# Novel Expanding Renal Cell Carcinoma Biomarkers

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## Abstract

Identification of reliable molecular biomarkers that can complement clinical practice represents a fascinating challenge in any cancer field. Renal tumors are usually asymptomatic and incidentally identified during imaging studies undertaken for unrelated causes. However, in 25% to 30% of patients the first diagnosis is accompanied by symptoms and associated with distant metastasis. Thus, early diagnosis may reduce the risk of disease progression also avoiding side effects of inadequate treatments. Moreover, the ability to categorize patients' risk of recurrence after radical treatment, or even predict benefit from a target therapy, represents a compelling challenge. Here we review the current state-of-the-art on RCC biomarkers, particularly focusing on the new approaches of genomics, liquid biopsy, proteomics, and metabolomics.

## Introduction

Renal cell carcinoma (RCC) is the third most common urological cancer in the United States, with an estimated 44 120 new cases in 2019[1]. Clear-cell renal cell carcinoma (ccRCC) is the most frequent subtype, accounting for approximately 75% to 80% of these tumors, and is responsible for the majority of kidney cancer deaths[2]. In this narrative review we present the current state-of-the-art on diagnostic and prognostic RCC biomarkers, particularly focusing on the new approaches of genomics, liquid biopsy, proteomics, and metabolomics. A MEDLINE/PubMed search was performed using individual or/and different combinations of terms including “renal cell carcinoma,” “biomarker,” “diagnosis,” “prognosis,” and “survival.” Only papers with the title and abstract in the English language were screened for eligibility. The full text of included papers was analyzed.

## Biomarkers in Early Detection and Diagnosis

Recent advances in diagnostic techniques have increased early ccRCC detection. Mortality rates, however, remain steady[3]. Imaging studies are still unable to differentiate histology, and renal mass biopsy has a 10% to 20% non-diagnostic rate[4]. Therefore, it is highly desirable to have novel and reliable biomarkers suitable for RCC screening and early detection, ensuring that the benefits of new technologies are fully realised (Table 1).

### Circulating cell-free DNA

Liquid biopsy assays, such as circulating tumor cells (CTCs) or circulating cell-free DNA (cfDNA), constitute promising and less invasive techniques that can overcome the limits related to conventional diagnostic methods[5]. cfDNA consist mostly of double-stranded molecules that circulate as nucleoprotein complexes[6].

Hauser et al. evaluated cfDNA from patients with RCC and from healthy individuals using quantitative real-time polymerase chain reaction (PCR). Two primer sets amplifying a sequence of the actin-beta gene (ACTB) were used: ACTB-106 detects fragmented cfDNA that results from apoptosis, and ACTB-384 detects long DNA fragments released by necrosis. In this analysis, DNA fragments were significantly increased in RCC patients compared to healthy controls[7]. Lu et al. evaluated cfDNA extracted from plasma of healthy controls and 229 ccRCC patients at stages M0 and M1. The 306 base pairs fragment was lower in RCC patients than in controls. Since cfDNA fragment sizes are indicators of the integrity of cfDNA molecules, the authors showed that the ratio of longer to shorter cfDNA

Key Words

Renal cell carcinoma, biomarker, diagnosis, prognosis, survival

Competing Interests

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## Abbreviations

| | |
|---|---|
| ACTB | actin-beta gene |
| ccRCC | clear-cell renal cell carcinoma |
| CSS | cancer-specific survival |
| CTCs | circulating tumor cells |
| cfDNA | circulating cell-free DNA |
| miRs | microRNAs |
| OS | overall survival |
| PCR | polymerase chain reaction |
| PFS | progression-free survival |
| RCC | renal cell carcinoma |

fragments was significantly improved in patients staged as M0 compared with those as M1 subgroup[8]. On plasma cfDNA, Yamamoto et al. showed that median levels of cfDNA and median size of fragments from RCC patients were significantly greater than those from controls. An optimal cut-off value of 2876 copies/mL was identified[9].

