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KIDNEY DISEASES (G CIANCIO, SECTION EDITOR)

Biomarkers of Renal Tumors: the Current State and Clinical Perspectives

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Abstract Renal cell carcinoma (RCC) ranks the first death rate among the urogenital tumors, whereas its incidence follows the incidences of prostate and bladder cancer. The diagnosis of RCC at early stages allows immediately undertaking appropriate treatment, which significantly increases patients' survival rate. Early and accurate diagnosis avoids inadequate treatment, provides the disease progression forecast, and permits to apply more efficient therapy. Unfortunately, the small renal tumors are usually asymptomatic resulting in the late diagnosis and, therefore, low efficacy of treatment. Thus, sensible and preventive biomarkers are essential for early RCC detection and monitoring of its progression. So far, many attempts were performed aimed at recognizing novel informative kidney tumor biomarkers applicable for early detection of the disease and possessing prognostic and predictive capabilities. This review summarizes recent advances in renal tumor biomarkers recognition, their diagnostic and prognostic values, and clinical feasibility.

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Introduction

So far, many types of distinct kidney tumors have been identified. More than 150 types among them are benign. Oncocytoma, adenoma, and angiomyolipoma are the most frequent benign kidney tumors, whereas leiomyoma, hemangioma, lipoma, and juxtaglomerular cell tumors are considered as rare ones. The three major types of malignant renal tumors are renal cell carcinoma, transitional cell cancer of the kidney occurring in adults, and Wilms' tumor occurring in children [1].

Renal cell carcinoma (RCC; ~90% of all malignant kidney tumors) usually represents one of distinct nephron malignant disorders, which may belong to different histological types, and differ in subsequent response to therapy [2]. As a result, RCC is one of the mostly common and deadly malignancies with approximately 61,560 new cases and 14,080 deaths estimated in the USA in 2015 [3]. Males suffer from RCC twice more frequently than females. The incidence peak occurs at age 50–70 years [4].

Early diagnosis of asymptomatic small renal tumors leads to better treatment results [5]. However, to date, over 50% of all cases of RCC are discovered by chance during imaging studies for other comorbidities [6]. Furthermore, initial evaluation of RCC in 25–30% of patients identifies the presence of distant metastases [7]. The role of the physical examination in the diagnosis of small renal tumors is insignificant. Widespread of radiological techniques (ultrasound diagnostics, computed tomography, magnetic resonance imaging) allowed detection of small asymptomatic tumors. This also results in increase of incidence of the so-called "false RCC".

Nonetheless, the incidence spread of advanced forms of "true" RCC increases worldwide indicating real disease incidence growth [4]. Imaging studies are quite expensive and may not always accurately distinguish the benign kidney tumor formation from the malignant one [5], whereas kidney biopsy is an invasive method of investigation associated with certain complications, which is applied only with clear indications for its implementation. Thus, the development and further introduction into clinical practice of novel methods for kidney tumors diagnosis, and, in particular, accurate early diagnostic tools for RCC are still extremely important to obtain better treatment results [5].

Currently, the characterized renal tumor biomarkers can be classified as (i) markers for early disease detection, (ii) diagnostic markers, (iii) prognostic markers, and (iv) predictive markers [8]. Furthermore, the new prognostic biomarker pointing to association of overall survival of RCC patients with its promoter region methylation have been recently reported [9••]. This review summarizes recent advances in characterization and evaluation of diagnostic potential of currently available biomarkers associated with renal tumors.

Biomarkers for Early Detection

Despite the active studies focused on recognition of novel universal diagnostic or prognostic markers suitable for RCC, the early detection and diagnosis of RCC remains a challenge to oncologists. The frequency of late RCC diagnosis is still relatively high in comparison to other urological tumors. This problem arises from the absence of symptoms characterizing the early stages of the disease [10]. Thus, an urgent need for biomarkers suitable for early RCC recognition still exists. Obviously, biomarkers presented in blood are preferable than markers obtained from other tissues [11]. Currently, a number of serological markers cause an outstanding interest in the case of RCC early diagnosis.

