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Review – Kidney Cancer

Diagnostic and Prognostic Molecular Markers for Renal Cell Carcinoma: A Critical Appraisal of the Current State of Research and Clinical Applicability

Christian Eichelberg [a,*], *Kerstin Junker* [b], *Börje Ljungberg* [c], *Holger Moch* [d]

[a] *Department of Urology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[b] *Laboratory of Molecular Biology, Department of Urology, Friedrich-Schiller-University, Jena, Germany*
[c] *Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden*
[d] *Institute for Surgical Pathology, University Hospital Zurich, Zurich, Switzerland*

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Abstract

Context: Earlier detection of renal cell carcinoma (RCC) and the recent expansion of treatment possibilities have positively influenced the outlook for patients with this disease. However, progression and treatment response are still not sufficiently predictable. Molecular markers could help to refine individual risk stratification and treatment planning, although they have not yet become clinically routine.

Objective: This review presents an overview of diagnostic and prognostic molecular markers for RCC and a subgrouping of these markers for different clinical issues.

Evidence acquisition: Literature and recent meeting abstracts were searched using these terms: *renal (cell) carcinoma, molecular/tumor markers, biopsy, blood, urine, disease progression/prognosis, immunohistochemistry, risk factors*, and *survival*.

Due to the resulting large number of articles, studies were subjectively selected according to the importance of a study on the field, number of investigated patients, originality, multivariate analyses performed, contrast with previously published data, actuality, and assumed clinical applicability of the described results. More then 90% of the selected studies originated from the past 10 yr; >50% of the articles were written in 2006 or later.

Evidence synthesis: These data were predominantly obtained via nonrandomized, retrospective, but often controlled studies. Thereby, the resulting level of evidence is 2A/2B. The broad spectrum of described molecular markers (MMs) for RCC consists of markers already extensively studied in other malignancies (eg, p53), as well as MMs typically associated with specific RCC-altered gene functions and pathways (eg, von Hippel–Lindau [VHL]). The main goal of using MMs is to refine the prediction of clinical end points like tumor progression, treatment response, and cancer-specific and/or overall survival. Further, MMs might facilitate the clinical work-up of undefined renal masses and prove to be more convenient tools for screening and follow-up in blood and urine.

* Corresponding author. University Hospital Hamburg-Eppendorf, Department of Urology, Martinistr. 52, Hamburg, 20246, Germany. Mobile: +49 (0)171 5217414.
E-mail address: c.eichelberg@uke.uni-hamburg.de (C. Eichelberg).

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Conclusions: Presently, there are a number of promising MMs for diverse clinical questions, but the available data are not yet valid enough for routine, clinical application. We should comply with the demand for large multicenter prospective investigations, stratified for RCC type and treatment modalities, to lift the use of molecular markers in RCC to a practical level, thereby providing a better consultation for our patients regarding diagnosis, treatment, and follow-up.

1. Introduction

Despite a stage migration to a higher proportion of localized renal cell carcinomas (RCC) [1], the demand for an individual aftercare of these patients is still foiled by the unpredictable course of localized RCC. Consequently, a suggested risk-stratification for follow-up schemes still has not yet achieved universal acceptance [2]. Simultaneously, the role of ablative treatments for small tumors is increasing, and there are a growing number of advocates of an ''active surveillance'' strategy for small renal masses. With even small tumors having metastatic potential and the fact that the overall RCC mortality has not yet dropped [3], markers for the individual aggressiveness of a tumor are desired.

The base of all prognostic models, the TNM system [4], is not yet optimal in predicting the long-term course of the disease: The overall concordance rate has been described to be 58–73% [5,6], and it seems to be not significant for e.g. papillary RCC (papRCC) [6]. Further, many authors demand a reclassification, especially for the overly global pT3–4 groups [5,7,8]. Integrated prognostic models seem to perform slightly better, but they are also based on the TNM system and have concordance rates between 60% and 85% [9–11].

For metastatic diseases, new therapeutic agents—so-called *targeted therapies*—have brought about a revolution in treatment strategies. However, unlike therapies in other cancers (eg, human epidermal growth factor receptor 2 [HER-2] in breast cancer [12]), the question of whether a tumor does have the target at which the therapy is aimed has not been raised. Moreover, with a lack of markers for response, we are unable to detect progress or to treat refractory cancer earlier than with radiologic evaluation. And finally, with the new drugs again posing the question of potential benefit of adjuvant therapies, an improved grouping is necessary to better determine the patients who are at high risk.

A broad variety of markers are described in literature. This review summarizes (pre-) clinically tested molecular markers (MMs) that might be applicable for above surrogates.

2. Evidence acquisition

Medline database searches were performed using the *terms renal (cell) carcinoma, molecular/tumor markers, biopsy, blood, urine, disease progression/prognosis, immunohistochemistry, risk factors*, and *survival*. Subsequent references to retrieved articles are additionally included. Furthermore, abstracts from the 2008 annual meetings of the European Association of Urology, the American Urological Association, and the American Society of Clinical Oncology were searched using the above-mentioned keywords.

Due to the resulting large number of articles, a subjective selection was based on the following: importance of a study on the field, large number of investigated patients, originality, multivariate analyses performed, contrast with previously published data, actuality, and assumed clinical applicability of the described results.

The selected articles were published between 1986 and 2008. More then 90% of the studies originated from the past 10 yr, and >50% of the articles were published in 2006 or later.

3. Results

3.1. Tissue markers

3.1.1. Primary tumor and/or resected metastases

This group includes the MMs, which have been investigated in either nephrectomy specimens or in resected metastases. Most of the studies are based on expression studies by high-throughput methods like tissue microarrays (TMAs). The achieved results were correlated in a mostly retrospective manner with clinical end points: progression-free survival (PFS), cancer-specific survival (CSS), and overall survival (OS).

We differentiated two subgroups: 1. MMs typically associated with RCC and 2. ''generic'' markers,