Nuzzo et al. performed cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq). cfMeDIP-seq is an enrichment-based method to comprehensively interrogate cfDNA methylation profile extracted from plasma and urine. The authors identified differentially methylated regions and selected the top rated between case and control samples. Samples from RCC patients were assigned a higher median methylation score than those from controls. Furthermore, the lowest methylation scores in RCC patients came from patients with small tumors. Thus the authors reported an accurate classification of patients across all stages of RCC in plasma cfDNA (AUC 0.99) and demonstrated the validity of this assay using urine cfDNA (AUC 0.86)[10].

The abundance and relative fragmentation of cfDNA in blood can be a universal marker for RCC7 yet the precise cfDNA metrics that are most clinically relevant remain controversial. Study results reported to date are limited by heterogeneity with respect to clinical stage, tumor pathology, blood sample processing, and methods of cfDNA analysis.

## Circulating tumor cells

CTCs are cells that have been shed into the vasculature or lymphatics from a primary tumor. The detection and analysis of CTCs can assist in determining patient prognosis and personalized treatments, as well as initial diagnostic and monitoring procedures. Moreover, CTCs are particularly suited to interrogate functional heterogeneity by combining genetic and transcriptomic assessment of single CTC[11] or by transcriptome and epigenome analysis[12].

It is difficult to assess the diagnostic value of CTCs in RCC due to the use of different methods of CTC collection and identification across studies[13]. The different techniques include epithelial or non-epithelial marker-dependent isolation, reverse transcription PCR-based methods, and morphological- and cell size-based methods[14]. Moreover, RCC cancer cells are inclined to the loss of their epithelial antigens through epithelial-to-mesenchymal transition, in which morphological transformation leads to acquisition of mesenchymal features[15]. Other surface markers have been developed to select RCC cancer cells in the blood (eg, CAIX). Adding this new set of cell surface markers including CAIX and CD147 to the conventional detection of CTCs through epithelial markers, such as the epithelial cell adhesion molecule (EpCAM), has shown better results[16].

## The role of microRNAs

MicroRNAs (miRs) are implicated in the regulation of processes such as proliferation, migration, invasion, and apoptosis, and are readily detectable in tissues and bodily fluids[17].

Wulfken et al. reported that the level of miR-1233 was significantly increased in patients with RCC compared with healthy controls. Thus, miR-1233 levels were investigated in an independent cohort confirming a higher mean value in RCC patients[18]. Zhao et al. found that miR-210 levels were higher in primary RCC tissues than in normal tissue. Furthermore, the serum level of miR-210 was significantly decreased in patients 7 days after nephrectomy; consequently, a potential combined role in early detection and monitoring after radical treatment could be proposed[19]. Iwamoto et al. confirmed at the serum level that the expression of miR-210 was significantly higher in RCC patients than in healthy controls[20]. In addition, a meta-analysis conducted by Chen et al. that included 7 studies, 570 RCC patients, and 415 healthy controls showed pooled sensitivity, specificity, and diagnostic OR to predict RCC of 74%, 76%, and 8.81, respectively[21].

Chen et al. evaluated the expression levels of miR-129-3p and miR-129-5p in 69 cases of paired renal tumors, healthy tissues, and conventional RCC cell lines. MiR-129-3p and miR-129-5p are 2 mature products of miR-129-2 known for its anti-tumor effects in various malignancies. They showed that miR-129-3p, but not miR-129-5p, was widely attenuated in human ccRCC, and chRCC, yielding a 73.5% accuracy in discriminating ccRCC from normal tissues. The relative miR-129-3p expression significantly differed between malignant and benign kidney tumors[22].

