Untargeted proteomics makes use of stable isotopes or synthetic stable isotopes, and can accordingly be categorized into stable isotope labeling by amino acids in cell culture (SILAC, metabolically labeled), isobaric tag for relative or absolute quantitation, tandem mass tags (iTRAQ/TMT, chemical labeled), and data-dependent acquisition/data-independent acquisition (DDA/DIA, label-free). Using the iTRAQ/TMT labeling method, White *et al.* identified 55 differentially expressed proteins between ccRCC and normal adjacent tissue samples in a total of 199 patients [35]. Five of these, ENO1, HSPB1, LDHA, and AHNAK, which are increased in the ccRCC, were confirmed further by immuno-blot and tissue microarray. Atrih *et al*. used label-free quantitative proteomics (LFQ) technology to examine the different protein spectra between ccRCC tissues and normal adjacent tissues (NAT) [36]. They showed that nearly 600 proteins were differentially expressed, and two of these, *CORO1A* and *ADFP*, were further validated in ccRCC samples. Using label-free DIA technique, Lin *et al.* analyzed samples from 31 ccRCC patients and 31 healthy volunteers [37]. Significant differences in serum peptide composition were noted between the two groups. This difference in the spectrum of serum peptidomes helps to distinguish ccRCC patients from healthy volunteers and demonstrates the great potential of serum peptidomes for the diagnosis of cancer.

Targeted proteomics refers primarily to parallel reaction monitoring (PRM) targeted quantitative proteomics, and thus is suitable for validation of biomarker candidates [34,38]. Di *et al.* used LFQ and PRM to analyze the role of urinary peptides in distinguishing early ccRCC from healthy controls and renal cell carcinoma. Nine peptides were identified with significantly increased expression levels in small renal masses compared to controls. Some other markers associated with ccRCC progression or shorter overall survival have also been identified [39]. This suggests the clinical significance of proteomic analysis of urinary peptides.

4. Application of metabolomics in renal cell carcinoma

Metabolomics is an emerging omics technology developed after genomics, transcriptomics, and proteomics, which mainly studies the comprehensive and dynamic metabolites and their changes produced by endogenous or exogenous stimuli of biological systems (including cells, tissues, blood, urine, saliva, feces, etc.) at a specific time and in a specific environment [40].

Untargeted metabolomics refers to the systematic and comprehensive analysis of the entire metabolome of a

sample or the comparison of the dynamic changes of all small-molecule metabolites before and after stimulation of an organism. Complement component 1q subcomponent binding protein (C1QBP) is a highly conserved multifunctional protein that plays a vital role in inflammation, infection, and cancer. *C1QBP* can inhibit the adhesion and metastasis of ccRCC cells, and the expression level is decreased in ccRCC [41]. Using untargeted metabolomics technology Wang *et al.* analyzed the differential metabolites of 200 major metabolites in *C1QBP*-overexpressing RCC cells [42]. Among 109 metabolites detected, 17 metabolites were changed significantly, including hypoxanthine. The data showed that *C1QBP* mainly promoted the catabolism of hypoxanthine by regulating the ROS generation mediated by xanthine dehydrogenase (*XDH*). Similarly, Feng *et al.* showed that knockdown of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (*PFKFB*) induced a significant reduction in multiple metabolites, among which the pentose phosphate pathway (PPP) was significantly enriched [43].

Targeted metabolomics refers essentially to the analysis of a specific substance or specific metabolite, such as a specific amino acid, short-chain fatty acid, or free fatty acid, and the quantification of the target metabolite concentration in the sample using a standard reference. *SETD2* is a histone H3 lysine trimethyl transferase, and its inactivation is associated with the recurrence of ccRCC. Liu *et al.* used GC-MS-based targeted metabolomics to find that the loss of *SETD2* increases the production of TCA cycle metabolites such as aspartate, malate, succinate, fumarate, and α-ketoglutarate in ccRCC cells, laying the foundation for further exploration of molecular mechanisms [44]. Amaro *et al*. used a GC-MS-based metabolic approach and analyzed a matched set of tissue and urine samples from a cohort of 18 patients with ccRCC. Data showed that the metabolic signature of the ccRCC tumors is reprogramming of amino acid, energy, sugar, and inositol phosphate metabolism, as well as a significant reduction in asparagine, proline, gluconate, 3-amino-butanate, 4-aminobutanoate, and urea [45].