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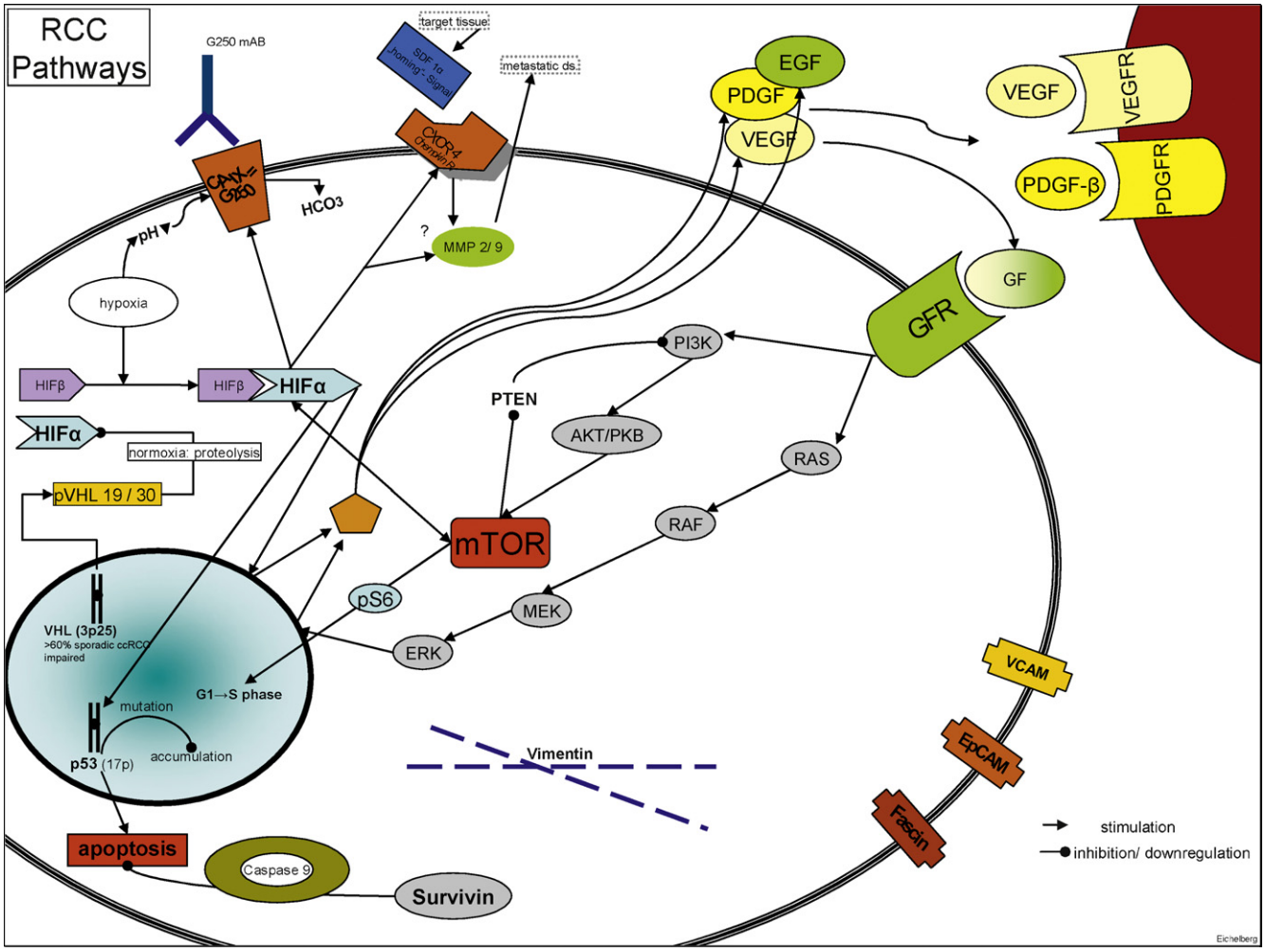

Fig. 1 – Pathways and markers in renal cell carcinoma.

which have been vastly investigated in other malignancies.

3.1.1.1. *Renal cell carcinoma–associated tumor markers*

3.1.1.1.1. *Von Hippel–Lindau pathway*

The understanding of the role of the von Hippel–Lindau (VHL) tumor suppressor gene in RCC has been one of the landmarks for the considerations about angiogenic pathways. It is inactivated in almost all RCC in patients with VHL syndrome.

Importantly, this tumor-suppressor gene on chromosome 3p was found to be also inactivated in about 70% of sporadic clear cell RCC (ccRCC), resulting in deficient protein isoforms pVHL19 and pVHL30.

One well-studied consequence of the deficient VHL proteins is an impaired degradation of hypoxia induced factor 1alpha (HIF-1α), which accumulates even under nonhypoxic conditions [13] (Fig. 1). However, the entire range of the regulative mechanisms controlled by pVHL goes far beyond this and includes regulation of cell-cycle arrest via p53 or deposition of extracellular matrix, closely linked to neoangiogenesis and tissue invasion [14].

The VHL gene's complex position might also explain why the prognostic role of VHL alterations is divergent. Yao et al found, on multivariate analysis of sex, age, grade, symptoms, that VHL mutation or hypermethylation strongly related to a better PFS and a CSS for stage I–III ccRCC [15]. Schraml et al reported that only ''loss-of-function'' mutations of VHL are associated with worse prognosis in univariate analyses, while tumor grade, stage, microvessel density, and tumor-cell proliferation were not associated with VHL mutations. They concluded that the regulation of angiogenesis and proliferation of RCC might not be directly influenced by VHL mutations [16].

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As for the therapeutically predictive value, VHL mutations or promoter methylations seem to have a modest positive correlation to anti–vascular endothelial growth factor (VEGF)–targeted therapy response, with a described objective response rate of 48% compared to 35% in patients with no VHL mutation or methylation [17].

3.1.1.1.2. *Hypoxia-induced factor 1alpha*

As described above, HIF-1α accumulates either in hypoxic cell conditions or when the pVHL is deficient. In a study by Wiesener et al, somatic mutations of the VHL gene were detected only in HIF-1α overexpressing ccRCC. Consequently, an increased expression of HIF-1α was found in 24 of 32 ccRCC tumors (75%), but only in three of eight non-ccRCC tumors. Moreover, none of the HIF-1α–negative ccRCCs displayed a VHL mutation [18].

Similar to VHL mutation, the prognostic value of an HIF-1α overexpression is also controversial: In a Western blot analyses of 66 ccRCCs, Lidgren et al showed that a high level of HIF-1α protein expression to be an independent, favorable, prognostic factor [19]. However, in the subsequent TMA study by the same group ($n = 176$), HIF-1α lost its significance on multivariate analysis [20].

Researchers from University of California–Los Angeles, however, showed that pHIF-1α expression was able to predict outcome in patients with metastatic disease. Patients with a high level of HIF-1α expression had a significantly worse survival than patients with a low level of expression (median survival: 13.5 vs 24.4 mo). Using multivariate analysis, HIF-1α expression and carbonic anhydrase 9 (CAIX) expression were shown to be the strongest prognostic factors in the study group of 141 patients with metastatic ccRCC [21].

This discrepancy nicely demonstrates the complexity of reasons that justify divergent results in the evaluation of potentially prognostic MMs, ranging from methodological divergences to an oversimplified understanding of an MM or to simply a biased or neglect able effect in a real-life clinical setting.

For HIF, a possible explanation might be related to the differences in staining and detection, for example, cytoplasmatic [20] versus nuclear [21]. With HIF being a transcription factor for many growth factors relevant for cancer development and progression, HIF might only be an active and thereby a negative predictor when translocated into the nucleus.

Furthermore, despite the fact that HIF-1α acts via transcriptional regulation of a number of factors involved in the downstream regulation of angiogenesis, glucose metabolism, and stimulation of growth factors, its complex intracellular signaling includes also the induction of apoptosis by stabilizing wild type p53, but it cannot interact with mutated p53 [22]. Therefore the p53 status might bias the results of the prognostic ability of HIF-1α, although p53 mutations are rare in ccRCC (see section 3.1.1.2.1.).

And finally, the excellent CSS rate of patients with early stage, nonmetastatic tumors after surgery could simply overexpose effects of HIF expression on survival. The proportion of N+ or M1 tumors in the Lidgren et al article [20] is not explicitly mentioned. However, 76 of 176 ccRCCs investigated were stage 1 or stage 2. In the article by Klatte et al [21], on the other hand, the correlation between HIF-1α and CSS was not significant for the 167 patients with localized disease.