In a prospective cohort, Yadav et al. found that use of serum miR-34a, miR-141, and miR-1233 was able

to diagnose ccRCC with a sensitivity of 80.76%, 75%, and 93.33%, and specificity of 80%, 73.33%, and 100%, respectively, when tumor pathologic was used as the reference. Moreover, a combined approach using a panel of 2 serum miRs (miR-141 and miR-1233), allowed a diagnosis of ccRCC with 100% sensitivity and 73.3% specificity[23].

Recently, Zhang et al. investigated whether miRNAs in serum exosomes can serve as biomarkers in ccRCC. Their findings showed that the expression levels of exosomal miR-210 and miR-1233 were significantly higher in RCC patients than in healthy individuals (both $P < 0.01$). ROC analysis demonstrated that exosomal expression levels distinguished RCC patients from healthy individuals with 70% sensitivity and 62.2% specificity for miR-210, and 81% sensitivity and 76% specificity for miR-1233[24].

### Metabolites as novel biomarkers of RCC

Metabolomic approaches have shown promising results in oncology, with the recognition of metabolic reprogramming as a hallmark of cancer. Globally, RCC metabolic signature of the tumor microenvironment is characterized by alterations in metabolites associated with energy metabolism, especially those involved in glycolysis, amino acid metabolism, and fatty acid catabolism pathways, which are essential for cell growth and proliferation[25].

Kim et al. first evaluated the utility of urine metabolomics analysis for metabolomic profiling. The authors identified a total of 212 molecules able to differentiate RCC presence. The rate of correct classification was 88%[26]. Ganti et al. showed differential urinary concentrations of several acylcarnitines as a surrogate of RCC status and grade, with most acylcarnitines being increased in RCC patients' urine. Furthermore, urinary acylcarnitines were increased in a grade-dependent fashion in RCC patients and likely emanated from the tumor tissues. Acylcarnitines have both cytotoxicity and immune modulatory properties and thus may play a role in decreasing the inflammatory response and providing a mechanism by which these cells are able to evade immune surveillance[27]. In the same field, Niziol et al. showed that hydroxybutyrylcarnitine, decanoylcarnitine, propanoylcarnitine, carnitine, dodecanoylcarnitine, and norepinephrine sulfate were found in much higher concentrations in both RCC tissues (compared with the paired normal tissue) and urine of cancer patients (compared with urine of control subjects)[28].

### Proteomics analysis

Proteomics offers a useful platform to study the complex molecular events of tumorigenesis. Upregulation in the glycolytic flux is a common pathway in cancer. Therefore, using isobaric tags for relative and absolute quantitation (iTRAQ) White et al. identified 55 proteins significantly dysregulated in RCC. Dysregulation of alpha-enolase (ENO1), L-lactate dehydrogenase A chain (LDHA), heatshock protein beta-1, known as Hsp27, mitochondrial (HSPE1) was confirmed in 2 independent sets of patients by western blot and immunohistochemistry (IHC). The expressions of AHNAK, ENO1, and Hsp27 were found to be significantly elevated in ccRCC compared with matched normal tissues. Whereas HSPE1 was significantly downregulated in RCC patients[29]. Zhang et al. recently found 16 significantly upregulated and 14 significantly downregulated in early-stage RCC compared with healthy controls. Serum heat shock cognate 71 (HSC71) was highly elevated in the RCC group compared with control group[30]. Kim et al. showed that RCC upregulated proteins were nicotinamide-N-methyltransferase (NNMT), secretagogin (SCGN), L-plastin, human neuron specific enolase (hNSE), nonmetastatic cell 1 (NM23A), ferritin light chain (FTL), and thioredoxin peroxidase (KIM2010). NNMT was the most commonly upregulated protein over all types of RCC, especially in comparison with normal tissues. SCGN was elevated in ccRCC samples but not in papillary, chromophobe, or normal tissue, while NM23A showed the same behavior, although the magnitude of changes was smaller than in the first 2 molecules[31].