Tu M2-PK

Dimeric type M2 form of pyruvate kinase (PK) represents one of the most promising biomarkers for renal cancer early detection. PK of healthy cell consists of four subunits and exists in several isoforms: L, R, M1, M2, which differ in their regulatory properties and localization. Type L is detected in liver and renal proximal tubules, type R—in erythrocytes, type M1—in muscle and brain, while type M2 —in the lungs [12]. However, only the type M2 PK is detectable in case of tumor cell, where it exists in the dimeric form. Thus, the dimeric M2-PK has been termed as Tumor M2-PK (Tu M2-PK). Several studies suggested that in the case of metastatic RCC, the level of Tu M2-PK in the patients' blood was significantly higher in comparison to those who have no metastases [13]. However, due to the low sensitivity (47%) at the early stages, it is not recommended to use this marker for primary diagnosis of RCC [14]. Importantly, in the study of Roigas et al. [13], the sensitivity was only 27.5% in non-metastatic RCC. Other authors have shown the prognostic role of Tu M2-PK with a significant correlation between the Tu M2-PK level and the RCC stage [14, 15]. The analysis of Tu M2-PK level changes in patients after surgical interference is of great interest. Thus, it was shown that after successful surgery of non-metastatic RCC, the increased level of Tu M2-PK normalized within 11 weeks, whereas it remained at the same level or even increased in the case of RCC-recurrence or metastasis [15].

VEGF

Overexpression of angiogenic factor, such as vascular endothelial growth factor (VEGF), induces the growth of new vessels. Consequently, VEGF is a key mediator in the invasion of RCC. There are few independent studies devoted to the diagnostic value of VEGF concentration alterations within the blood of patients with renal cell carcinoma. Thus, it was shown that plasma VEGF correlates with the grade and stage of RCC, as well as metastasis [16]. Plasma VEGF concentrations were significantly higher in patients with clear cell RCC than in the control group. The authors of another work found that the increased VEGF levels in the patients' serum correlated with shorter recurrence-free period and with reduction of these patients' survival rate [17]. The study by Negrier et al. investigated the interrelation between the survival of 302 patients and metastatic RCC after surgical treatment and the serum VEGF levels [18]. Thus, it was shown that the initial VEGF levels determine the progression-free survival value and the overall survival value in patients after surgical treatment of metastatic RCC. However, the use of VEGF as an independent prognostic marker is complicated by the fact that VEGF is normally present at high concentrations in platelets. Therefore, during platelet lysis, the serum VEGF concentration increases and the determination of non-platelet VEGF specific for tumor angiogenesis processes gets complicated [19].

TATI

The work by Paju et al. [20] shows that an increase of tumor-associated trypsin inhibitor (TATI) concentration in the blood is observed in 48% of patients with renal cell carcinoma. It was found that healthy kidney tissues also produced this tumor biomarker, while its increased concentrations in the blood serum was due to the activation of TATI synthesis by tumor cells [21]. Moreover, the correlation of TATI increased concentrations with clinical stage and tumor cell differentiation degree was found. It was also reported that patients with

increased concentrations of the marker had significantly lower survival than patients with its normal level [20].

Other Markers

High concentration of carbonic anhydrase 9 (CA9) in blood shows the presence of clear cell RCC with 86% confidence [22]. The attempts of renal cell carcinoma diagnosis also included the following tumor markers: carcinoembryonic antigen (CEA) and carbohydrate antigens CA15-3, CA125, and CA19-9, which have sensitivity rates of 5% and 10, 13, and 5%, respectively [23]. Serum protein M65 levels were significantly elevated (almost two-fold) in patients with metastatic RCC compared with healthy individuals [24].