5. Joint analysis of proteomics and metabolomics in renal cell carcinoma

Compared to single-omics analysis methods, joint analysis of proteomics and metabolomics of biological samples can explore the biological molecular functions and regulatory mechanisms related to diseases more systematically and comprehensively. Wettersten *et al.* conducted a joint proteomics and untargeted metabolomics analysis of RCC tissues of different grades based on the Fuhrman grading standard [46]. The study found that the TCA cycle was significantly downregulated in RCC tissues, glycolysis and tryptophan metabolic pathways were significantly upregulated in high-grade RCC tissues, fatty acid β-oxidation was significantly downregulated in high-grade RCC tissues, and the glutamine pathway was reprogrammed into the GSH/GSSG antioxidant system in high-grade RCC. This study revealed the grade-dependent metabolic reprogramming regulated by RCC-related metabolic pathways, which will help clinical personalized treatment of RCC patients of different grades and provide potential targets for new drug development.

With joint analysis of proteomics and metabolomics, Yuan *et al.* found that the anti-tumor effect of *SLC39A1* may be related to changes in purine and pyrimidine metabolism, glutathione metabolism, and iron poisoning, ROS generation, PI3K-AKT, cAMP-Epac, and PPAR signaling pathways [47]. Solute carrier family 39 member 1 (*SLC39A1*), also known as ZIP1, is responsible for transferring zinc ions into cells.

The results of multi-omics analysis showed a more comprehensive picture of *SLC39A1* molecular perturbations, providing new insights into the occurrence and development of RCC. Moreover, Li *et al.* used multi-omics (histopathology, proteomics, single-cell sequencing, phosphorylation proteomics, tumor metabolomics, and tumor-specific glycoproteomics) technology to integrate histopathology, proteomics, and metabolomics data from

305 tumors to comprehensively characterize the complexity and heterogeneity of ccRCC [48].

6. Renal cell carcinoma-related biomarkers

Tumor biomarkers are molecules synthesized by tumor cells and related cells, and have important biological significance in tumor occurrence, development, and treatment. Detecting tumor marker levels can help with early diagnosis, precise diagnosis, treatment, and prognosis of tumors.

As highly sensitive, highly accurate, and high-throughput systematic research methods, proteomics and metabolomic approaches have been used to discover complex protein biomarkers in various tumors, such as lung cancer, cervical cancer, colorectal cancer, thyroid cancer, ovarian cancer, and renal cell carcinoma [49–54].

6.1. Urinary biomarkers

Urine collection is simple, non-invasive, and the amount is relatively abundant compared to other biological fluids [55]. Monteiro *et al.* analyzed volatile organic compounds in the urine and found that the combination of 21 metabolites can effectively distinguish RCC patients from non-RCC volunteers. 2-oxopropanal and 2,5,8-trimethyl-1,2,3,4-tetrahydronaphthalene-1-ol are expected to be potential biomarkers for the diagnosis of RCC [56].

Liu *et al.* analyzed urine samples from 100 RCC patients and some controls and found that *PKHD1L1*, *UGTL6*, *FAP4*, and *C3* can assist in the diagnosis of ccRCC [57]. In addition, they suggested that N-formyl kynurenine can be used as a potential diagnostic biomarker. Morozumi *et al.* showed that the combination of lactate, glycine, 2-hydroxyglutaric acid, succinic acid, and pyrimidine acid is a potential predictive model [58]. Oto *et al.* demonstrated that the p-cresol glucuronide can be used as a diagnostic marker, while isobutyl-L-carnitine and L-proline betaine can be used as potential prognostic markers for RCC [59]. Moreover, Yang *et al.* identified 133 proteins that were differentially expressed in the urine of patients with ccRCC, including 85 upregulated and 48 downregulated proteins [60]. They further showed that *VSIG4*, *HLA-DRA*, *SERPINF1*, and *IGLV2-23* were statistically significant, and this prognostic model applies to patients with ccRCC, but further clinical studies are needed to confirm its effectiveness.

6.2. Blood-based biomarkers

Serum tumor markers are important methods for early detection of tumors, monitoring tumor progression, and evaluating treatment efficacy.