## Prognostic Biomarkers

Although most biomarkers for early detection and diagnosis remain at an early stage, more advances have been made with prognostic biomarkers for RCC. To date, few biomarkers have been taken beyond single studies, thus none are yet ready for routine clinical practice. Furthermore, emerging and promising approaches can serve as new platform in which novel potential biomarkers can be found. Following any type of surgical treatment of RCC, there is a need for risk stratification aiming to enable personalized outcome prediction. The major endpoints evaluated and predicted using prognostic biomarkers across the studies referred to in the following sections of this paper are disease/progression/recurrence-free survival (D/P/RFS), overall and cancer-specific survival (OS, CSS), and correlations with clinicopathological features that might influence the prognosis among these patients[32] (Table 2).

### cfDNA

One of the most promising uses of liquid biopsy is to determine the risk of recurrence after curative treatment. Wan et al. measured plasma levels of cfDNA before and after surgery for localized disease. Mean preoperative level of plasma cfDNA in patients who developed recurrent disease was significantly higher than in those

**TABLE 1.**

Novel potential candidates biomarkers in diagnosis and early detection of renal cell carcinoma

| Biomarker | Source | Trend | Correlation/Use | Reference |
|---|---|---|---|---|
| cfDNA | plasma<br>serum | increased ↑ | RCC and mRCC detection, association with histotype, monitoring after curative surgery | Hauser et al. (2010)[7]<br>de Martino et al. (2012)[51]<br>Lu et al. (2016)[8]<br>Yamamoto et al. (2018)[9]<br>Nuzzo et al. (2020)[10] |
| CTCs | blood | increased ↑ | RCC detection and monitoring | Allard et al. (2004)[52]<br>Li et al. (2005)[53]<br>Liu et al. (2016)[16]<br>Broncy et al. (2018)[54] |
| **miRNA** | | | | |
| miR-1233 | serum | increased ↑ | RCC detection | Wulfken et al. (2011)[18]<br>Zhang et al (2018)[24]<br>Yadav et al. (2017)[23] |
| miR-451 | serum | decreased ↓ | RCC detection | Redova et al. (2012)[55] |
| miR-378 | serum | increased ↑ | RCC detection | Redova et al. (2012)[55] |
| miR-21 | tissue | increased ↑ | RCC detection, differential diagnosis between ccRCC, pRCC and chRCC and oncocytoma | Faragalla et al. (2012)[56] |
| miR-15a | tissue<br>urine | both<br>increased ↑ | RCC detection, differential diagnosis between malignant and benign renal tumors | von Brandestain et al. (2012)[57] |
| miR-210 | tissue<br>urine<br>serum | increased ↑<br>increased ↑<br>increased ↑ | RCC detection and disease monitoring after local treatment | Zhao et al. (2013)[19]<br>Iwamoto et al. (2014)[20]<br>Zhang et al. (2018)[24]<br>Chen et al. (2018)[21] |
| miR-129-3p | tissue | decreased ↓ | RCC detection, differential diagnosis between malignant and benign renal tumors | Chen et al. (2014)[22] |
| miR-34a | serum | decreased ↓ | RCC detection | Yadav et al. (2017)[23] |
| miR-141 | serum | decreased ↓ | RCC detection | Yadav et al. (2017)[23] |

*Abbreviations: cfDNA, cell-free DNA; ccRCC, clear-cell renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; CTCs, circulating tumor cells; ENO1, alpha-enolase; FINC, fibronectin-1; HSC71, heat shock cognate 71; HSPE1, heat shock protein family E member 1; miRNA, microRNA; mRCC, metastatic renal cell carcinoma; NNMT, nicotinamide N-methyltransferase; pRCC, papillary renal cell carcinoma; RCC, renal cell carcinoma; S100A8, S100 calcium-binding protein A8; S100A9, S100 calcium-binding protein A9; Tu M2-PK, tumor M2-PK.*