Diagnostic Biomarkers

While the few characterized markers for early detection in general are low effective, a large number of diagnostic markers of renal cancer have been already implemented in clinical practice. Currently, there is no single marker specific to a particular subtype of CCR. Therefore, the diagnostic information could be obtained by analyzing expression profiles of several proteins. A number of antigens that can be diagnostically useful in the case of RCC belong to the following groups: enzymes (carbonic anhydrase 9 (CA9), alpha-methylacyl-COA racemase (AMACR)), cytoskeleton proteins (vimentin, ae1/AE3 keratins, cytokeratins (CK7, CK19, CK20)), intercellular adhesion proteins (E-cadherin, kidney-specific cadherin (kds-cadherin)), cluster of differentiation proteins (CD10, CD15, C-kit), transcription factors (box Pax2 (paired) and Pax8), Ca^{2+}-binding proteins (recoverin, S100, parvalbumin), lectins (galectin-3), glycoproteins (EMA), scaffold proteins (caveolin-1), and immunoglobulins (RCC marker—a monoclonal antibody against a normal renal proximal tubule antigen) [9••, 25–33, 34•]. The general use of these markers is confined to differential diagnostics of RCC subtypes, which are clear cell (CC-RCC), papillary (Pap-RCC), and chromophobe (Ch-RCC) tumors. Hereinafter, the immunohistochemical profiles required for the diagnosis of these subtypes will be summarized (see also Table 1).

Clear Cell RCC

Approximately 75% of malignant renal tumors are qualified as CC-RCC [35]. In the gross examination, this type of tumor has a yellow color, may be homogeneous, sometimes with cystic changes and hemorrhage. Microscopic studies usually reveal solid or acinar growth pattern of CC-RCC, but in some cases, these tumors may have cystic, solid, papillary, or tubular patterns. CC-RCC tumors are normally positive for recoverin, vimentin, AE1/AE3 keratins, EMA, CK18, CD10, RCC marker, caveolin-1, S100, PAX2, PAX8, and CA9 and negative for C-kit, kds-cadherin, E-cadherin, parvalbumin, CK7, CK19, CK20, and AMACR [28–33, 34•]. Since progression of CC-RCC is similar to Pap-RCC in terms of mechanisms and involved components, discrimination of each of these subtypes of renal cancer often requires specific makers. For this case, the simultaneous expression of CK7 with AMACR is used as a sign of solid forms of Pap-RCC [36]. Finally, several marker combinations were suggested for distinguishing CC-RCC from Ch-RCC. CC-RCC is positive for RCC marker, vimentin, S100A1, caveolin-1, and PAX2 and negative for parvalbumin, C-kit, E-cadherin, kds-cadherin, and CK7, while Ch-RCC tumors exhibit exactly the opposite behavior. Thus, parvalbumin, C-kit, and kds-cadherin have the highest sensitivity and specificity for Ch-RCC [37] and vimentin is one of the most notable markers in differential diagnostic of CC-RCC since Ch-RCC is nearly always negative for it [38].

Papillary RCC

Pap-RCC is the second most common subtype after CC-RCC. The incidence of this subtype reaches 10% of all RCCs. Pap-RCC is a yellow-brownish tumor with fibrous pseudocapsule, homogenous structure and sometimes with hemorrhage and necrosis focuses. Microscopically Pap-RCC has papillary, trabecular-papillary, or solid-papillary growth pattern. Based on the cytologic features, Pap-RCC has been subdivided into types 1 and 2. Type 1 (also called "basophilic") tumors consists of small cells with poor cytoplasm, while type II (also called "eosinophilic") is a less common variant representing large cells with voluminous and eosinophilic cytoplasm [39]. The most selective diagnostic marker of both types of Pap-RCC is racemase AMACR, as its expression in other kidney tumors has never been detected. Type 1 papillary RCC is usually positive for recoverin, vimentin, AE1/AE3 keratins, CK7, S100, EMA, CD10, AMACR, and RCC marker but negative for C-kit, kds-cadherin, and parvalbumin. Yet, type 2 Pap-RCC exhibits versatile immunoprofile thereby limiting its immunodetection [28–33, 34•].