Liu *et al.* used early ccRCC as a model to explore the proteomic relationship between tissue, plasma, and urine. They demonstrated that three plasma proteins (FGFR1, GOT1, FGFBP2) and three urinary proteins (CETP, SEZ6L2, COX5B) have good performance for ccRCC prediction [61]. Zheng *et al.* proposed that two tumor metabolic derivatives, succinylated adenosine and succinate cysteine, could be excellent early-detection biomarkers in RCC cells lacking fumarate hydratase (FH) [62]. It is well-known that FH deficiency leads to abnormal metabolic re-programming that may lead to malignant transformation of the RCC. Furthermore, Wang *et al.* showed that 3-β-D-galactosyl-sn-glycerol, 7,8-dihydroneopterin, lysophosphatidylcholine (LPC), and γ-aminobutyryllysine can be used as biomarkers to distinguish patients with RCC from healthy controls or with benign renal tumors [63].

Wolrab *et al.* showed that seven lipids, including cholesterol ester (CE) 16:0, ceramide (Cer) 42:1,

lysophosphatidylcholine (LPC) 18:2, phosphatidylcholine (PC) 36:2, PC36:3, sphingomyelin (SM) 32:1 and SM41:1, were potential biomarkers for RCC, as well as for breast cancer and prostate cancer [64]. However, they can only be used for cancer screening and need to be further verified in prospective studies.

Furthermore, Zheng *et al.* found that alanine, creatine, choline, isoleucine, lactate, leucine, and valine in serum can be used as prognostic markers of metabolic recovery in RCC patients after nephrectomy [65].

6.3. Other biomarkers

Tumor interstitial fluid (TIF) is not only a transport medium for secreted proteins, nutrients, and waste between cells and capillaries, but also a rich source of candidate markers due to its proximity to tumors [66].

Teng *et al.* analyzed TIF from 10 patients with ccRCC and matched NATs and found that the TIF proteome was primarily composed of shed or secreted proteins that were eventually found in the circulation. The serum levels of eight proteins (NNMT, ENO2, TSP1, CD14, LGALB1, TBG [SERPINA7], ANXA4, and FT H1) were elevated in patients [67].

Compared with body fluid samples such as urine and blood, tissue samples contain richer protein metabolism information. Sato *et al.* used liquid chromatography-mass spectrometry to analyze cancer tissues and normal renal tissues of 20 ccRCC patients and found that a total of 58 metabolites were significantly elevated in tumor tissues, of which 34 showed potential for early diagnosis [68]. They also demonstrated some of the characteristic signaling pathways for malignant RCC, namely the TCA cycle, the TCA intermediates, the nucleotide sugar pathway, and the inositol pathway. Similarly, Niziol *et al.* found that the concentrations of acetyl carnitine in the lipid metabolism pathway and glutamine in the amino acid metabolism pathway were significantly increased in tumor tissues, which can be used as potential diagnostic biomarkers [69].

In addition to the aforementioned biomarkers based on proteomics and metabolomics methods, other molecules play an important role in the diagnosis, treatment, and prognosis of RCC. Miikkulainen *et al.* found that high expression of prolyl hydroxylase-3 (*PHD3*) in ccRCC can maintain high expression of *HIF2α* and its target genes, thereby enhancing the invasiveness of cancer cells [70]. In addition, two studies showed that serum *PHD3* is a new serological diagnostic biomarker for RCC [71,72]. Furthermore, kidney injury molecule-1 (*KIM-1*) can also be used as a potential diagnostic marker, as its expression level is significantly correlated with kidney injury status and increases with the stage of the disease [73,74].

In addition, Koh *et al.* showed that patients with reduced ctDNA mutation abundance had better progression-free survival (PFS, $P = 0.0441$) than those with increased mutation abundance. Therefore, early ctDNA dynamics can be used as a predictive biomarker for ICI treatment response in patients with metastatic RCC [75]. Nuzzo *et al.* used a cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) technology to screen markers for the whole genome methylation profile of ctDNA in plasma samples from 99 patients with stage I-IV RCC and 28 healthy cancer-free controls [76]. The top 300 differentially methylated regions (DMRs) are capable of discriminating between plasma RCC and other samples. **Table 1** shows major omics-based biomarkers in renal cell carcinoma.

