Original Study

Synchronous Metastatic Clear-Cell Renal Cell Carcinoma: A Distinct Morphologic, Immunohistochemical, and Molecular Phenotype

Solene-Florence Kammerer-Jacquet,[1,2] Angelique Brunot,[3] Adelaide Pladys,[4] Guillaume Bouzille,[5] Julien Dagher,[1] Sarah Medane,[2] Benoit Peyronnet,[6] Romain Mathieu,[6] Gregory Verhoest,[6] Karim Bensalah,[6] Julien Edeline,[3] Brigitte Laguerre,[3] Alexandra Lespagnol,[7] Jean Mosser,[7] Frederic Dugay,[8] Marc-Antoine Belaud-Rotureau,[2,8] Nathalie Rioux-Leclercq[1,2]

Abstract

In order to compare synchronous and metachronous metastatic clear cell renal cell carcinoma, we performed a pathologic, immunohistochemical, and molecular study on primary tumors in a retrospective series of 48 consecutive patients with up to 10 years of follow-up. Synchronous metastatic clear cell renal cell carcinoma had a distinct phenotype that may explain their worse prognosis.

Introduction: Clear cell renal cell carcinomas (ccRCCs) are highly metastatic tumors with metastases detected at diagnosis (synchronous) or during follow-up (metachronous). To date, there have been no reports comparing primary ccRCC of patients with synchronous and metachronous metastases, who are different in terms of prognosis. Determining whether there is a phenotypic difference between these 2 groups could have important clinical implications. **Patients and Methods:** In a retrospective consecutive cohort of 98 patients with ccRCC, 48 patients had metastases, including 28 synchronous and 20 metachronous presentations, with a follow-up of 10 years. For each primary tumor in these metastatic patients, pathologic criteria, expression of vascular endothelial growth factor, partitioning-defective 3, CAIX, and programmed death ligand 1 as detected by immunohistochemistry, and complete *VHL* status were analyzed. Univariate analysis was performed, and survival was assessed using Kaplan-Meier curves compared by log-rank test. **Results:** Compared with primary ccRCC in patients with metachronous metastases, primary ccRCC in patients with synchronous metastases were significantly associated with a poorer Eastern Cooperative Oncology Group performance ($P = .045$), higher pT status ($P = .038$), non-inactivated *VHL* gene ($P = .01$), sarcomatoid component ($P = .007$), expression of partitioning-defective 3 ($P = .007$), and overexpressions of vascular endothelial growth factor (> 50%) ($P = .017$) and programmed death ligand 1 ($P = .019$). Patients with synchronous metastases had a worse cancer-specific survival than patients with metachronous metastases even from metastatic diagnosis (median survival, 16 months vs. 46 months, respectively; $P = .01$). **Conclusion:** This long-term study is the first to support the notion that synchronous m-ccRCC has a distinct phenotype. This is probably linked to the occurrence of oncogenic events that could explain the worse prognosis. These particular patients with metastases could benefit from specific therapy.

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[1]Department of Pathology
[5]Department of Clinical Investigation
[6]Department of Urology
[7]Department of Molecular Genetics
[8]Cytogenetic and Cellular Biology Laboratory, University Hospital, Rennes, France
[2]CNRS, UMR6290, Institut de Génétique & Développement de Rennes, Rennes 1 University, Rennes, France
[3]Department of Medical Oncology, Centre de Lutte Contre le Cancer, Rennes, France
[4]Department of Epidemiology, Ecole des Hautes Études en Santé Publique, Rennes, France

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Address for correspondence: Solene-Florence Kammerer-Jacquet, MD, Service d'Anatomie Pathologique, CHU Pontchaillou, 2 rue Henri Le Guilloux, 35 033 Rennes Cedex 9, France
E-mail contact: jacquet.sf@gmail.com

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common histologic subtype of renal cell carcinoma.[1] Tumor cells are characterized by inactivation of the *VHL* gene leading to hypoxia-inducible factors stabilization, which induces the transcription of genes such as vascular endothelial growth factor (*VEGF*).[2] As a consequence, the tumor microenvironment is highly vascularized. Another critical component of the tumor microenvironment is the immune system, as some tumors have a high density of tumor infiltrating lymphocytes.[3] Moreover, tumor cells were shown to express programmed death ligand 1 (PD-L1) to escape the immune system.[4,5]

With approximately 40% of patients dying of metastases, ccRCC are highly aggressive tumors.[6] The most common sites of metastasis are the lungs, distant lymph nodes, liver, bones, brain, and adrenal gland. Twenty percent to 30% of patients are diagnosed with metastatic disease (synchronous presentation), whereas 20% of patients with non-metastatic disease at diagnosis will later develop metastases during follow-up (metachronous presentation).[7] Metastases generally arise in the first 6 years after surgery.[8]

Synchronous and metachronous ccRCC have different prognoses. Currently, one of the criteria of the Memorial Sloan Kettering Cancer Center (MSKCC) and Heng risk criteria models for predicting survival in metastatic patients is a time from initial diagnosis (including original localized disease) to treatment of less than 1 year.[9-11] This risk factor includes patients with synchronous metastases. The difference in prognosis between the 2 groups of patients may be linked to their primary tumors having different phenotypes. To our knowledge, there are no reports comparing primary ccRCC in patients with synchronous and metachronous metastases. Determining whether there is a phenotypic difference between these 2 groups could have important clinical implications.