3.1.1.1.3. *Vascular endothelial growth factor*

The idea of either the direct inhibition of VEGF or the blocking of its signaling cascade confounded the ''therapeutic revolution'' for metastatic RCC and is principle of most of today's approved targeted therapies. Not surprisingly, VEGF has also been widely studied as an MM.

VEGF production is significantly increased in RCC with VHL gene alterations and raised HIF-1α protein expressions. Furthermore, it is associated with a more aggressive tumor phenotype [23]. Several groups could show that a raised VEGF expression is a significant predictor for outcome, and in some studies showed this correlation even using multivariate analyses together with stage and grade [24–26].

With the close relationship between VHL and HIF-1α, one might expect a raised VEGF expression to be an exclusive feature of ccRCC. However, a study of 300 RCCs demonstrated no difference between the RCC types [25]. Yildiz et al reported elevated VEGF expression in 29% of ccRCCs and, surprisingly, in 67% of papRCCs [27]. This might be another example that theoretically linear and logical pathways are just not that plain in real life. Further confirmation of this comes from the downstream VEGF-signaling via phospho-extracellular signal-regulated kinase (pERK), which theoretically should be lower under anti-VEGF therapy. As Murphy et al stated in a recent publication, ''assessing endothelial cell ERK activation in tumor biopsies may allow monitoring of sorafenib activity in patients in clinical trials'' [28]. Data from the large TARGET trial, however, revealed that pERK-staining levels were not predictive of sorafenib therapy results [29].

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3.1.1.1.4. *Grawitz 250 or carbonic anhydrase 9*

As early as 1986 Oosterwijk et al described Grawitz 250 (G250) as an RCC-specific antibody [30]. It took several years to merge these findings with the parallel investigations on an RCC-related carbonic anhydrase 9 (CAIX) [31], later identified as identical targets. G250 and/or CAIX have been shown to be unique HIF-1α target genes in ccRCC [32]. In contrast to normal kidney tissue, 95% of ccRCCs are CAIX and/or G250 positive [33].

High CAIX expression levels in primary tumors, as well as in resected lung metastases, were associated with improved prognosis in advanced ccRCC [34,35]. Bui et al identified CAIX as an independent predictor of survival, even when analyzed together with stage, grade, nodal status, metastatic status, and performance status [36]. This result was questioned by Leibovich et al in a large analysis of 730 patients that also found low CAIX expression univariately associated with increased risk of RCC death (risk ratio: 1.65), but not at multivariate analysis [37].

Beside the ccRCC specificity and the prognostic value of CAIX, it seems to predict outcome of therapy with interleukin 2, with more responding patients having high CAIX expressing tumors compared with nonresponders (78% vs 51%) [38]. These data might help to optimize the selection of patients eligible for the toxic but potentially curative IL-2 therapy and might thereby help to preserve this therapeutic option in today's era of targeted therapies. Results of the SELECT trial (www.ClinicalTrials.gov identifier: NCT00554515) are eagerly awaited here.

Furthermore, the high specificity of CAIX qualifies it as a potential therapeutic target for monoclonal antibody therapy [39]. Results of its use in an adjuvant setting are expected (Adjuvant RENCAREX Immunotherapy Phase 3 [ARISER] trial, www.ClinicalTrials.gov identifier: NCT00087022).

3.1.1.1.5. *Mammalian target of rapamycin pathway*

The mammalian target of rapamycin (mTOR) pathway has been shown to be upregulated in many human cancers. As for RCC, it symbolizes the second major pathway of today's targeted therapy options, with a proven efficiency of the mTOR inhibitors temsirolimus (PFS and OS [40]) and everolimus (PFS only, [41]).

The literature about the prognostic role of mTOR as an MM is sparse. In a very recent study, Youssif et al reported positive cytoplasmatic mTOR staining in metastatic specimens to be correlated with improved CSS in 132 specimens [42], although it is unclear whether these results depended on a previous temsirolimus therapy.

Downstream, the mTOR-targeted, phosphorylated S6 ribosomal protein (pS6) did show cytoplasmatic staining in 85% of 375 investigated RCCs, and it was significantly increased in higher stages and grades and in metastatic tumors. Using multivariate analyses, pS6 came out as the strongest predictor of disease-specific survival (DSS) [43]. Cho et al published data showing that a high level of pS6 staining correlates with temsirolimus response. Additionally, none of the patients without a high level of expression of pS6 experienced an objective tumor response [44]. If these results could be confirmed, pS6 could help to better select patients of the poor risk group for temsirolimus therapy, or otherwise prevent them from weekly intravenous therapy, considering in their limited life expectancy.

Upstream to mTOR, the phosphatase PTEN is regulating the mTOR-pathway by inhibiting AKT phosphorylation by PI3K. Although PTEN mutation is reported to be a rare event in RCC, PTEN deletion correlates with a poor prognosis [45], and decreased staining has been associated with a nonsignificant trend toward shorter OS [6].

3.1.1.2. *"Generic" tumor markers*

This group includes those MMs that have been extensively described and investigated in other malignancies. We therefore abstained from a further characterization of each marker.

3.1.1.2.1. *p53*

Overall, p53 positivity seems to be a rare event in RCC [46] and is probably more frequent in metastases than in primary tumors [47]. As for the different RCC types, p53 overexpression was found to be more frequent in non-ccRCCs, and especially in papRCC [47,48].

However, with regard to the predictive value of a positive p53 staining, the literature is again divided. A reason for this could be the heterogeneous p53 staining within tumors [47], which could enable sampling errors. On the other hand, non-ccRCC subgroup analyses typically lack large numbers, with the potential risk of a model being overly fitted. In summary, p53 overexpression seems to be correlated with poorer prognoses, as displayed in a selection of recent literature in Table 1.

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Table 1 – Selection of recent literature investigating overexpression of p53 in renal cell carcinoma (RCC)

RCC subtype	Central statement about correlation for p53 overexpression	*n*	p53 positive, % (all subtypes)	Year	Citation
All	PFS decrease but significant only for ccRCC	240	23 (localized) 52 (metastasized)	2004	Zigeuner et al [47]
All	CSS decrease but only in pap and chromRCC, not ccRCC	90	19	2001	Rioux-Leclerq et al [49]
N.d.	PFS decrease in localized RCC	193	7	2005	Shvarts et al [50]
ccRCC	CSS decrease, both non- and metastatic RCC	119	59	2007	Klatte et al [51]
ccRCC	OS decrease	73	17	2000	Staller et al [52]
ccRCC	OS decrease	50	16	1997	Chawla et al [53]
papRCC	OS decrease, more common in type II papRCC (>36%)	50	12 (type 1) 36 (type 2)	2008	Kallakury et al [54]

PFS = progression-free survival; CSS = cancer-specific survival; ccRCC = clear cell renal cell carcinoma; ccRCC = chromophobe renal cell carcinoma; N.d. = not described; OS = overall survival; papRCC = papillary renal cell carcinoma.