Chromophobe RCC

Ch-RCC shares only 5% of renal epithelial tumors. It is a heterogeneous tumor of low malignant potential that macroscopically looks like solid well-circumscribed node with light brown or beige color. Microscopically Ch-RCC exhibits solid pattern with thin fibrovascular septa and usually consists of large polygonal or round cells with eosinophilic cytoplasm and prominent cell membrane [40]. Ch-RCC was first described in 1985 and from that moment was considered as benign tumor [41]. The ways of progression and molecular

Table 1 Immunohistochemical profile of the different renal cell carcinoma subtypes

Renal carcinoma subtype	Immunohistochemical markers	
	Positive	Negative
Clear cell RCC	Recoverin, vimentin, AE1/AE3 keratins, EMA, CK18, CD10, RCC marker, caveolin-1, S100, PAX2, PAX8, CA IX	C-kit, kds-cadherin, E-cadherin, parvalbumin, CK7, CK19, CK20, AMACR
Papillary RCC	Recoverin, vimentin, AE1/AE3 keratins, CK7, S100, EMA, CD10, AMACR, and RCC marker	C-kit, kds-cadherin, parvalbumin
Chromophobe RCC	Recoverin, kds-cadherin, E-cadherin, caveolin-1, parvalbumin, C-kit, EMA, AE1/AE3 keratin, CD15 and CK7	vimentin, CA IX, CD15, S100, PAX2, RCC marker, AMACR
Oncocytoma	Recoverin, AE1/AE3 keratin parvalbumin, S100A1, C-kit, caveolin-1, E-cadherin and kidney-specific cadherin, PAX2, PAX8, EMA, galectin-3, CD15, BCA2	AMACR, CAIX, RCC marker, vimentin and caveolin-1

features of Ch-RCC are close to those of eosinophilic variant of CC-RCC as well as of renal oncocytoma—another benign tumor which often needs to be diagnostically distinguished form Ch-RCC. Ch-RCC is positive for recoverin, kds-cadherin, E-cadherin, caveolin-1, parvalbumin, C-kit, EMA, AE1/AE3 keratin, CD15, and CK7 and usually negative for vimentin, CA9, CD15, S100, PAX2, RCC marker, and AMACR [28–33, 34•]. The immunodiagnostic approach for discriminating Ch-RCC form CC-RCC is given above (section "Clear Cell RCC") while specific markers distinguishing Ch-RCC from oncocytoma will be described in the next section.

Oncocytoma

Renal oncocytoma is a benign tumor that may form solid nests, acini, tubules, or microcysts with abundant hyalinized stroma. Its cells are often round, sometimes cuboid form with granular eosinophilic cytoplasm. Necrosis focuses and mitotic figures are usually not seen in this case [42]. Oncocytoma can be immunostained for recoverin, AE1/AE3 keratin, parvalbumin, S100A1, C-kit, caveolin-1, E-cadherin and kidney-specific cadherin, PAX2, PAX8, EMA, galectin-3, CD15, and BCA2. AMACR, CA9, RCC marker, vimentin, and caveolin-1 are usually not detected [28–33, 34•, 43]. As mentioned above, renal oncocytoma can occasionally be confused with Ch-RCC. However, despite of their similar immunoprofiles, there are several antigens which may help to differentiate these subtypes. Thus, a combination of CK7 with PAX2 is useful in this respect since oncocytoma is positive for PAX2 and negative for CK7, whereas Ch-RCC is characterized by the opposite situation [36]. In addition, two independent studies revealed that expression of cell adhesion protein claudin-7 is the hallmark of both oncocytoma and Ch-RCC, and therefore, this protein could be employed for distinguishing these two benign tumors from malignant variants of RCC with high sensitivity and specificity [44].

It should be noted that alongside with tumor-specific proteins, a new group of nucleic acid-based diagnostic markers for renal tumors was recently discovered. These are miR, small regulatory microRNAs controlling expression of various proteins on the post-transcriptional level. Youssef et al. on the basis of miR-profile research provided for 94 patients have developed an original four-stage method for diagnostics of various RCCs with subsequent histological verification. The method thread consists in estimation of correlation between concentrations of certain miRs peculiar to different RCC subtypes [45].

