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Molecular Subtypes of Clear-cell Renal Cell Carcinoma are Prognostic for Outcome After Complete Metastasectomy

Annelies Verbiest[a], *Gabrielle Couchy*[b], *Sylvie Job*[c], *Laure Caruana*[b], *Evelyne Lerut*[d], *Raymond Oyen*[e], *Aurélien de Reyniès*[c], *Lorenzo Tosco*[f,g], *Steven Joniau*[f], *Hendrik Van Poppel*[f], *Dirk Van Raemdonck*[h], *Kathleen Van Den Eynde*[d], *Agnieszka Wozniak*[a], *Jessica Zucman-Rossi*[b], *Benoit Beuselinck*[a,b,i,*]

[a] *Laboratory of Experimental Oncology, Department of Oncology, KU Leuven, Leuven, Belgium;* [b] *Inserm, UMR-1162, Génomique Fonctionnelle des Tumeurs Solides, IUH, Paris, France;* [c] *Programme Cartes d'Identité des Tumeurs, Ligue Nationale Contre le Cancer, Paris, France;* [d] *Department of Pathology, University Hospitals Leuven, Leuven, Belgium;* [e] *Department of Radiology, University Hospitals Leuven, Leuven, Belgium;* [f] *Department of Urology, University Hospitals Leuven, Leuven, Belgium;* [g] *Department of Imaging and Pathology, KU Leuven, Leuven, Belgium;* [h] *Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium;* [i] *Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven, Belgium*

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Abstract

Background: Metastasectomy is routinely performed in selected patients with metastatic clear-cell renal cell carcinoma (ccRCC) as an alternative to systemic therapy. In the absence of randomized trials, the benefit and best way of patient selection remain unclear. Earlier, we described four molecular ccRCC-subtypes (ccrcc1–4) that have a prognostic and predictive value upon first-line sunitinib or pazopanib.
Objective: Assess the prognostic value of ccrcc1–4 subtypes after complete metastasectomy. (1) Compare outcomes of good-prognosis ccrccc2&3-tumors with intermediate/poor-prognosis ccrcc1&4-tumors. (2) Compare outcomes of the four subtypes separately.
Design, setting, and participants: Single-center retrospective study (1995–2017), assessing 43 ccRCC patients undergoing complete metastasectomy without systemic treatment.
Intervention: Molecular subtype determined with established 35-gene expression classifier.
Outcome measurements and statistical analysis: Median disease-free survival (DFS), time to systemic therapy, cancer-specific (CSS) and overall survival (OS) from metastasectomy, estimated with Kaplan-Meier method and tested against other predictors with multivariable Cox regression.
Results and limitations: Median DFS was 23 mo for ccrcc2&3-tumors versus 9 mo for ccrcc1&4-tumors ($p = 0.011$, hazard ratio [HR] = 2.6). Median time to systemic therapy was 92 mo versus 28 mo ($p = 0.003$, HR = 3.3). Median CSS was 133 mo versus 50 mo ($p < 0.001$, HR = 2.7). Median OS was 127 mo versus 50 mo ($p = 0.011$, HR = 2.5). The classification remained independent upon multivariable analysis. Outcomes remained significantly different when comparing four subtypes separately. The intrinsic heterogeneity of expression profiles is a limitation of this approach.
Conclusion: Even after clinical patient selection, patients with a ccrcc1- or ccrcc4-tumor are at a higher risk of relapse after complete metastasectomy. Patients with a ccrcc2- or ccrcc3-tumor usually experience a long DFS. These results need validation in a larger cohort to establish the subtypes as prognostic marker.
Patient summary: Metastasectomy is recommended for some patients with metastatic clear-cell kidney cancer; however, we do not know who will benefit the most. We show that molecular subtypes increase the possibility to predict which patients are at risk for early relapse after metastasectomy and who may benefit more from other treatment options.

* Corresponding author. Department of General Medical Oncology, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. Tel. (secr) +32 16 34 69 00; Fax: +32 16 34 69 01.
E-mail address: benoit.beuselinck@uzleuven.be (B. Beuselinck).

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1. Introduction

Clear-cell renal cell carcinoma (ccRCC) accounts for the majority of primary kidney cancers, which are responsible for about 35 000 deaths per year in Europe [1]. Metastatic ccRCC is preferentially treated in first-line with an angiogenesis inhibitor (sunitinib or pazopanib) in International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) good risk patients, or immune checkpoint inhibitors (nivolumab-ipilimumab) in IMDC intermediate/poor risk patients [2]. However, in selected patients, complete metastasectomy is recommended with the aim of prolonging survival or at least postponing systemic therapy [3]. The intervention is mainly offered to fit patients presenting with a limited tumor load after a long disease-free interval [4].