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with localized disease or controls[33]. Analyzing the genomic and mitochondrial cfDNA concentrations, Lu et al. developed 2 models that incorporated clinicopathological features to specific expression patterns among cfDNA fragments. Particularly, *APP* gene, the *Alu* sequences, and the mitochondrial DNA fragments showing significant correlation in terms of OS and RFS[8]. In terms of quantitative measurement,

**TABLE 1.**

Novel potential candidates biomarkers in diagnosis and early detection of renal cell carcinoma, *Cont'd*

| Biomarker | Source | Trend | Correlation/Use | Reference |
|---|---|---|---|---|
| **Metabolomics and Proteomics** | | | | |
| Acetylcarnitines | urine<br>tissue | increased ↑ | RCC detection, grade-dependent behavior | Ganti et al. (2012)[27]<br>Niziol et al. (2018)[28] |
| Tu M2-PK | plasma | increased ↑ | RCC and mRCC detection | Roigas et al. (2001)[58]<br>Weinberger et al. (2007)[59] |
| AHNAK | tissue | increased ↑ | RCC detection | White et al. (2014)[29] |
| ENO1 | tissue | increased ↑ | RCC detection | White et al. (2014)[29] |
| HSPE1 | tissue | decreased ↓ | RCC detection | White et al. (2014)[29] |
| NNMT | tissue | increased ↑ | RCC detection | Kim et al. (2010)[31] |
| HSC71 | serum | increased ↑ | RCC detection | Zhang Y et al. (2015)[30] |
| S100A8 | serum | increased ↑ | RCC detection | Zhang L et al. (2015)[60]<br>Zhang L et al. (2016)[61] |
| S100A9 | serum | increased ↑ | RCC detection | Zhang L et al. (2015)[60]<br>Zhang L et al. (2016)[61] |
| FINC | plasma | increased ↑ | RCC detection | Yokomizo et al. (2011)[62] |

*Abbreviations: cfDNA, cell-free DNA; ccRCC, clear-cell renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; CTCs, circulating tumor cells; ENO1, alpha-enolase; FINC, fibronectin-1; HSC71, heat shock cognate 71; HSPE1, heat shock protein family E member 1; miRNA, microRNA; mRCC, metastatic renal cell carcinoma; NNMT, nicotinamide N-methyltransferase; pRCC, papillary renal cell carcinoma; RCC, renal cell carcinoma; S100A8, S100 calcium-binding protein A8; S100A9, S100 calcium-binding protein A9; Tu M2-PK, tumor M2-PK.*

Yamamoto et al. divided their cohort into 2 subgroups according to the length of cfDNA fragments. Their results showed that cfDNA fragment size was significantly associated with progression-free survival (PFS). Although cfDNA fragmentation correlated with poorer outcomes, cfDNA plasma levels were not associated with any of survival outcomes[9]. Evaluating plasma circulating tumor DNA (ctDNA) as a subset of cfDNA, Bacon et al. reported that only 33% of patients had detectable ctDNA. Among ctDNA-positive patients the most commonly mutated genes were *VHL*, *BAP1*, and *PBRM1*. Moreover, ctDNA-positive patients had shorter OS and PFS on first-line therapy[34].

## CTCs identification

An initial experience using a RT-PCR assay to detect CTCs in peripheral blood of patients at different stages of RCC reported a different rate of positivity on localized and metastatic RCC (mRCC)[35]. Developing a new set of cell surface markers including CAIX and CD147, Liu et al. showed a significant association of CTC number/CTC expression status of vimentin, with disease progression[16]. Wang et al. investigated the relationship of dynamic changes of CTCs and Beclin-1 expression of CTCs and RCC prognosis. CTCs were divided into epithelial, mesenchymal, and mixed phenotype-based surface biomarkers. For the metastatic group, the number of mixed CTCs at 12 months was significantly higher than mixed preoperatively and 6 months CTCs. Of note, the number of preoperative Beclin-1 positive CTCs was significantly higher in the metastatic group. Thus, variation trend of CTCs and Beclin-1 expressive CTCs was significantly associated with the onset of metastatic disease[36]. Moreover, in a prospective cohort of 60 patients who underwent surgical treatment with curative intent, Haga et al. evaluated CTCs drawn from a peripheral artery collected just before and immediately after surgery. The authors showed that open