Prognostic Biomarkers

After any type of surgical treatment of renal tumors, there is a need for monitoring further progression of the disease and survival rate of the patient. The group of prognostic markers that have been considered suitable for these purposes include the following proteins: phosphatase PTEN, carbonic anhydrase CA9, beta-1,4-galactosyltransferase B4GALT1, metallopeptidase ADAM17, trifunctional enzyme HADHA, lactate dehydrogenase LDHA, thioredoxin TXNDC5, cyclin-dependent kinase inhibitor P27, cluster of differentiation proteins CD151, CD82, CD105 and DDR1 (CD167a), regulator of apoptosis Bcl-2, inhibitor of apoptosis survivin, proliferation marker Ki-67, cell adhesion proteins claudins 1–4, intercellular adhesion protein ICAM-1, cytoskeleton protein vimentin, RNA-binding protein SAM68, and Ca^{2+}-binding protein S100A11. The main parameters that could be monitored using prognostic markers are the stage, grade and size of the tumor, disease-free survival (DFS), recurrence-free survival (RFS), overall survival (OS), progression-free survival (PFS), cause-specific survival (CSS), as well as susceptibility to invasion and metastasis. The correlations between expression of the markers and clinicopathological characteristics of

the renal tumors are summarized in Table 2. For some of the above-listed proteins, the additional back-up information is presented below.

Bcl-2

Bcl-2 is a ubiquitous regulator of apoptosis, overexpression of which was recorded in more than 50% of patients with RCC [46, 47]. Yet, different studies report conflicting data about correlations between the levels of this marker and clinicopathological characteristics of the disease. For instance, Vasavada et al. using a small group of 28 patients revealed a statistically significant correlation between the expression level of bcl-2 and tumor grade but did not find any correlations with the possibility of relapse, metastasis, and OS [48]. By contrast, another work subjecting a group of 101 patients with localized form of the RCC revealed that increased expression of bcl-2 was associated with low staging and grading and correlated with better survival of the patients [49]. Virman et al. investigated expression of bcl-2 in parallel with Ki-67—another protein involved in apoptosis regulation. They demonstrated that high expression of Ki-67 with low expression of bcl-2 is associated with poor survival compared with the reverse situation [50].

Ki-67

In addition to joint use of Ki-67 with bcl-2, the ability of the former to discriminate RCC characteristics was evaluated in combination with CA9. Based on the data obtained, RCC tumors were divided into three groups, which were characterized by different survival: a group with low risk (low level Ki-67 or high level of CA9), a group with medium risk (high level of Ki-67 or low level of CA9), and a group with high risk (high level of Ki-67 and low level of CA9). OS in these groups was 101, 31, and 9 months, respectively [49, 53, 55, 56, 75, 76].

miR

As in the case of diagnostic markers, miRs are becoming increasingly important for monitoring clinicopathological characteristics of RCCs. Slaby et al. established the value for miR-106b as a prognostic marker of early metastases after nephrectomy. Thus, the expression levels of this RNA were significantly lower in tumors of patients who developed metastasis. In general, these patients additionally exhibited reduction of miR-155 and miR-106a, but only changes in concentration of miR-106b were considered statistically significant.

Table 2 Correlations of renal prognostic marker status with clinicopathological characteristics and survival

Marker	Stage	Grade	Size	DFS	RFS	OS	PFS	CSS	INV	MET	Ref.
CA9	– [51] n [52]	– [51] n [52]	– [51]	nd	nd	– [51] p [52]	nd	nd	– [51]	n [52]	[53]
B4GALT1	p	p	nd	nd	nd	n	nd	nd	nd	nd	[54]
Ki-67	p	p	p	n	n	n	nd	n	p	p	[53, 55, 56]
ADAM17	–	–	nd	nd	nd	nd	n	nd	nd	–	[57]
Vimentin	p	p	nd	nd	nd	n	nd	nd	nd	p	[58]
P27	nd	p	p	nd	nd	–	nd	n	nd	p	[59]
Survivin	p	p	p	n	–	n	nd	n	p	p	[60, 61]
CD151	p	p	p	nd	nd	nd	n	n	nd	p	[62]
CD82	p	p	nd	nd	nd	n	n	n	nd	nd	[63]
CD105	n	n	–	nd	nd	nd	nd	nd	nd	nd	[64]
LDHA	p	p	p	n	nd	n	nd	nd	nd	nd	[65]
DDR I	p	nd	nd	nd	nd	nd	nd	nd	p	p	[66]
TXNDC5	–	n	–	nd	nd	n	nd	nd	p	nd	[67]
SAM68	p	p	nd	nd	nd	n	nd	nd	nd	p	[68, 69]
HADHA	n	n	n	nd	nd	nd	nd	p	nd	n	[70]
S100A11	p	p	p	n	nd	–	nd	nd	nd	nd	[71•]
Claudin 1	–	n	nd	nd	nd	–	nd	nd	nd	nd	[72, 73]
Claudin 2	–	p	nd	nd	nd	–	nd	nd	nd	nd	[72, 73]
Bcl-2	n [49]	p [48] n [49]	nd	nd	nd	– [48] p [49]	nd	nd	nd	– [48]	[48, 49]
PTEN	n	n	–	nd	nd	p	nd	nd	nd	nd	[74]

