# The Presence of Vascular Mimicry Predicts High Risk of Clear Cell Renal Cell Carcinoma after Radical Nephrectomy

Lin Zhou,* Yuan Chang,* Le Xu,* Zheng Liu, Qiang Fu, Yuanfeng Yang, Zongming Lin† and Jiejie Xu†

*From the Department of Urology, Zhongshan Hospital (LZ, YC, YY, ZL) and Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences (ZL, QF, JX), Fudan University and Department of Urology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (LZ, LX), Shanghai, People's Republic of China*

**Purpose:** Vascular mimicry is a type of tumor cell plasticity. The aim of this study was to determine the prognostic value of vascular mimicry in patients with clear cell renal cell carcinoma.

**Materials and Methods:** We performed a retrospective cohort study in 387 patients with clear cell renal cell carcinoma who underwent radical nephrectomy at Zhongshan Hospital, Fudan University between 2008 and 2009. Pathological features, baseline patient characteristics and followup data were recorded. Vascular mimicry in clear cell renal cell carcinoma tissue was identified by CD31-periodic acid-Schiff double staining. Univariate and multivariate Cox regression models were used to analyze the impact of prognostic factors on recurrence-free survival. The concordance index and the Akaike information criterion were used to assess the predictive accuracy and sufficiency of different models.

**Results:** Positive vascular mimicry staining occurred in 25 of 387 clear cell renal cell carcinoma cases (6.5%) and it was associated with an increased risk of recurrence (log-rank $p < 0.001$). Incorporating vascular mimicry into pT stage, Fuhrman grade and Leibovich score helped refine individual risk stratification. Moreover, vascular mimicry was identified as an independent prognostic factor ($p = 0.001$). It was entered into a nomogram together with pT stage, Fuhrman grade, tumor size and necrosis. In the primary cohort the Harrell concordance index for the established nomogram to predict recurrence-free survival was slightly higher than that of the Leibovich model (0.850 vs. 0.823), which failed to reach statistical significance ($p = 0.158$).

**Abbreviations and Acronyms**

ccRCC = clear cell RCC
C-index = concordance index
ECOG-PS = Eastern Cooperative Oncology Group performance status
PAS = periodic acid-Schiff
RCC = renal cell carcinoma
RFS = recurrence-free survival
RN = radical nephrectomy
SSIGN = Mayo Clinic stage, size, grade and necrosis
UISS = UCLA Integrated Staging System
VM = vascular mimicry

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* Equal study contribution.

† Correspondence: Department of Urology, Zhongshan Hospital, Fudan University, 180 Fenglin Rd., Shanghai 200032, People's Republic of China (telephone: +86 21 65643671; FAX: +86 21 64437203; e-mail: zongminglin@gmail.com) and Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University, 138 Yixueyuan Rd., Mailbox 103, Shanghai 200032, People's Republic of China (telephone: +86 21 54237332; FAX: +86 21 64437203; e-mail: jjxufdu@fudan.edu.cn).

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**Conclusions:** Vascular mimicry could be a potential prognosticator for recurrence-free survival in patients with clear cell renal cell carcinoma after radical nephrectomy. Further external validation and functional analysis should be pursued to assess its potential prognostic and therapeutic values for clear cell renal cell carcinoma.

**Key Words:** carcinoma, renal cell; veins; prognosis; neoplasm recurrence, local; mortality

Clear cell RCC, a hypervascular tumor, represents the most common subtype of adult kidney cancer.[1] Due to widespread use of abdominal imaging the detection of organ confined tumors has increased those that are seemingly curable by surgical resection. Despite this up to 30% of early stage cases of ccRCC after curative nephrectomy relapse with metastasis due to undetectable micrometastases.[2]

VM, in which tumor cells differentiate into endothelial-like cells and form tubular structures rich in extracellular matrix, was first reported in 1999 in melanoma.[3] These tubular structures, which are rich in extracellular matrix, including laminin, collagens IV and VI, and heparin sulfate proteoglycans, allow the perfusion of blood and fluid throughout tumor tissues.[3,4] Subsequent studies involving a combination of intravenous tracers together with confocal and immuno-electron microscopy have shown that fluid can be conducted by the endothelium-lined vasculature as well as extravascularly along the channel-like spaces created by PAS and laminin positive patterned loops and networks that encase clusters of tumor cells.[5–7]

Since this discovery, VM formation has been observed in various malignant tumors, including RCC.[8–10] Interestingly, in the placenta literature the term vasculogenic mimicry has been used to describe the process of trophoblast cells invading endothelial cell-lined spiral arteries and expressing several molecules characteristic of endothelial cells.[11,12] Recently, Wagenblast et al traced the spread of aggressive mouse breast cancer cells and found that 2 proteins, Serpine2 and Slpi, promoted metastasis by stimulating vascular mimicry.[13] A meta-analysis of the 22 studies showed that VM is associated with advanced stage disease and a poor clinical outcome.[14] However, other studies have shown that VM was not significantly associated with tumor prognosis, although the studies indicated that patients with VM positive cancer had shorter survival than patients with VM negative cancer.[15–17] Thus, the impact of VM on the prognosis in patients with cancer remains controversial.

In this study we analyzed the impact of VM on RFS in a large cohort of patients with T1-T3N0M0 ccRCC who underwent RN.

## PATIENTS AND METHODS

### Patient Selection

This study was approved by the clinical research ethics committee of Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China. Written informed consent was obtained from each patient. The study database included 387 patients with T1-3N0M0 ccRCC from Zhongshan Hospital, Fudan University. Study inclusion criteria were 1) histopathologically proven ccRCC and 2) RN performed between January 7, 2008 and December 23, 2009. Clinical information was collected from patient records.

Histological subtypes were reassigned according to 2014 EAU (European Association of Urology) guidelines.[18] TNM stage was reclassified according to the 2010 AJCC (American Joint Committee on Cancer) TNM classification.[19] Fuhrman grade and necrosis were reported according to the 2012 ISUP (International Society of Urological Pathology) consensus.[20] ECOG-PS was prospectively recorded and re-archived as previously described.[21] UISS, SSIGN and Leibovich scores were applied to all valid cases according to the original scoring algorithm.[22–24]

After surgery patients were evaluated by physical examination, laboratory studies, chest imaging and abdominal ultrasound or computerized tomography every 6 months for the first 2 years and annually thereafter. RFS was calculated from the date of surgery to the date of recurrence. Followup data were updated in March 2015.

### CD31-PAS Double Staining

Microarray development and immunohistochemistry were performed according to previously applied methods[25] using appropriate antibodies after control staining (anti-CD31 antibody, diluted 1:40, Code M0823, Dako, Glostrup, Denmark). Slides were stained with the Periodic Acid-Schiff (PAS) Kit (Sigma®) according to manufacturer instructions. PAS staining, hematoxylin and eosin staining and CD31 immunohistochemistry were used to evaluate the presence and extent of mimicry as previously described.[4,13,14] Samples of ccRCC with high VM formation served as the positive control (supplementary figure, http://jurology.com/). Negative control sections were treated with normal nonimmune IgG instead of with primary antibody (supplementary figure, http://jurology.com/).

Small vessel-like structures in the tumor that were PAS positive but CD31 negative were presumed to be VM channels, particularly if they contained red blood cells. All quantification was performed while blinded.

### Statistical Analysis

The chi-square or Fisher exact test was used for categorical variables. The t-test or Wilcoxon rank-sum test was used