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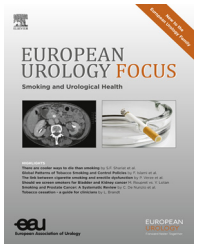

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Kidney Cancer

Serum Adiponectin Predicts Cancer-specific Survival of Patients with Renal Cell Carcinoma

Michela de Martino [a,†], *Carmen V. Leitner* [a,†], *Sebastian L. Hofbauer* [a], *Ilaria Lucca* [a], *Andrea Haitel* [b], *Shahrokh F. Shariat* [a,c,d], *Tobias Klatte* [a,*]

[a] *Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Vienna General Hospital, Vienna, Austria;* [b] *Clinical Institute of Pathology, Medical University of Vienna, Vienna General Hospital, Vienna, Austria;* [c] *Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA;* [d] *Department of Urology, Weill Cornell Medical College, New York Presbyterian Hospital, New York, NY, USA*

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Abstract

Background: Prediction of outcomes in patients with renal cell carcinoma (RCC) is crucial for clinical decision-making. The limited accuracy of conventional prognostic factors such as stage and grade may be increased by the use of biomarkers.
Objective: To evaluate the association of serum adiponectin and leptin and polymorphisms in the leptin and leptin receptor genes with RCC histopathology and prognosis.
Design, setting, and participants: Adiponectin and leptin levels were measured in preoperative serum samples from 131 consecutive patients with sporadic unilateral RCC. The polymorphisms G–2548A (rs7799039) in the leptin gene (*LEP*) and Gln223Arg (Q223R, A668G, rs1137101) in the leptin receptor gene (*LEPR*) were genotyped in 233 patients.
Outcome measurements and statistical analysis: Multivariable associations with RCC-specific survival were analyzed using Cox models.
Results and limitations: Median preoperative serum adiponectin was 15.8 μg/ml (interquartile range 10.0–23.1). Adiponectin was lower in patients with distant metastases ($p = 0.017$) or histologic tumor necrosis ($p = 0.015$). On multivariable analysis adjusted for the effects of variables in the Karakiewicz nomogram, each 1-μg/ml increase in adiponectin was associated with a 8% decrease in the hazard of death from RCC (hazard ratio 0.92, 95% confidence interval 0.86–0.98; $p = 0.007$). The discrimination of the Karakiewicz nomogram increased by 0.6% on inclusion of adiponectin. Leptin levels, *LEP* G–2548A and *LEPR* Q223R were not associated with either RCC pathology or outcomes. Limitations include the retrospective study design, the low numbers of patients, and a lack of standardized follow-up.
Conclusions: This study suggests that lower preoperative serum adiponectin is associated with features of biologically aggressive RCC, metastasis, and survival.
Patient summary: We assessed the relationship between outcomes and blood levels of adiponectin and leptin and genetic changes in leptin and leptin receptor genes. We found that patients with lower adiponectin levels have more aggressive tumors and poorer survival.

† These authors contributed equally to this work.
* Corresponding author. Department of Urology, Comprehensive Cancer Center, Medical University of Vienna, Vienna General Hospital, Währinger Gürtel 18–20, A-1090 Vienna, Austria.
Tel. +43 1 4040026020; Fax: +43 1 4040023320.
E-mail address: tobias.klatte@meduniwien.ac.at (T. Klatte).

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1. Introduction

Renal cell carcinoma (RCC) is the leading malignant tumor of the kidney, with more than 330 000 cases diagnosed annually [1]. At initial diagnosis, approximately 20% of patients have metastatic disease, and another 10–20% of patients undergoing potentially curative surgery develop metastases during follow-up, leading to poor prognosis [2,3]. Established clinical and pathologic prognostic factors for RCC include symptoms, tumor stage, size, and subtype, lymph node metastasis, distant metastasis, and grade [3–7]. Prognostic factors have been combined in multivariable prognostic models, but their accuracy is still not optimal [3]. It has been shown that preoperative biomarkers detected in peripheral blood such as genetic and protein markers increase the accuracy of established prognostic factors [2,8], yet none is routinely used in clinical practice.

Lipid metabolism plays an important role in RCC development and progression, although the exact biological pathways remain poorly understood [9]. A higher body mass index (BMI) has been linked to a higher risk of developing RCC [10]. Data also indicate that higher preoperative cholesterol levels are associated with a more favorable prognosis [11,12]. Other potential surrogate biomarkers of lipid metabolism are the adipocytokines adiponectin and leptin, which are secreted by adipocytes [13]. Besides regulating food intake, they influence a variety of neoplastic mechanisms such as inflammation, insulin resistance, and cell growth and proliferation [14,15]. Several case-control studies demonstrated a relationship between adiponectin [9,16] and leptin [17] and RCC, and two small studies showed an association with distant metastases [18] and vascular invasion [19], suggesting a possible role as prognostic factors. Furthermore, the polymorphisms G–2548A (rs7799039) in the leptin gene (*LEP*) and Gln223Arg (Q223R, A668G, rs1137101) in the leptin receptor (*LEPR*) gene are biomarkers of lipid metabolism that have been linked to prognosis in various types of cancer [20,21], but there are no data for RCC.