Although retrospective series typically show favorable outcomes after metastasectomy, it remains an open question to what extent surgery provides actual benefit, rather than the indolent tumor biology in these highly selected patients. In non-small-cell lung carcinoma, a recent randomized controlled trial pointed toward a progression-free survival benefit in patients who received local treatment of metastases (mostly stereotactic body radiotherapy) [5]. In other tumor types, randomized trials are lacking. A recent systematic review on metastasectomy in RCC identified only 15 comparative studies; however, all were retrospective in nature and with a high risk of bias [4,6]. Although most studies pointed toward the benefit of complete metastasectomy, a multivariable analysis was performed in only eight to correct for a limited set of confounders. Moreover, in a large patient series at our own center, we found that the baseline prognostic characteristics of patients who undergo metastasectomy are too favorable to allow for a liable comparison of outcomes with patients who do not undergo metastasectomy, even by multivariable analysis (A. Verbiest, unpublished data, 2017). Importantly, no clear guidelines exist to aid patient selection for metastasectomy.

Briefly, an important knowledge gap remains to the extent to which intrinsic tumor biology explains the favorable outcomes after metastasectomy and to the selection of patients that will benefit from it. This study aimed to resolve the second question.

Earlier, unsupervised cluster analysis of whole genome mRNA expression data has revealed distinct molecular subtypes of ccRCC. These subtypes, based on intrinsic tumor biology, are correlated with prognosis. In the post-nephrectomy setting, Brannon et al. [7] and the Cancer Genome Atlas project (TCGA) [8] have identified two (ccA and ccB) and four (m1–m4) subtypes, respectively, that show different survival after nephrectomy. In the metastatic setting, we have identified four subtypes, named ccrcc1–4, that differ in terms of mRNA expression, methylation status, mutation profile, cytogenetic anomalies, and immune infiltrate [9]. These subtypes are not only correlated with overall survival (OS) but also with response rate and progression-free survival on the angiogenesis inhibitors sunitinib and pazopanib. Subtypes ccrcc2&3 have a favorable prognosis and high response rate to sunitinib and pazopanib [9,10]. Ccrcc2-tumors display relative upregulation of genes implicated in angiogenesis, whereas ccrcc3-tumors exhibit an expression profile similar to that of the normal kidney tissue [9,11]. In contrast, subtypes ccrcc1&4 have an intermediate and poor prognosis, respectively, and rarely show durable responses to sunitinib or pazopanib [9,10]. Both show relative upregulation of Myc-targets. However, ccrcc1-tumors have an immune-cold phenotype with virtual absence of tumor-infiltrating lymphocytes, whereas ccrcc4-tumors show an immune-inflamed phenotype, with an abundant but exhausted immune infiltrate and relative upregulation of genes involved in inflammation, migration, and immune response [9,12].

The molecular subtypes as described by Brannon et al [7], TCGA [8], and Beuselinck et al [9] are overlapping. As they have a prognostic value in the post-nephrectomy and metastatic setting, we hypothesized that they would also be able to predict outcome after metastasectomy.

2. Materials and methods

2.1. Patient population

At University Hospitals Leuven, a systematic record from 1995 to present is maintained of RCC patients who undergo metastasectomy. We selected patients who met the following inclusion criteria: clear-cell histology, resection of the primary tumor, complete metastasectomy, no prior or adjuvant systemic treatment, no other metastatic cancer, availability of fresh frozen primary tumor tissue, and at least one follow-up computed tomography (CT) scan after metastasectomy. Clear-cell histology was confirmed by an expert genitourinary pathologist. Ipsilateral adrenal lesions that were spatially separated from the primary tumor were considered metastases, whereas continuous growth into the adrenal gland was not. Follow-up policy was left to the discretion of the treating physician.

The protocol was approved by the local medical ethics review boards, and signed consent was obtained from all patients in accordance with Belgian legislation. Frozen biological material from deceased patients was used when prior agreement for such use was given by the institutional review board.

2.2. Sample classification

Extraction of mRNA from fresh frozen primary tumor tissue was performed using the Maxwell 16 LEV simplyRNA tissue kit according to the manufacturer's protocol (Promega). Quantification was performed using the NanoDrop spectrophotometer (ThermoFisher) and quality control by gel electrophoresis (E-gel 48 1% agarose and Mother E-base by Invitrogen). Using the High-Capacity Transcription kit from ThermoFisher, 500 ng of total RNA was reverse transcribed in a final volume of 50 μl. Preamplification of 6 ng cDNA/sample was performed using PreAmp Master Mix and the specific primers of the classifier as well as 18s. Preamplified cDNA were diluted 1:5 in DNAse/RNAse free water before qRT-PCR. qRT-PCR reactions were performed using the high throughput BioMark qRT-PCR system (Fluidigm) according to the manufacturer's protocol. Expression data (CT values) were analyzed using the Fluidigm qRT-PCR Analysis software version 4.1.3. 18S was used for normalization. The tumors were classified into the ccrcc1–4 molecular classification by our 35-gene classifier algorithm as described earlier [9].

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