3.1.1.2.2. Ki 67

Ki-67 has been described as a multivariate independent negative predictor of OS [36,49] and PFS [50]. In the study by Bui et al the combination of Ki67 and CAIX was even able to displace nuclear grade in a multivariate analysis of 224 patients [36].

3.1.1.2.3. CXCR3

CXCR3 is a receptor for cytokines induced by interferon, and among its assumed functions are the deterrence of angiogenesis and the promotion of cellular immunity. It is also expressed by tumor-infiltrating lymphocytes. A ''protective'' effect has recently been reported, since CXCR3, in a multivariate mode, was an independent predictor for PFS after nephrectomy in 154 localized ccRCC [51].

3.1.1.2.4. CXCR4

Cancer cells expressing the chemokine receptor CXCR4 regularly metastasize to organs expressing its specific ligand: stromal cell-derived factor-1alpha (SDF-1α). The expression of CXCR4 in RCC has been demonstrated to be pVHL and HIF dependent. This resulted in a correlation of strong CXCR4 expression with a poor rate of CSS in ccRCC [21,52]. Moreover, high levels were seen in locally recurrent and high grade tumors and in bone and lung metastases [53].

3.1.1.2.5. Matrix metalloproteinase 2 and matrix metalloproteinase 9

Matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) are widespread in human malignancies and are known to facilitate tumor expansion and promotion of metastasis by mediating the degradation of basement-membrane and connective-tissue barriers. In RCC, MMP-2 and MMP-9 overexpression were found in 67–76% and 43% of tumors, respectively, and more frequently in non-ccRCC tumors [13,54]. Elevations of MMP correlated with aggressiveness [55], grade, and survival [54], and even with early symptoms in localized tumors [56].

MMP-2 and MMP-9 are often coexpressed with CXCR4 and are assumed to be likewise coregulated by pVHL and HIF. It remains unclear, however, whether there is a direct induction of MMP-expression through CXCR4 [13].

3.1.1.2.6. Insulin-like growth factor II mRNA-binding protein

The oncofetal RNA-binding protein IMP3 (insulin-like growth factor II mRNA-binding protein) is assumed to regulate transcription of insulin-like growth factor II mRNA. Its reappearance after embryogenesis has been observed in a number of other solid tumors to be a negative predictor. For RCC, there are validated data [57] showing that IMP correlate with higher stage, grade, sarcomatoid differentiation and decreased CSS. Moreover, there is a profound correlation with decreased PFS in localized tumors with a 4–17-fold lesser probability for a metastasis-free survival, both in ccRCC [58], as in papRCC, and in chromophobe RCC (chRCC) [59].

3.1.1.2.7. Epithelial cell adhesion molecule

An infrequent (10%) positive epithelial cell adhesion molecule (EpCAM) staining in ccRCC has been reported to be a independent predictor of both recurrence-free survival (RFS) and DSS [50,60]. Kim et al found a trend to better survival in cases of EpCAM-positive tumors, which however, was not significant according to univariate or multivariate

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analyses [6]. Similar results were reported by Went et al [61].

3.1.1.2.8. Vimentin

Vimentin expression was predominantly seen in ccRCC and papRCC (51% and 61%, respectively), but only rarely in chRCC (4%) and oncocytomas (12%). The authors observed that the Vimentin expression was significantly associated with a poor prognosis independent of grade and stage [62].

3.1.1.2.9. Fascin

Fascin expression seems to be associated with negative tumor attributes since it was reported to correlate with higher grade, higher stage, larger tumor size, and sarcomatoid transformation. Moreover, it was positive in 46% of metastases, compared to only 10% in primary tumors. Seventy-seven percent of the fascin-positive patients investigated by Zigeuner et al had a metastatic course of disease, compared with 21% of the fascin-negative [63].

3.1.1.2.10. Livin

Wagener et al reported the antiapoptotic livin/melanoma inhibitor of apoptosis proteins (ML-IAP) gene as a potentially new target for therapy, with a significantly increased level of livin expression in RCC compared with normal tissue [64]. However, in a more recent study livin expression levels did not correlate with either pathologic parameters, clinical parameters, or disease end points [65].

3.1.1.2.11. Survivin

An inhibition of apoptosis using survivin with a consecutive progression and recurrence was reported: a positive survivin staining was independently associated with higher stage, with higher grade, and with a significantly lower CSS [66,67]. Parker et al reported 43.0% and 87.2% 5-yr CSS rates for patients with high versus low levels of survivin expression, respectively [68].

3.1.1.3. Conclusion with regard to renal cell carcinoma–associated tumor markers

The potential of MMs suggested by clinical research is encouraging. Knowledge of various pathways will facilitate creation of systems of biomarkers that are predictive of individual response to therapy. Useful biomarkers may have potential as therapeutic targets. Undoubtedly studies on MMs will provide many new opportunities for the discovery in urology that will benefit our patients.

3.1.2. Molecular markers for biopsy specimens

The following section summarizes MMs as potentially supportive in the work up of biopsy specimens in terms of identification of malignant cells and of tumor type.

3.1.2.1. G250/CAIX

As stated above, CAIX is frequently expressed (95%) and highly specific for ccRCC [34]. In CAIX RNA analyses of fine-needle–aspiration biopsies of ccRCC and papRCC tissue versus benign tumor tissue, the positive predictive values and the negative predictive values were reported to be 100% and 45%, respectively [69]. Similar results were achieved by CAIX immunostaining of fine-needle–aspiration biopsies, where a perfect discrimination between ccRCC and benign lesions was seen in the 22 patients investigated [33].

3.1.2.2. CD70

The transmembrane CD70 protein might be involved in immune hideout mechanisms of solid tumors. In a set of 41 patients, Junker et al reported 100% positivity by immunostaining for CD70 in ccRCC specimens, whereas the other RCC types were only rarely positive. Furthermore, all nonmalignant kidney samples were negative for CD70 [70].

3.1.2.3. Papillary renal cell carcinoma

In a very recent study, Per et al found that cytokeratin 7 and mucin 1 (MUC-1) were more frequent in papRCC type 1 than in papRCC type 2 (84% and 76% vs 32% and 28%), whereas papRCC type 2 tumors were more often were positive for p53 (36% vs 12%) [71].

3.1.2.4. Chromophobe renal cell carcinoma and oncocytoma

3.1.2.4.1. KIT gene products

The diagnosis of chRCC and oncocytoma are challenging, and immunohistochemical detection of the *KIT* gene product on the cell membrane might be of a specificity similar to G250 in ccRCC: While all chRCC were *KIT* positive, in the report by Yamazaki et al none of the ccRCC or non-neoplastic kidney tissues showed detectable expression of KIT [72].

Pan et al showed similar results by analyzing 379 benign and malignant kidney tumors. They found

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KIT expression to be typically associated with chRCC (83% positive) and oncocytomas (71% positive), whereas none of the other types of renal masses expressed *KIT* [73]. Kruger et al confirmed these results and suggested that KIT reactivity be used as a supplementary diagnostic criteria to differentiate chRCC from other RCC types [74].