Table 1. Major omics-based biomarkers in renal cell carcinoma

Sample type	Sample size	Markers/Models	Diagnostic performance	Function	Reference
Urine	30 RCC patients and 37 non-renal cancer volunteers	2,5,8-trimethyl-1,2,3,4-tetrahydronaphthalene-1-ol	Sensitivity: 73.9%; Specificity: 79.0%	Diagnosis	Monteiro [56]
	100 patients with RCC, 34 patients with benign renal tumors, and 129 healthy volunteers	N-formyl kynurenine	Sensitivity: 84.8%; Specificity: 83.8%; AUC = 0.808	Diagnosis	Liu [57]
	56 patients with RCC, controls: 27 patients with T1ARCC and 10 patients with benign tumors	(lactic acid, glycine, 2-hydroxyglutarate, succinic acid, and kynurenic acid) combined model (prediction of recurrence)	Sensitivity, Specificity, AUC: 88.9%, 88.0%, 0.894	Predicting recurrence	Morozumi [58]
	23 patients with RCC (14 ccRCC and 9 PRCC) and 23 healthy controls	Diagnostic markers: p-cresol glucuronide Prognostic markers: isobutyl-l-carnitine and l-proline betaine	Fold change = 2.922, $P = 0.012$; Fold change = 2.098, $P = 0.004$; Fold change = 3.328, $P = 0.004$)	Diagnosis and prognostic	Oto [59]
	12 patients with ccRCC and 11 non-neoplastic patients without urinary tract disease	Combined model of polycystic kidney disease-like 1 (PKHD1L1), angiopoietin-like protein 6 (ANGPTL6), fatty acid binding protein 4 (FABP4), and complement C3	AUC = 0.835	Diagnosis and prognostic	Yang [60]
Blood	45 healthy controls, 40 patients with benign renal tumors, and 46 patients with RCCs	3-β-D-Galactosyl-sn-glycerol, 7,8-Dihydroneopteri, lysophosphatidylcholine (LPC), and γ-Amino butyryl-lysine	RCC group: Healthy control group: AUC = 0.990, 0.916, 0.909, 0.962; Sensitivity: 97.73%, 97.73%, 93.18%, 86.36%; Specificity: 100.00%, 73.33%, 80.00%, 95.56%)	Diagnosis	Wang [63]
	37 RCC patients	Combined model of cholesterol ester, ceramide, lysophosphatidylcholine, phosphatidylcholine, PC36:3, sphingomyelin, and SM41:1	Sensitivity, specificity, and accuracy: 85%, 95%, and 92%	Diagnosis	Wolrab [64]
	104 RCC patients and healthy volunteers	(alanine, creatine, choline, isoleucine, lactate, leucine, and valine) combined model	Accuracy: 94.74%	Diagnosis	Zheng [65]
	99 ccRCC patients, 14 patients with benign renal tumors, and 29 healthy controls	KIM-1	Sensitivity and specificity for stage I: 81% and 83%; sensitivity for stages II to IV: 97%	Diagnosis	USHLINSKII [74]
	56 patients who underwent radical or partial nephrectomy, 13 patients with benign renal tumors, and 56 healthy controls	PHD3	AUC = 0.668, sensitivity, specificity, positive predictive value, and negative predictive value: 66.1%, 68.1%, 28.8%, and 37.3%	Diagnosis	Kim [72]
Blood and urine	27 patients with stage T1-2 ccRCC and 27 healthy volunteers	plasma proteins (FGFR1, GOT1, FG*FBP2*), urinary proteins (CETP, SEZ6L2, COX5B)	Blood or Urine: Specificity: 92.6%/92.6%, Sensitivity: 96.3%/92.6%	Diagnosis	Liu [61]
Tumor interstitial fluid (TIF)	10 ccRCC patients and matched NAT	thrombospondin-1 (TSP1), thyroxine-binding globulin (TBG), CD14, enolase 2 (ENO2), nicotinamide N-methyltransferase (NNMT), Annexin A41b, Ferritin 1, galectin-1	SRM peak area fold change: 2.9 ± 2.9, 1.7 ± 0.9, 2.9 ± 1.7, 11.7 ± 18.8, 11.7 ± 22.9, 9.9 ± 14.7, 2.4 ± 2.0, 1.8 ± 1.3	Diagnosis	Teng [67]

7. Conclusion

RCC is one of the common cancers of the urinary system. Its main abnormal metabolic characteristics, such as the Warburg effect, increased fat deposition, and mitochondrial dysfunction, can cause various changes in proteins and metabolites.

The abnormal metabolic characteristics of ccRCC determine the application of proteomics and metabolomics technologies in it. With the development of the omics-based technologies, a variety of potential biomarkers have been found that are expected to be used for early diagnosis, treatment, or prediction of prognosis. However, the accuracy of biomarkers identified by these technologies needs to be improved, and the specific molecular mechanisms of the identified biomarkers in tumors need to be further explored.

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