**TABLE 2.**
Novel potential candidate biomarkers in prognosis of renal cell carcinoma

| Biomarker | Source | Outcomes correlated | Reference |
|---|---|---|---|
| CTCs | peripheral blood | RFS, OS | Bluemke et al. (2009)[63]<br>Liu et al. (2016)[16]<br>Wang et al. (2019)[36] |
| Beclin-1-positive CTCs | peripheral blood | RFS | Wang et al. (2019)[36] |
| cfDNA | plasma | RFS, OS | de Martino et al. (2011)[51]<br>Wan et al. (2013)[33]<br>Lu et al. (2016)[8]<br>Yamamoto et al. (2018)[9]<br>Bacon et al. (2020)[34] |
| **miRNA** | | | |
| miR-378 | serum | DFS, clinical stage | Fedorko et al. (2015)[64] |
| miR-221 | serum | OS, CSM,<br>lymphovascular invasion | Teixeira et al. (2014)[65]<br>Vergo et al. (2014)[66] |
| miR-150 | serum | DSS, clinical stage | Chanudet et al. (2017)[67] |
| miR-451 | serum | clinical stage | Redova et al. (2012)[68] |
| miR-21 | tumor tissue | CSS, OS, DFS, clinical stage, tumor grade, tumor size | Faragalla et al. (2012)[56]<br>Tang et al. (2015)[38]<br>Vergho et al. (2014)[69]<br>Vergho et al. (2014)[66] |
| miR-126 | tumor tissue | DFS, CSS, OS | Vergho et al. (2014)[69]<br>Vergho et al. (2014)[66]<br>Khella et al. (2015)[70] |
| miR-106b | tumor tissue | PFS | Slaby et al. (2010)[71] |
| miR-27a-3p | tumor tissue | PFS | Nakata et al. (2015)[72] |
| miR-210 | tumor tissue | CSS | Tang et al. (2015)[38] |
| miR-141 | tumor tissue | CSS | Tang et al. (2015)[38] |
| miR-200c | tumor tissue | CSS | Tang et al. (2015)[38] |
| miR-429 | tumor tissue | CSS | Tang et al. (2015)[38] |
| miR-486 | tumor tissue | CSM, clinical stage | Goto et al. (2013)[73] |
| miR-23b | tumor tissue | OS | Ishihara et al. (2014)[74] |

*Abbreviations: cfDNA, cell-free DNA; CSM, cancer-specific mortality; CSS, cancer-specific survival; CTCs, circulating tumor cells; DFS, disease-free survival; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; OS, overall survival; PFS, progression-free survival; PKM2, pyruvate kinase-muscle-2; RCC, renal cell carcinoma; RFS, recurrence-free survival; S100A8, S100 calcium-binding protein A8; TK1, thymidine kinase 1; Tu M2-PK, tumor M2-P*