3.1.2.4.2. Epithelial cell adhesion molecule

Despite an assumed association to a favorable prognosis in ccRCC (*see* section 3.1.1.2.7.), EpCAM expression patterns might also serve to discriminate chRCC and oncocytomas. Went et al reported a strong and homogeneous positivity on large sections of 90% of chRCC ($n = 20$), whereas in oncocytomas ($n = 15$) only single tumor cells or small clusters were EpCAM positive [61].

3.1.2.4.3. Kangai 1 and loss of chromosomes y and 1p

Kangai 1 (KAI-1) could be a promising marker to differentiate chRCC from oncocytomas. In an investigation of 152 tumors, Kauffmann et al reported a KAI-1 positivity in 87% of chRCC, compared with 7% of 28 oncocytomas. Only one ccRCC showed a low level of staining, and none of the papRCC were KAI-1 positive [75]. If the resulting sensitivity and specificity rates of 90% and 83%, respectively, could be confirmed in further studies, use of KAI-1 could be a real breakthrough in this diagnostic pitfall.

Another possible way to differentiate these two renal tumor entities was recently reported by Klatte et al, where a concomitant loss of chromosome y and chromosome 1p was reported to be diagnostic for oncocytoma in men. While 62.5% of the oncocytomas simultaneously showed the genetic aberrations, none of the chRCCs had loss of both 1p and y [76].

3.1.2.5. Conclusion with regard to molecular markers for biopsy specimens

Improvements in the clinical work-up of patients with undefined renal masses are one part of the scientific endeavor important for the urologists. Putative markers for diagnostic or differential diagnostic purposes, such as CAIX, CD70, KIT, EpCAM, and KAI-1, need to be validated in large clinical patient investigations.

3.2. Molecular markers in blood and serum

A reliable serum marker would obliviously ease screening and follow-up of patients with RCC. There are some promising reports describing potential markers in peripheral blood:

3.2.1. G250 and/or CAIX

McKiernan et al detected CAIX mRNA by reverse transcriptase polymerase chain reaction (RT-PCR) in preoperative blood samples of patients with localized tumors and healthy donors. They found CAIX mRNA in 86% of the patients with ccRCC, compared to 0% in patients with benign lesions, and 1.8% in the healthy control group [77]. A follow-up of the same 41 patients with RCC showed a 5-yr PFS of 88% and 40% for the preoperatively CAIX-negative and CAIX-positive patients, respectively [78].

3.2.2. Vascular endothelial growth factor

High serum levels of VEGF have been reported to predict stage, grade, and OS, especially in patients with pT3b–c and in clinical stages 1–3 [79,80]. Perioperatively, high venous VEGF levels before and immediately after surgery significantly correlated with higher tumor grade, larger tumor size, level of vascular invasion, and short DSS [81].

In a study of 302 patients treated by cytokine therapy, pretreatment VEGF blood levels were independently prognostic for OS and PFS in multivariate analyses [82].

As for the targeted therapies aiming at VEGF, the data are somewhat surprising. Subgroup analysis from the AVOREN trial using the anti-VEGF therapy with bevacizumab, demonstrated similar PFS benefit independent of the baseline VEGF levels in 85 cases [83].

In a biomarker analysis derived from the TARGET trial, high levels of blood VEGF were associated with a shorter PFS in the placebo group, but VEGF levels were not predictive for PFS or for response to sorafenib therapy in the treatment arm [29].

3.2.3. Serum amyloid A

Serum amyloid A (SAA) blood levels have been reported to correlate with distant metastases. In an analysis of SAA concentrations in healthy controls and in patients with localized tumors, there were no significant differences (median: 3 mg/l and 4 mg/l). In M1 patients, however, a nine-fold increase in the median level was observed, and SAA levels were an independent predictor of OS [84].

A protein pattern, including SAA-1 identified by surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS)

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analysis of serum samples of 50 ccRCC patients and 50 volunteers was able to discriminate the two groups with a sensitivity of 70–78% and a specificity of 82–92%, respectively [85].

3.2.4. *Insulin-like growth factor-1*

In a series of 256 RCC patients, Rasmuson et al found a positive correlation between IGF-1 serum levels and survival. At multivariate analysis, a serum level above median IGF-1 levels and tumor stage were independent predictors of OS in the investigated cohort [86].

3.2.5. *Conclusions regarding molecular markers in blood and serum*

Such promising research is important to retain expertise and research money within urological departments of urology. Given a proven reliability of blood MMs like CAIX and IGF-1, they could serve to give specific fingerprints that can be used for screening and classification but also follow-up of patients with RCC.

3.3. *Molecular markers in urine*

The idea to follow localized tumors or to monitor drug-based therapy results by simply analyzing tumor-specific markers in the easily available excretory product of the kidney is desirable. However, there is only scant literature on urine markers for RCC.

3.3.1. *Urinary nuclear matrix protein 22*

Aside from prostate-specific antigen (PSA) for prostate carcinoma, urinary nuclear matrix protein 22 (NMP 22) is the only Federal Drug Administration (FDA)–approved screening marker. It is known to be specific for transitional cell carcinoma, and it is available as a flow-through rapid diagnostic test. There are some reports suggesting that NMP 22 might also work as an RCC screening marker. In a study of 41 patients, 60% of the RCC patients had a positive urinary NMP 22 test, compared with only 13% in the control group [87]. Similarly, Ozer et al and Huang et al reported preoperatively urinary NMP-22 levels significantly higher in each of their respective RCC groups than in healthy volunteers [88,89].

3.3.2. *Others*

A protein profile set detected in urine of RCC patients by SELDI demonstrated excellent sensitivity and specificity to discriminate among the simultaneously investigated volunteers in an initial ''blind'' set but declined in a ''coin drop'' in a second larger set [90]. Some other authors have reported marker sets achieved by SELDI analysis of urinary proteins to discriminate RCC patients from healthy controls [91,92].

Although their patient number was quite small, Teratani et al reported fatty acid–binding protein (FABP) cDNA to be amplified in preoperative urine of RCC patients but not postoperatively or in healthy controls [93].

4. Discussion

Although there are many promising studies of MMs in RCC, after >10 yr of investigation, MMs have not yet made their way into clinical practice; the results of the studies are sometimes contradictory. When analyzing the reasons for this, some pitfalls of the existing data become obvious:

1. Many authors continue to mix different RCC types in their analyses, even though different RCC types represent genetically different tumor entities with different biological behaviors [94].
2. Differences in handling tissue samples, in staining techniques, and in molecular methods might give differing results, making interpretation difficult.
3. Depending on the further treatment modalities applied in the investigated patient cohorts, an investigated MM might just be a marker of response for a particular therapy but not a prognostic marker for the tumor itself. This would make a marker's universal validity questionable, since there are significant variations of therapeutic schemes both regionally (high dose intravenous IL-2 in the United States vs INF/IL-2 subcutaneous in Europe) and over time (immune modulation vs targeted therapies).
4. Tumor necrosis, tumor heterogeneity, large tumor size, and variations in hypoxic time during surgery (eg, clamping time) might artificially activate relevant pathways due to hypoxia or other factors which result in an inhomogeneous distribution pattern within a tumor. For HIF-1α, at least, it has been shown that levels within specimens remain constant for 60 min [18].