This is the first study to conduct an in-depth analysis of primary ccRCC in correlation with pathologic criteria, *VHL* status, and long-term clinical outcome with a view to seeking differences depending on synchronous or metachronous metastatic status.

Materials and Methods

Patients

Between 2002 and 2005, 98 consecutive patients were operated for sporadic ccRCC in the Department of Urology at Rennes University Hospital. Twenty-eight patients had synchronous metastases at initial diagnosis and underwent nephrectomy in accordance with international guidelines.[12] They received no prior therapy. Twenty patients had a nephrectomy before developing metastases that were detected during follow-up (first computed tomography scan 6 months after surgery) and defined as metachronous. Consequently, a total of 48 patients presented with metastases between 2002 and 2012 and were included in our study. Patient charts were retrospectively reviewed to assess pretreatment Eastern Cooperative Oncology Group (ECOG) performance status, methods of detection (incidental or symptomatic), involved metastatic sites with retrieved first staging computed tomography scan, MSKCC score, Heng criteria, and therapies.[9,11] The outcome was specific death, which we assessed with a 10-year follow-up. The study protocol was approved by the local advisory board, and informed consent was obtained from each patient for the study.

Tissue Sample Management

Tumor samples were obtained from the processing of biological samples by the Rennes Biological Resources Center-Health (CRB-Health) (BB-0033-00056). The research protocol was conducted under French legal guidelines and fulfilled the requirements of the local institutional ethics committee. All consecutive ccRCC and paired renal cortex samples were analyzed. Immediately after macroscopic examination, small samples were collected from surgical specimens, frozen in liquid nitrogen, and stored at −80°C until DNA extraction. Genomic DNA was extracted from 25 to 35 mg of frozen tissue sections using a QIAamp DNA minikit (Qiagen, Courtaboeuf, France). DNA quantity and quality were estimated by optical density (OD 260/280) measurement and 0.8% agarose gel electrophoresis using standard protocols.

Pathologic Analysis

After fresh tissue sampling, surgical specimens were formalin-fixed. Paraffin sections were stained with hematoxylin and eosin-safran for light microscopy. All slides were reviewed by a dedicated uropathologist (N.R.L.). The macroscopic and histologic parameters analyzed were: tumor size, multifocality, nucleolar grade according to the International Society of Urological Pathology grading system, sarcomatoid component, tumor necrosis, granular component, lymphocyte infiltrate, and microvessel invasion.[13] Sarcomatoid component was defined as more than 10% involvement of the tumor. Tumor stage was defined by the latest International Union Against Cancer classification (2009).[14]

Immunohistochemistry (IHC)

For each ccRCC case, a representative slide of the tumor with the highest nucleolar grade and the corresponding paraffin block were selected. VEGF (anti-VEGF antibody, sc-152, dilution 1/100; Santa Cruz Biotechnology, Santa Cruz, CA), CAIX (anti-CAIX antibody, ab15086, dilution 1/1500, Abcam, Cambridge, UK), partitioning-defective 3 (PAR-3) (anti-PAR-3, HPA0300443, dilution 1/50, Sigma-Aldrich, St Louis, MO), and PD-L1 (anti-PD-L1 antibody, clone 130021, dilution 1/200, R&D Systems, Minneapolis, MN) expression was assessed by IHC as previously described.[15-17] The cut-off for positive cases was 85% of tumor cells for CAIX as described in a previous study.[16,18] The percentage of tumor cells for VEGF was reported. Only cytoplasmic PAR-3 expression in tumor cells was considered positive.[17] PD-L1 was overexpressed when intensity of membranous or cytoplasmic staining in tumor cells was moderate to strong, as previously described.[19] Regarding tumor infiltrating lymphocytes, CD3 (anti-CD3 antibody, clone SP7, dilution 1/100; Thermo Scientific, Waltham, MA) and CD20 (anti-CD20 antibody, clone L26, dilution 1/25; Dako, Glostrup, Denmark) expression was assessed. The inflammatory extent was coded as 1 (few sparse lymphocytes in the tumor) or 2 (marked dense lymphocytes or lymphoid nodules). IHC scoring was independently assessed by 2 pathologists (S.-F.K.-J. and N.R.L.) blinded to the clinical grouping of the specimens. Discordant cases were reevaluated collegially to reach a consensus score.

VHL Gene Analysis

We determined the complete *VHL* status for each tumor by analyzing *VHL* gene mutation, deletion, and promoter methylation. *VHL* mutations were detected by sequencing using denaturing