p positive correlation, *n* negative correlation, – no correlation, *nd* not determined, *DFS* disease-free survival, *RFS* recurrence-free survival, *OS* overall survival, *PFS* progression-free survival, *CSS* cause-specific survival, *INV* invasion, *MET* metastasis

It was also found that the contents of miR-210 and miR-141 in RCC compared with healthy kidney parenchyma were 60 times higher and 15 times lower, respectively [77].

Predictive Biomarkers

Traditionally, RCC was regarded as a disease resistant to medical and radiation therapy. Furthermore, although RCC was recognized as one of the few tumors that respond to immunotherapy, the clinical results of this approach were also rather unpromising. For instance, only 10–15% of patients responded to cytokine (interferon and interleukin-2) treatment [78]. Apparent increase in the lifetime of the majority of patients with RCC became possible only after implementation of targeted pharmaceuticals into the chemotherapeutic practice. Since angiogenesis is a key mechanism of propagation of neoplastic process, the targeted therapy of RCC was focused on angiogenesis. Consistently, vascular endothelial growth factor (VEGF), endothelial growth factor/platelet-derived growth factor receptors (VEGFRs/PDGF-R) and their alternative ligands circulating in blood, as well as mammalian target of rapamycin (mTOR) protein kinase (also involved in angiogenesis) became the main targets of novel pharmaceuticals. Up to the moment, the indications for selection of targeted treatment method are not yet clearly defined. In this regard, the predictive markers, the presence of which in tumor and/or serum of the RCC patients would correlate with efficiency of the targeted treatment, are of high demand. Indeed, the analysis of expression of these markers would forecast the patient's response to the therapy and consequently determine appropriate targeted drug choice. Based on the results of clinical trials, seven targeted pharmaceuticals namely sunitinib, pazopanib, bevacizumab, sorafenib, axitinib, temsirolimus, and everolimus were approved for use in patients with RCC in different countries (Fig. 1) [79•]. The current section will review the existing data on predictive potential of different protein markers during targeted therapy employing each of these drugs.

Sorafenib

Sorafenib is a multitarget drug, which has protein kinase inhibitory activity that suppresses cell proliferation and angiogenesis. It inactivates receptor tyrosine kinases (VEGFR-2, VEGFR-3, PDGF-в, RET, C-kit) and serine/threonine kinases (C-Raf, B-Raf) in tumor cells and tumor vascular cells [81]. In early studies, it was shown that high expression level of marker CA9 correlated with PFS and tumor shrinkage in sorafenib-treated patients with RCC. However, in the subsequent study involving 133 patients with CC-RCC, no correlation between CA9 expression status and PFS was found in 66 patients, who received sorafenib [82].