5. Conclusions

Presently, there are a number of promising molecular markers that address several clinical questions, but the available data are not valid enough for

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routine, clinical application. Further well-performed, reproducible studies are needed to display markers useful in the clinical work-up of patients with RCC. Consequently, we should comply with the demand for large, multicenter, prospective investigations that are stratified for RCC type and treatment modalities. This would lift molecular markers in diagnosis and treatment of RCC to a practical level, helping therapists to refine and economize their follow-up and to individualize treatment strategies. Furthermore, use of MMs could prevent patients from unnecessary radiological exposure, psychological pressure, and too-late detection of progression and could also spare patients from ineffective therapies.

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Study concept and design: Eichelberg, Junker, Ljungberg, Moch.
Acquisition of data: Eichelberg, Junker, Ljungberg, Moch.
Analysis and interpretation of data: Eichelberg, Junker, Ljungberg, Moch.
Drafting of the manuscript: Eichelberg, Junker, Ljungberg, Moch.
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References

[1] National Cancer Institute. Surveillance, Epidemiology, and End Results (SEER). http://www.seer.cancer.gov.
[2] Ljungberg B, Hanbury DC, Kuczyk MA, et al. Renal cell carcinoma guideline. Eur Urol 2007;51:1502–10.
[3] Hollingsworth JM, Miller DC, Daignault S, Hollenbeck BK. Rising incidence of small renal masses: a need to reassess treatment effect. J Natl Cancer Inst 2006;98:1331–4.
[4] Sobin LH, Wittekind CH, editors. International Union Against Cancer (UICC) TNM Classification of Malignant Tumours. 6th ed. New York, NY: Wiley-Liss; 2002 . p. 193–5.
[5] Margulis V, Tamboli P, Matin SF, Meisner M, Swanson DA, Wood CG. Redefining pT3 renal cell carcinoma in the modern era. Cancer 2007;109:2439–44.
[6] Kim HL, Seligson D, Liu X, et al. Using protein expressions to predict survival in clear cell renal carcinoma. Clin Cancer Res 2004;10:5464–71.
[7] Ficarra V, Novara G, Iafrate M, et al. Proposal for reclassification of the TNM staging system in patients with locally advanced (pT3–4) renal cell carcinoma according to the cancer-related outcome. Eur Urol 2007;51:722–31, discussion 729–31.
[8] Thompson RH, Cheville JC, Lohse CM, et al. Reclassification of patients with pT3 and pT4 renal cell carcinoma improves prognostic accuracy. Cancer 2005;104:53–60.
[9] Hupertan V, Roupret M, Poisson JF, et al. Low predictive accuracy of the Kattan postoperative nomogram for renal cell carcinoma recurrence in a population of French patients. Cancer 2006;107:2604–8.
[10] Galfano A, Novara G, Iafrate M, et al. Mathematical models for prognostic prediction in patients with renal cell carcinoma. Urol Int 2008;80:113–23.
[11] Cindolo L, Patard JJ, Chiodini P, et al. Comparison of predictive accuracy of four prognostic models for nonmetastatic renal cell carcinoma after nephrectomy: a multicenter European study. Cancer 2005;104:1362–71.
[12] Ross JS, Fletcher JA, Bloom KJ, et al. HER-2/neu testing in breast cancer. Am J Clin Pathol 2003;120(Suppl):S53–71.
[13] Struckmann K, Mertz K, Steu S, et al. pVHL co-ordinately regulates CXCR4/CXCL12 and MMP2/MMP9 expression in human clear-cell renal cell carcinoma. J Pathol 2008; 214:464–71.
[14] Frew IJ, Krek W. pVHL: a multipurpose adaptor protein. Sci Signal 2008;1:pe30.
[15] Yao M, Yoshida M, Kishida T, et al. VHL tumor suppressor gene alterations associated with good prognosis in sporadic clear-cell renal carcinoma. J Natl Cancer Inst 2002;94: 1569–75.
[16] Schraml P, Struckmann K, Hatz F, et al. VHL mutations and their correlation with tumour cell proliferation, microvessel density, and patient prognosis in clear cell renal cell carcinoma. J Pathol 2002;196:186–93.
[17] Rini BI, Jaeger E, Weinberg V, et al. Clinical response to therapy targeted at vascular endothelial growth factor in metastatic renal cell carcinoma: impact of patient characteristics and Von Hippel-Lindau gene status. BJU Int 2006;98:756–62.
[18] Wiesener MS, Munchenhagen PM, Berger I, et al. Constitutive activation of hypoxia-inducible genes related to overexpression of hypoxia-inducible factor-1alpha in clear cell renal carcinomas. Cancer Res 2001;61:5215–22.