We hypothesized that preoperative serum levels of adiponectin and leptin and the genetic polymorphisms *LEP* G–2548A and *LEPR* Q223R differ among patients with differential tumors and outcomes. We tested this hypothesis in a consecutive cohort of patients with RCC.

2. Patients and methods

2.1. Patient selection

The aim of this study was to assess the association between biomarkers involved in lipid metabolism and RCC pathology and survival. We screened 301 consecutive patients referred for treatment of a solid renal tumor between 2008 and 2011 for study inclusion. Patients with benign renal tumors ($n = 46$), unclassified or collecting duct RCC ($n = 4$), malignant renal tumors other than RCC ($n = 2$), those who were not treated surgically ($n = 4$), and those with hereditary renal tumor syndromes ($n = 2$) or bilateral disease (n = 10) were excluded, leaving 233 patients as the study cohort. The study was approved by the institutional review board.

2.2. Staging and study variables

Clinical, pathologic, and follow-up data were abstracted from patient charts. Clinical data included age, gender, and BMI. Staging was performed according to the 2010 TNM classification. For clinical staging, a computed tomography (CT) scan of the abdomen and a CT scan or an X-ray of the chest was performed. Further imaging was only carried out if the patient was symptomatic or for surgeon preference. Patients with clinically positive retroperitoneal nodes (size >1 cm on imaging, enlarged or palpable nodes during surgery) underwent node dissection. All N+ cases reported in this study had pathologic confirmation. All other cases were pNx, but clinically N0. M stage was assigned clinically. Radical and partial nephrectomy was performed in 122 (52.4%) and 111 (47.6%) patients, respectively. Pathologic data included T stage, nuclear grade according to Fuhrman criteria, histologic tumor necrosis, and histologic subtype (clear cell RCC vs non–clear cell RCC) as evaluated by a dedicated genitourinary pathologist (A.H.). Postoperative surveillance in nonmetastatic disease followed guideline recommendations. No patient received neoadjuvant or adjuvant targeted therapy or immunotherapy.

2.3. Analysis of adiponectin and leptin

A preoperative serum sample for analysis of adiponectin and leptin was collected from 131 patients. Peripheral venous blood was drawn into a serum Vacuette tube without anticoagulant (Greiner Bio-One, Kremsmünster, Austria). Total serum adiponectin and total serum leptin were determined using enzyme-linked immunosorbent assays according to the manufacturer's instructions (adiponectin, #K1001-1, B-Bridge International, Cupertino, CA, USA; leptin, #KAC2281, Life Technologies, Grand Island, NY, USA). Each sample was analyzed in duplicate and the average value was used for final analysis. The coefficient of variation between the two measurements was <5%.

2.4. Analysis of LEP G–2548A and LEPR Q223R

LEP G–2548A and *LEPR* Q223R were genotyped in genomic DNA from the 233 patients using polymerase chain reaction (PCR)/restriction fragment length analysis. Genomic DNA was extracted from 10 ml of whole blood using a kit (Wizard Genomic DNA Purification, Promega, Madison, WI, USA). DNA was stored at 4 °C until final analyses. The primer pair was 5′-TTT CCT GTA ATT TTC CCG TGAG-3′ (forward) and 5′-AAA GCA AAG ACA GGC ATA AA-3 (reverse) for *LEP* G–2548A, and 5′-TCC TGC TTT AAAA GCC TAT CCA GTA TTT-3′ (forward) and 5′-AGC TAG CAA ATA TTT TTG TAA GCA AT-3′ (reverse) for *LEPR* Q223R. Genotyping was successful in all patients for *LEP* G–2548A and in 230 patients for *LEPR* Q223R.

For the PCR mixture, we used 10× PCR buffer, 0.5 mM $MgCl_2$, 0.3 mM dNTP, 30 pmol of primers, 0.3 U of Platinum Taq DNA polymerase (Life Technologies), and 50 ng of genomic DNA in DNase/RNase-free H_2O. The PCR program comprised 10 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at the appropriate annealing temperature for each primer pair, 40 s at 72 °C, and 7 min at 72 °C on a Veriti 96-well thermal cycler (Life Technologies). PCR products were then incubated in a water bath for 2 h at 37 °C with the restriction endonucleases HinP1I and HpaII (New England Biolabs, Ipswich, MA, USA) for *LEP* G–2548A and *LEPR* Q223R, respectively. The fragments were visualized on 2% agarose gel stained with ethidium bromide following electrophoretic separation. Genotyping was based on fragment length. For quality control, 50 randomly selected samples were analyzed via Sanger sequencing, and the results were in concordance with the restriction fragment length polymorphism analysis.

2.5. Statistical analysis

Shapiro-Wilk statistics showed that adiponectin and leptin levels were not normally distributed, so Mann-Whitney *U* tests were used for

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