Sunitinib

Sunitinib represents a low-molecular tyrosine kinase inhibitor exhibiting activity towards VEGFR and PDGFR. Reduced levels of VEGFR-3 and VEGF-C were correlated with better response in RCC patients that were medicated with sunitinib after ineffective treatment using bevacizumab [83]. High HIF-2α, CD31, and CA9 status in combination with low VEGFR1 and PDGFRB status evidenced benefit of sunitinib treatment compared with sorafenib treatment [84]. MMP-9 and tumor necrosis factor alpha (TNFα) were elevated in mRCC patients

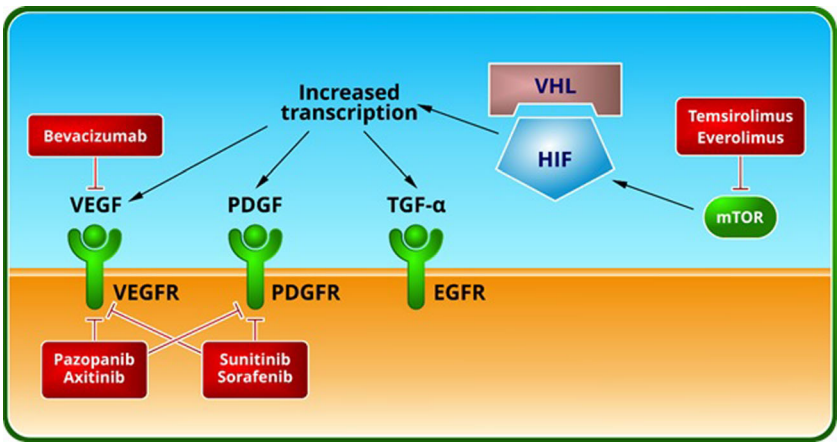

Fig. 1 Mechanism of action of targeted drugs in renal cancer therapy. In renal cancer cells, von Hippel–Lindau (VHL) tumor suppressor (VHL) protein is incapable to promote ubiquitinylation of hypoxia-inducible factor 1 (HIF1) resulting in marked accumulation of the latter and overexpression of VEGF, PDGF, and TGF-a, thereby stimulating angiogenesis. Mammalian target of rapamycin (mTOR) also promotes angiogenesis by increasing the expression of HIF1. The anticancer effect of the targeted drugs is produced by suppressing angiogenesis via inhibition of growth factors or their receptors (bevacizumab, sunitinib, pazopanib, sorafenib, axitinib) or blocking mTOR activity (temsirolimus and everolimus) [80]

who were treated with sunitinib but showed progression of the disease [85].

Pazopanib

Pazopanib is a tyrosine kinase inhibitor that suppresses angiogenesis by interacting with PDGFR-alpha and PDGFR-beta, and C-kit—receptors of VEGF-1, VEGF-2, and VEGF-3. The increased plasma levels of IL-8 (interleukin-8), HGF (hepatocyte growth factor), osteopontin, and TIMP-1 (tissue inhibitor of metalloproteinases-1) in metastatic RCC patients treated with pazopanib indicated therapeutic failure with significant reduction in PFS [86]. By contrast, reduction of VEGF-2 content in blood serum of such patients on the fourteenth day of pazopanib administration evidenced for more favorable forecast with tendency for slowed disease progression and increased OS [87].

Everolimus and Temsirolimus

One of the major targets of antineoplastic therapy in RCC patients is serine/threonine kinase mTOR (mammalian target of rapamycin), which plays an important role in regulation of cellular growth and proliferation. mTOR is known to increase expression of the HIF1 thereby stimulating angiogenesis. Everolimus is the first oral drug belonging to the group mTOR inhibitors. Li et al. revealed that the effectiveness of everolimus treatment in patients with mRCC can be predicted by analyzing expression levels of phospho-mTOR and phospho-S6RP (S6 ribosomal protein). Thus, higher levels of these proteins were associated with better clinical benefit of the drug administration and increased PFS [88]. Temsirolimus is another selective inhibitor of kinase mTOR. It binds to intracellular protein FKBP-12 (tacrolimus (FK506) binding protein) and the resulting complex inhibits activity of mTOR thereby indirectly controlling cell division. It was found that expression of phospho-Akt and phospho-S6 ribosomal protein had positive correlation with response to temsirolimus treatment [89]. In addition, increased serum levels of LDH in temsirolimus-treated patients with metastatic RCC were associated with longer OS while patients with decreased LDH demonstrated worse prognosis [90].