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[19] Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Vasko J, Ljungberg B. The expression of hypoxia-inducible factor 1alpha is a favorable independent prognostic factor in renal cell carcinoma. Clin Cancer Res 2005;11:1129–35.
[20] Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Bergh A, Ljungberg B. Hypoxia-inducible factor 1α expression in renal cell carcinoma analyzed by tissue microarray. Eur Urol 2006;50:1272–7.
[21] Klatte T, Seligson DB, Riggs SB, et al. Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma. Clin Cancer Res 2007;13:7388–93.
[22] An WG, Kanekal M, Simon MC, Maltepe E, Blagosklonny MV, Neckers LM. Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. Nature 1998;392:405–8.
[23] Na X, Wu G, Ryan CK, Schoen SR, di'Santagnese PA, Messing EM. Overproduction of vascular endothelial growth factor related to von Hippel-Lindau tumor suppressor gene mutations and hypoxia-inducible factor-1 alpha expression in renal cell carcinomas. J Urol 2003;170:588–92.
[24] Yildiz E, Gokce G, Kilicarslan H, Ayan S, Goze OF, Gultekin EY. Prognostic value of the expression of Ki-67, CD44 and vascular endothelial growth factor, and microvessel invasion, in renal cell carcinoma. BJU Int 2004;93: 1087–93.
[25] Jacobsen J, Grankvist K, Rasmuson T, Bergh A, Landberg G, Ljungberg B. Expression of vascular endothelial growth factor protein in human renal cell carcinoma. BJU Int 2004;93:297–302.
[26] Paradis V, Lagha NB, Zeimoura L, et al. Expression of vascular endothelial growth factor in renal cell carcinomas. Virchows Arch 2000;436:351–6.
[27] Yildiz E, Ayan S, Goze F, Gokce G, Gultekin EY. Relation of microvessel density with microvascular invasion, metastasis and prognosis in renal cell carcinoma. BJU Int 2008;101:758–64.
[28] Murphy DA, Makonnen S, Lassoued W, Feldman MD, Carter C, Lee WM. Inhibition of tumor endothelial ERK activation, angiogenesis, and tumor growth by sorafenib (BAY43-9006). Am J Pathol 2006;169:1875–85.
[29] Bukowski R, Heng D, Eisen T, et al. Sorafenib in advanced renal cell carcinoma (RCC): survival and biomarker results from a phase III trial. Eur Urol Suppl 2008;7:245.
[30] Oosterwijk E, Ruiter DJ, Hoedemaeker PJ, et al. Monoclonal antibody G 250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. Int J Cancer 1986;38:489–94.
[31] Pastorek J, Pastorekova S, Callebaut I, et al. Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment. Oncogene 1994;9:2877–88.
[32] Grabmaier KA, de Weijert MC, Verhaegh GW, Schalken JA, Oosterwijk E. Strict regulation of CAIX(G250/MN) by HIF-1alpha in clear cell renal cell carcinoma. Oncogene 2004;23:5624–31.
[33] Liao SY, Aurelio ON, Jan K, Zavada J, Stanbridge EJ. Identification of the MN/CA9 protein as a reliable diagnostic biomarker of clear cell carcinoma of the kidney. Cancer Res 1997;57:2827–31.
[34] Bui MH, Seligson D, Han KR, et al. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 2003;9:802–11.
[35] Tennstedt P, Schneider P, Oosterwijk E, et al. Investigation of Ca9 expression in pulmonal metastatic lesions from patients with clear cell renal cell carcinoma. J Urol 2008;179:136.
[36] Bui MH, Visapaa H, Seligson D, et al. Prognostic value of carbonic anhydrase IX and KI67 as predictors of survival for renal clear cell carcinoma. J Urol 2004;171:2461–6.
[37] Leibovich BC, Sheinin Y, Lohse CM, et al. Carbonic anhydrase IX is not an independent predictor of outcome for patients with clear cell renal cell carcinoma. J Clin Oncol 2007;25:4757–64.
[38] Atkins M, Regan M, McDermott D, et al. Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer. Clin Cancer Res 2005;11: 3714–21.
[39] Oosterwijk E, Debruyne FM, Schalken JA. The use of monoclonal antibody G250 in the therapy of renal-cell carcinoma. Semin Oncol 1995;22:34–41.
[40] Hudes G, Carducci M, Tomczak P, et al. A phase 3, randomized, 3-arm study of temsirolimus (TEMSR) or interferon-alpha (IFN) or the combination of TEMSR + IFN in the treatment of first-line, poor-risk patients with advanced renal cell carcinoma (adv RCC) [abstract]. J Clin Oncol 2006;24(Suppl):LBA4.
[41] Motzer RJ, Escudier B, Oudard S, et al. RAD001 vs placebo in patients with metastatic renal cell carcinoma (RCC) after progression on VEGFr-TKI therapy: results from a randomized, double-blind, multicenter phase-III study [abstract]. J Clin Oncol 2008;26(Suppl):LBA5026.
[42] Youssif T, Tanguay S, Alam-Fahmy M, Koumakpayi I, Sircar K. Expression of PI3K/AKT/mTOR pathway in renal cell carcinoma metastases: correlation with pathologic findings and survival. J Urol 2008;179:210.
[43] Pantuck A, Seligson DB, Klatte T, et al. Prognostic relevance of the mTOR pathway in renal cell carcinoma. Cancer 2007;109:2257–67.
[44] Cho D, Signoretti S, Dabora S, et al. Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer 2007;5:379–85.
[45] Velickovic M, Delahunt B, McIver B, Grebe SK. Intragenic PTEN/MMAC1 loss of heterozygosity in conventional (clear-cell) renal cell carcinoma is associated with poor patient prognosis. Mod Pathol 2002;15:479–85.
[46] Sejima T, Miyagawa I. Expression of Bcl-2, p53 oncoprotein, and proliferating cell nuclear antigen in renal cell carcinoma. Eur Urol 1999;35:242–8.
[47] Zigeuner R, Ratschek M, Rehak P, Schips L, Langner C. Value of p53 as a prognostic marker in histologic subtypes of renal cell carcinoma: a systematic analysis of primary and metastatic tumor tissue. Urology 2004; 63:651–5.
[48] Mombini H, Givi M, Rashidi I. Relationship between expression of p53 protein and tumor subtype and grade in renal cell carcinoma. J Urol 2006;3:79–81.

Please cite this article in press as: Eichelberg C, et al. Diagnostic and Prognostic Molecular Markers for Renal Cell Carcinoma: A Critical Appraisal of the Current State of Research and Clinical Applicability, Eur Urol (2009), doi:10.1016/j.eururo.2009.01.003

