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ORIGINAL ARTICLE

# Factor-inhibiting hypoxia-inducible factor expression in patients with high-risk locally advanced renal cell carcinoma and its relationship with tumor progression

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**Abstract** Hypoxia-inducible factor (HIF) plays an important role in renal cell carcinoma (RCC) associated with angiogenesis. Factor-inhibiting HIF (FIH), which is the upstream mediator protein of HIF, is receiving more attention today. In the present study, the role of FIH expression in high-risk locally advanced renal cell carcinoma (LARCC) was explored. Eighty-eight high-risk LARCC cases were divided into two groups based on their prognosis. Using immunohistochemical staining, the correlations of FIH expression along with clinicopathological factors, progression-free survival (PFS), and overall survival (OS) were analyzed. FIH was mainly located in the cytoplasm (34/88) and nucleus (31/88) of the renal tumor cell. Nuclear negative expression or cytoplasmic positive expression of FIH were associated with an increased risk of disease progression ($p = 0.007$ and $p < 0.001$, respectively) and worse OS ($p = 0.020$ and $p = 0.008$, respectively). Using the group with nuclear and cytoplasmic FIH negative expression as reference, further stratified analysis found that the exclusive nuclear FIH expression group had a better PFS and OS [hazard ratio (HR) = 0.153, $p = 0.07$ and HR = 0, $p = 0.961$, respectively], and the exclusive cytoplasmic FIH positive group experienced the worst PFS and OS (HR = 2.876, $p = 0.005$ and HR = 2.799, $p = 0.034$, respectively). In addition, nuclear negative expression of FIH was associated with a significant negative predictive value for the effect of interferon-alpha (IFN-$\alpha$) on PFS ($p = 0.045$). The nuclear negative and cytoplasmic positive expressions of FIH were identified not only as risk factors for disease progression in high-risk LARCC postoperative patients, but also to be associated with poor OS.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Furthermore, the nuclear negative expression of FIH may be a promising biomarker for postoperative adjuvant therapy.


## Introduction

Renal cell carcinoma (RCC) accounts for 80% of the carcinoma of the kidney and causes 102,000 deaths per year [1,2]. Based on the current clinical stage, about 70% of carcinoma patients can be diagnosed to have localized or locally advanced renal cell carcinoma (LARCC). Similar to other malignant tumors, surgical excision is usually considered the first treatment option. After radical or partial nephrectomy, nearly 20—40% of the cases with no original evidence of metastases experience recur rence and metastasis [3]. In the case of metastasis, the prognosis is poor and the 2-year survival rate is 20% [4]. These data suggest the importance of accurate means for forecasting tumor progression in individuals with LARCC.

Currently, UISS (UCLA Integrated Staging System), (the score based on stage, size, grade, and necrosis) SSIGN, and Leibovich algorithm [5—7] are the widely used integrated staging systems for predicting the outcome of patients with clear cell renal cell carcinoma (ccRCC). However, these systems are based on the tumor, lymph nodes, and metastasis staging system (TNM) classification issued by American Joint Committee on Cancer (AJCC) in 2002 [8]. Other variables, such as molecules, can also act as prognostic factors for evaluating the recurrence or progression of the disease. Examples of these include alfa-fetoprotein (AFP) levels in hepatocellular carcinoma [9], prostate-specific antigen (PSA) levels in prostate cancer [10], and so on. Although several molecules, including Neutrophil gelatinase-associated lipocalin (NGAL) [11], B7-H1 [12], and vascular endothelial growth factor (VEGF) [13], have been linked with the clinical outcomes of RCC, none of them have been used in clinical decision making.

As the hub in the signaling pathways for angiogenesis in solid tumorigenesis, VEGF is regulated by hypoxia-inducible factor (HIF), which is a heterodimer composed of $\alpha$ and $\beta$ subunits [13,14]. The level of $\alpha$ subunit in a tumor dictates HIF's function. Currently, there are two mechanisms that utilize it. Although the regulatory mechanisms are different, prolyl hydroxylase domain enzymes (PHDs) and factor-inhibiting hypoxia-inducible factor (FIH) act in accordance with HIF-1$\alpha$ expression [15,16]. Notably, FIH plays a more prominent role in inhibiting HIF-1$\alpha$ transcriptional activity [17].

Recently, a report stated that low nuclear expression of FIH is a strong independent prognostic factor for a poor overall survival (OS) in ccRCC [18]. The Leibovich algorithm is a prediction model to forecast the disease progression of ccRCC, based on tumor stage, regional lymph node status, tumor size, nuclear grade, and histologic tumor necrosis [7]. Patients undergoing radical nephrectomy can be stratified into three groups based on the risk for metastases according to Leibovich scores, and patients belonging to groups with high scores ($\geq$ 6) face greater risks than the others [7]. This study explores whether FIH expression is a useful factor in predicting high-risk LARCC progression after surgical resection.

## Materials and methods

### Patients and follow-up

Under the ethical guidelines and after gaining informed consent, pathological sections and associated clinical information were prospectively collected from 88 patients with RCC from 2001 to 2006. Patients with chromophobe RCC and collecting system tumor other than ccRCC were excluded. The median follow-up of the cohort was 59 months. According to the Leibovich integrated stratification system, all the cases were regarded to be at high risk for progression.

Detailed follow-up data were available for all the patients. On the final cutoff day, a total of 39 patients had disease progression and 21 patients died of cancer-related diseases. Demographic, clinical, and pathological data on age, sex, tumor site, primary size, TNM classification, clinical staging, Fuhrman grading, tumor necrosis, Leibovich score, and postoperative adjuvant therapy were collected in detail. Progression-free survival (PFS) and OS were the two final indicators in this study.

### Immunohistochemical analysis

Immunohistochemical staining was performed using a monoclonal antibody against FIH-1. Formalin-fixed, paraffin-embedded tissue sections (4 $\mu$m) of microarrayed ccRCC were dewaxed in xylene and then rehydrated in graduated ethanol solutions. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 45 minutes. The primary antibodies, namely, FIH-1 antibody (clone FIH162c, mouse IgG1 monoclonal antibody; ABCAM, Cambridge, MA, USA), were incubated for 16—18 hours at 4 °C. Phosphate-buffered saline was used as a negative control, and breast carcinoma tissue was used as a positive control.

Two pathologists, who were unaware of the relevant clinical data, viewed and scored the FIH expression independently. When the scores differed, disagreements were adjudicated by a third observer. The percentage of positively stained nuclei and cytoplasmic staining was assessed based on a semiquantitative scoring system. The scoring system for the percentage of stained nuclei was as follows: 0, $<$25% cells staining positive; 1, 30—50% positive cells; and 2, $>$50% positive cells. The scoring system for nuclei and cytoplasmic staining was the following: 0, negative; 1, weak; 2, moderate; and 3, strong. Tumor cells expressing FIH with intensity $\geq$2 and the percentage of stained nuclei $\geq$30% were defined as positive expressions.