Axitinib and Bevacizumab

Axitinib and bevacizumab represent another targeted drugs dealing with tumor angiogenesis. Axitinib possesses a selective inhibitory activity towards VFGFR [1–3] and PDGFR whereas bevacizumab is a recombinant monoclonal antibody that selectively binds and suppresses activity of VEGF. At the moment, no evidence for predictive markers for monitoring treatment of RCC patients using these drugs was found.

Paraneoplastic Antigen Recoverin as a New Biomarker of RCC

The common property of the majority of the described marker proteins is their expression both in tumors and in normal renal tissue. Currently, the growing evidence is indicative of another type of potential tumor biomarkers—onconeural antigens, representing neuronal proteins that are aberrantly expressed in cancer cells. Their unique features are specificity for respective cancer cells as well as immunogenic activity resulting in generation of the serum autoantibodies. These features potentiate application of onconeural antigens in diagnostics and, furthermore, in immunotherapeutic assays aimed to stimulate the immune response against cancer cells of various tumors [91, 92]. For instance, for renal tumors, they can be used for early serological detection, discrimination of malignant and benign tumors, and prognostic purposes.

A striking example of onconeural antigen is neuronal Ca^{2+}-binding protein recoverin, which normally localizes in photoreceptor cells but can be aberrantly expressed in malignant tumors. In some cases, recoverin expression is accompanied by generation of ant-recoverin autoantibodies and development of paraneoplastic syndrome (cancer-associated retinopathy) [93–96]. A role of recoverin in tumor cells remained unspecified, but a number of studies revealed this protein in lung cancer, melanoma, gastrointestinal, breast and gynecological cancers, and others [97–99]. Recently, this list was supplemented by renal cancers. Thus, recoverin expression was found in about 68% of patients with different subtypes of RCC and oncocytoma. In the latter case, expression of recoverin was higher (91.7%) compared to renal chromophobe adenocarcinoma (50%). Expression of recoverin has no correlation with OS of patients with renal tumors, but it had a tendency to positively correlate with tumor size (9). It was demonstrated that expression of recoverin in some tumors is mediated by demethylation of the certain CpGs of the recoverin gene region overlapping the promoter up-stream of the first exon and the first exon itself [100]. Consistently, it was found that up to 86% renal tumor samples that have demethylated CpGs in the recoverin gene promoter region were positive for recoverin. Importantly, that methylation of the recoverin promoter at position −80 positively correlated with OS of the examined patients (9). Taken together, these data suggest recoverin as the first onconeural antigen that has potential as diagnostic and/or prognostic marker for renal tumors if used alone or in combination with other biomarkers.

Conclusions

RCC is still considered as one of the most unfavorable malignant urological diseases in terms of prognoses. Furthermore, the absence of symptoms characterizing early stages of RCC

makes its detection and diagnosis a challenging problem. Although recent efforts resulted in identification of more than 40 various biomarkers for RCC, their efficiency remains quite different. Thus, reliable biomarkers for early detection of kidney tumors still do not exist since the available antigens are insufficient in sensitivity and/or specificity. By contrast, several marker combinations are suggested for various diagnostic purposes such as differential diagnosis of the subtypes of RCC. Moreover, a great number of prognostic and predictive RCC biomarkers make possible monitoring and prediction of the clinicopathological characteristics of the disease (such as chance of a recurrence and metastasis, survival, therapy efficacy, etc.) before treatment, after surgery, or during chemotherapy including administration of novel-targeted drugs. Yet, despite some progress in this field, further studies aimed at accumulating more clinical data for existing RCC markers, searching for new antigens, and revealing new correlations are required to obtain more accurate diagnostic impacts. In addition, elucidation of the roles of the identified biomarker proteins in RCC pathogenesis seems a promising research line since these data may be useful not only for diagnostics but also for the development of new strategies of treatment of this lethal disease.

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Compliance with Ethical Standards

Conflict of Interest Marina O. Golovastova, Dmitry O. Korolev, Larisa V. Tsoy, Vladimir A. Varshavsky, Andrey Z. Vinarov, and Evgeni Yu. Zernii each declare no potential conflicts of interest.

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- •• Of major importance

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