[49] Rioux-Leclercq N, Turlin B, Bansard J, et al. Value of immunohistochemical Ki-67 and p53 determinations as predictive factors of outcome in renal cell carcinoma. Urology 2000;55:501–5.
[50] Shvarts O, Seligson D, Lam J, et al. p53 is an independent predictor of tumor recurrence and progression after nephrectomy in patients with localized renal cell carcinoma. J Urol 2005;173:725–8.
[51] Klatte T, Seligson DB, Leppert JT, et al. The chemokine receptor CXCR3 is an independent prognostic factor in patients with localized clear cell renal cell carcinoma. J Urol 2008;179(Suppl):61–6.
[52] Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature 2003; 425:307–11.
[53] Chawla R, Kramer M, Siva H, Tunuguntla GR, Jorda M, Lokeshwar BL. Is CXCR-4 a new prognostic and metastatic marker in renal cell carcinoma? J Urol 2008; 179 (Suppl):139.
[54] Kallakury BV, Karikehalli S, Haholu A, Sheehan CE, Azumi N, Ross JS. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clin Cancer Res 2001;7:3113–9.
[55] Kugler A, Hemmerlein B, Thelen P, Kallerhoff M, Radzun HJ, Ringert RH. Expression of metalloproteinase 2 and 9 and their inhibitors in renal cell carcinoma. J Urol 1998;160:1914–8.
[56] Kawata N, Nagane Y, Igarashi T, et al. Strong significant correlation between MMP-9 and systemic symptoms in patients with localized renal cell carcinoma. Urology 2006;68:523–7.
[57] Hoffmann NE, Sheinin Y, Lohse CM, et al. External validation of IMP3 expression as an independent prognostic marker for metastatic progression and death for patients with clear cell renal cell carcinoma. Cancer 2008;112: 1471–9.
[58] Jiang Z, Chu PG, Woda BA, et al. Analysis of RNA-binding protein IMP3 to predict metastasis and prognosis of renal-cell carcinoma: a retrospective study. Lancet Oncol 2006;7:556–64.
[59] Jiang Z, Lohse CM, Chu PG, et al. Oncofetal protein IMP3: a novel molecular marker that predicts metastasis of papillary and chromophobe renal cell carcinomas. Cancer 2008;112:2676–82.
[60] Seligson DB, Pantuck AJ, Liu X, et al. Epithelial cell adhesion molecule (KSA) expression: pathobiology and its role as an independent predictor of survival in renal cell carcinoma. Clin Cancer Res 2004;10: 2659–69.
[61] Went P, Dirnhofer S, Salvisberg T, et al. Expression of epithelial cell adhesion molecule (EpCam) in renal epithelial tumors. Am J Surg Pathol 2005;29:83–8.
[62] Moch H, Schraml P, Bubendorf L, et al. High-throughput tissue microarray analysis to evaluate genes uncovered by cDNA microarray screening in renal cell carcinoma. Am J Pathol 1999;154:981–6.
[63] Zigeuner R, Droschl N, Tauber V, Rehak P, Langner C. Biologic significance of fascin expression in clear cell renal cell carcinoma: systematic analysis of primary and metastatic tumor tissues using a tissue microarray technique. Urology 2006;68:518–22.
[64] Wagener N, Crnkovic-Mertens I, Vetter C, et al. Expression of inhibitor of apoptosis protein livin in renal cell carcinoma and non-tumorous adult kidney. Br J Cancer 2007;97:1271–6.
[65] Kempkensteffen C, Hinz S, Christoph F, et al. Expression of the apoptosis inhibitor livin in renal cell carcinomas: correlations with pathology and outcome. Tumour Biol 2007;28:132–8.
[66] Kosari F, Parker AS, Kube DM, et al. Clear cell renal cell carcinoma: gene expression analyses identify a potential signature for tumor aggressiveness. Clin Cancer Res 2005;11:5128–39.
[67] Byun SS, Yeo WG, Lee SE, Lee E. Expression of survivin in renal cell carcinomas: association with pathologic features and clinical outcome. Urology 2007;69:34–7.
[68] Parker AS, Kosari F, Lohse CM, et al. High expression levels of survivin protein independently predict a poor outcome for patients who undergo surgery for clear cell renal cell carcinoma. Cancer 2006;107:37–45.
[69] Li G, Cuilleron M, Cottier M, et al. The use of MN/CA9 gene expression in identifying malignant solid renal tumors. Eur Urol 2006;49:401–5.
[70] Junker K, Hindermann W, von Eggeling F, Diegmann J, Haessler K, Schubert J. CD70: a new tumor specific biomarker for renal cell carcinoma. J Urol 2005;173: 2150–3.
[71] Perret AG, Clemencon A, Li G, Tostain J, Peoc'h M. Differential expression of prognostic markers in histological subtypes of papillary renal cell carcinoma. BJU Int 2008;102:183–7.
[72] Yamazaki K, Sakamoto M, Ohta T, Kanai Y, Ohki M, Hirohashi S. Overexpression of KIT in chromophobe renal cell carcinoma. Oncogene 2003;22:847–52.
[73] Pan CC, Chen PC, Chiang H. Overexpression of KIT (CD117) in chromophobe renal cell carcinoma and renal oncocytoma. Am J Clin Pathol 2004;121:878–83.
[74] Kruger S, Sotlar K, Kausch I, Horny HP. Expression of KIT (CD117) in renal cell carcinoma and renal oncocytoma. Oncology 2005;68:269–75.
[75] Kauffman EC, Barocas DA, Yang XJ, Liu H, Scherr DS, Tu JJ. KAI1 is a novel biomarker for chromophobe renal cell carcinoma. J Urol 2008;179:134.
[76] Klatte T, Nagesh Rao P, Martino MD, et al. Gender specific cytogenetic signatures can distinguish between oncocytoma and chromophobe renal cell carcinoma. J Urol 2008;179(Suppl):168.
[77] McKiernan JM, Buttyan R, Bander NH, et al. The detection of renal carcinoma cells in the peripheral blood with an enhanced reverse transcriptase-polymerase chain reaction assay for MN/CA9. Cancer 1999;86:492–7.
[78] Gilbert SM, Whitson JM, Mansukhani M, et al. Detection of carbonic anhydrase-9 gene expression in peripheral blood cells predicts risk of disease recurrence in patients with renal cortical tumors. Urology 2006;67:942–5.

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[79] Jacobsen J, Rasmuson T, Grankvist K, Ljungberg B. Vascular endothelial growth factor as prognostic factor in renal cell carcinoma. J Urol 2000;163:343–7.
[80] Rioux-Leclercq N, Fergelot P, Zerrouki S, et al. Plasma level and tissue expression of vascular endothelial growth factor in renal cell carcinoma: a prospective study of 50 cases. Hum Pathol 2007;38:1489–95.
[81] Klatte T, Bohm M, Nelius T, Filleur S, Reiher F, Allhoff EP. Evaluation of peri-operative peripheral and renal venous levels of pro- and anti-angiogenic factors and their relevance in patients with renal cell carcinoma. BJU Int 2007;100:209–14.
[82] Negrier S, Chabaud S, Escudier B, et al. Serum level of vascular endothelial growth factor (VEGF) as an independent prognostic factor in metastatic renal cell carcinoma (MRCC) [abstract]. J Clin Oncol 2007;25(Suppl):5044.
[83] Escudier B, Ravaud A, Négrier S, et al. Update on AVOREN trial in metastatic renal cell carcinoma (mRCC): efficacy and safety in subgroups of patients (pts) and pharmacokinetic (PK) analysis [abstract]. J Clin Oncol 2008; 26(Suppl):5025.
[84] Ramankulov A, Lein M, Johannsen M, et al. Serum amyloid A as indicator of distant metastases but not as early tumor marker in patients with renal cell carcinoma. Cancer Lett 2008;28:269, 85–92.
[85] Junker K, von Eggeling F, Muller J, Steiner T, Schubert J. Identification of biomarkers and therapeutic targets for renal cell cancer using ProteinChip technology [in German]. Urologe A 2006;45:305–6, 308, 310–2.
[86] Rasmuson T, Grankvist K, Jacobsen J, Olsson T, Ljungberg B. Serum insulin-like growth factor-1 is an independent predictor of prognosis in patients with renal cell carcinoma. Acta Oncol 2004;43:744–8.
[87] Kaya K, Ayan S, Gokce G, Kilicarslan H, Yildiz E, Gultekin EY. Urinary nuclear matrix protein 22 for diagnosis of renal cell carcinoma. Scand J Urol Nephrol 2005;39:25–9.
[88] Ozer G, Altinel M, Kocak B, Yazicioglu A, Gonenc F. Value of urinary NMP-22 in patients with renal cell carcinoma. Urology 2002;60:593–7.
[89] Huang S, Rhee E, Patel H, Park E, Kaswick J. Urinary NMP22 and renal cell carcinoma. Urology 2000;55:227–30.
[90] Rogers MA, Clarke P, Noble J, et al. Proteomic profiling of urinary proteins in renal cancer by surface enhanced laser desorption ionization and neural-network analysis: identification of key issues affecting potential clinical utility. Cancer Res 2003;63:6971–83.
[91] Holcakova J, Hernychova L, Bouchal P, et al. Identification of alphaB-crystallin, a biomarker of renal cell carcinoma by SELDI-TOF-MS. Int J Biol Markers 2008;23:48–53.
[92] Wu DL, Zhang WH, Wang WJ, Jing SB, Xu YM. Proteomic evaluation of urine from renal cell carcinoma using SELDI-TOF-MS and tree analysis pattern. Technol Cancer Res Treat 2008;7:155–60.
[93] Teratani T, Domoto T, Kuriki K, et al. Detection of transcript for brain-type fatty acid-binding protein in tumor and urine of patients with renal cell carcinoma. Urology 2007;69:236–40.
[94] Moch H, Gasser T, Amin MB, Torhorst J, Sauter G, Mihatsch MJ. Prognostic utility of the recently recommended histologic classification and revised TNM staging system of renal cell carcinoma: a Swiss experience with 588 tumors. Cancer 2000;89:604–14.

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