continued on **page 38**

**TABLE 2.**
Novel potential candidate biomarkers in prognosis of renal cell carcinoma, *Cont'd*

| Biomarker | Source | Outcomes correlated | Reference |
|---|---|---|---|
| miR-27b | tumor tissue | OS | Ishihara et al. (2014)[74] |
| lnc-ZNF180-2 | tumor tissue | PFS, CSS, OS, clinical stage | Ellinger et al. (2015)[40] |
| lnc-NBAT-1 | tumor tissue | OS | Xue et al. (2015)[75] |
| **Metabolomics** | | | |
| Creatine | tumor tissue | Advanced Tumor Stages (T3-4) | Gato et al. (2012)[42] |
| Glutamate | tumor tissue | Advanced Tumor Stages (T3-4) | Gato et al. (2012)[42] |
| Glutamine | tumor tissue | Advanced Tumor Stages (T3-4) | Gato et al. (2012)[42] |
| GAPDH | tumor tissue | High Grade | Wettersten et al. (2015)[43] |
| Enolase-2 | tumor tissue | High Grade | Wettersten et al. (2015)[43] |
| PKM2 | tumor tissue | High Grade | Wettersten et al. (2015)[43] |
| L-Lactate | tumor tissue | High Grade | Wettersten et al. (2015)[43] |
| Glutathione | tumor tissue | Advanced Stages | Hakimi et al. (2016)[44] |
| TuM2-PK | serum | RFS | Nisman et al. (2010)[76]<br>Gayed et al. (2015)[77] |
| TK1 | serum | RFS | Nisman et al. (2010)[76] |
| cathepsin D | urine | OS | Vasudev et al. (2009)[78] |
| S100A8 | tissue | DFS, tumor grade, stage | An et al. (2019)[79] |

*Abbreviations: cfDNA, cell-free DNA; CSM, cancer-specific mortality; CSS, cancer-specific survival; CTCs, circulating tumor cells; DFS, disease-free survival; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; OS, overall survival; PFS, progression-free survival; PKM2, pyruvate kinase-muscle-2; RCC, renal cell carcinoma; RFS, recurrence-free survival; S100A8, S100 calcium-binding protein A8; TK1, thymidine kinase 1; Tu M2-PK, tumor M2-P*

nephrectomy resulted in a significantly greater number of postoperative CTCs. At multivariate level that the surgical approach was significantly correlated with the number of postoperative CTCs ($P = 0.016$) and the perioperative change in CTCs ($P = 0.01$). Thus, especially after open surgery more cancer cells can be expelled into the bloodstream, suggesting a careful follow-up for these patients[37].

## miRNA

In a comprehensive meta-analysis of 29 published studies reporting miRNA signatures in RCC, Tang et al. identified a robust meta-signature of miRNAs as a prognostic biomarker. They reported that high expression of miR-21, miR-210, and low expression of miR-141, miR-200c, and miR-429 were associated with worse CSS following RCC resection[38]. Similarly,

Gu et al. conducted a meta-analysis of 27 published studies and found that elevated expression of miR-21, miR-1260b, miR-210, miR-100, miR-125b, miR-221, miR-630, and miR-497 was associated with a poor prognosis in RCC patients. Conversely, decreased expression of miR-106b, miR-99a, miR-1826, miR-215, miR-217, miR-187, miR-129–3p, miR-23b, miR-27b, and miR-126 was associated with a worse prognosis. Importantly, the results from this meta-analysis confirmed that elevated miR-21 expression was associated with shorter OS, CSS, and DFS. The decreased expression of miR-126 was associated with shorter CSS, OS, and DFS[39].

Also, results were promising in a study by Ellinger et al. regarding specific circulating long non-coding (lnc) RNAs, defined as RNA transcripts longer than 200 nucleotides that are not transcribed into a protein. The authors next validated the expression profile of 6 lncRNAs transcripts (lnc-ACO1625, lnc-CYP4A22-2/3, lnc-PEAK1.1-1, lnc-PCYOX1L, lnc-VCAN-1, lnc-ZNF180-2) with potential prognostic interest. A significant increase of lnc-ZNF180-2 expression in advanced RCC tissue compared with localized RCC was observed. Furthermore, lnc-ZNF180-2 expression levels were an independent predictor of PFS, CSS, and OS[40]. Qu et al. built a model named RCClnc4 based on 4 lncRNAs to improve postoperative risk stratification after radical treatment. Stratifying patients into high-risk versus low-risk groups in terms of clinical outcomes, RCClnc4 remained as an independent prognostic factor, achieving a higher accuracy than clinical staging systems like TNM and SSIGN score[41].

## Prognostic value of metabolomic approaches

Analyzing tumors and their matched tissue, Gao et al. studied the metabolomic RCC profile. Creatine, glutamate, and glutamine were found at higher concentrations in tissues of tumors at T3-4 stages[42]. The glycolysis-relevant metabolites are significantly increased in high-grade disease, suggesting that glucose metabolism is more prominent with increasing tumor grade. Consequently, glyceraldehyde 3-phosphate dehydrogenase, enolase 2, and pyruvate kinase-muscle-2 are increased in tumor tissue as compared with normal tissues. L-lactate follows the same tendency in a grade-dependent manner. Also levels of carnitine and acyl/acetyl-carnitines were associated with grade, suggesting how the combination of these metabolites can predict the biological aggressiveness of RCC and thus influence its prognosis[43]. A study by Hakemi et al. showed increased levels of glutathione were also grade- and stage-dependent[44]. Thus, the upregulation of antioxidant capacity in adaptation to intrinsic oxidative stress is indeed a common event in RCC, especially in the advanced stages[45].

## Epigenetic and DNA methylation biomarkers

Epigenetic variations play an important role in renal carcinogenesis and progression. DNA methylation is defined as a covalent addition of a methyl group to cytosines that precede a guanosine which are mainly clustered as CpG islands in the promoter region of genes bringing a functional silencing[46]. Furthermore, DNA methylation alterations are often shown to be associated with clinicopathological features and RCC patient survival or both[47]. CpG island methylation markers reflect tumor biology, allowing the identification of patients with "high epigenetic risk" who can benefit from tailored management to improve survival outcomes.

In a recent systematic review, Joosten et al. described 9 genes (*SFRP1, BNC1, GREM1, RASSF1A, PCDH8, SCUBE3, GATA5, LAD1,* and *NEFH*), associated with patient survival. Their prognostic value was independently validated in other studies[48]. To develop a 5-CpG-based assay for ccRCC prognosis, a panel composed by methylation of *PITX1, FOXE3, TWF2, RIN1,* and *EHBP1L*, was validated in 3 independent sets from China, the United States, and the Cancer Genome Atlas (TCGA) database. Stratifying patients into 2 groups from this 5-CpG panel, Wei et al. defined low- and high-risk categories. An important correlation between the high-risk group and poorer OS[49] was demonstrated. With the same endpoint, Chen et al. identified 7 specific prognosis-subgroups based on the DNA methylation spectrum of RCC from the TCGA database. The specific DNA methylation patterns reflected differentially in the clinical index, including TNM classification, pathological grade, clinical stage, and age. In addition, 437 CpGs corresponding to 477 genes of 151 samples were identified as specific hyper/hypomethylation sites for each specific subgroup. The authors then constructed a Bayesian classifier to determine the function of the prognosis prediction model, with 437 specific CpG sites as characters (AUC 0.95)[50].

## Conclusions

Cancer biomarkers have shifted treatment and management of patients with many cancer types. Although "personalized" medicine is becoming more common in our daily practice, none of the RCC biomarkers discussed are in routine clinical use. Metabolomics and proteomics studies have shown excellent potential in terms of diagnostic accuracy, but research in these areas still appears to be hypothesis-generating. Most of publications mentioned above aimed to understand tumor biology due to the high heterogenicity of RCC.

Circulating biomarkers have attracted a lot of interest; however, the great diversity of techniques precludes any further conclusions. The growing use of liquid biopsy, popularized by the easy accessibility of samples, and the accompanying standardization of methods of analysis and quantification of CTCs, cfDNA and miRNAs will continue to provide promising results. Particularly, NGS cfDNA is a novel technology that can complement tumor tissue biopsy. It has demonstrated its potential role across the diagnostic and prognostic fields of both localized and metastatic RCC. Single molecule validations are being replaced by multipanel biomarkers to provide improved validation results. It also reflects the role of molecular biology in current clinical nomograms as a transition tool from bench to bedside.

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