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Poor prognosis and advanced clinicopathological features of clear cell renal cell carcinoma (ccRCC) are associated with cytoplasmic subcellular localisation of Hypoxia inducible factor-2α

Nils Kroeger [a,b,1], David B. Seligson [c,1], Sabina Signoretti [d], Hong Yu [c], Clara E. Magyar [c], Jiaoti Huang [c], Arie S. Belldegrun [a], Allan J. Pantuck [a,*]

[a] *Institute of Urologic Oncology, Department of Urology, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA, USA*
[b] *Department of Urology, University Medicine Greifswald, Germany*
[c] *Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles,CA, USA*
[d] *Department of Pathology, Dana Farber Cancer Institute, Harvard School of Medicine, Boston, MA, USA*

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Abstract ***Background:*** Pre-clinical studies have implicated hypoxia inducible factor (HIF)-2α as an important oncogene for clear cell renal cell carcinoma (ccRCC). Generally considered to act as a nuclear transcription factor, a recent study has also implicated HIF-2α as a protein translational initiation complex function within the cytoplasm (Uniacke et al., 2012). We hypothesised that both the absolute expression as well as the sub-cellular localisation of HIF-2α would predict clinicopathological features and cancer specific survival (CSS) in ccRCC.
Methods: A tissue microarray (TMA) study was conducted on three hundred and eight ccRCC patients. Survival differences were investigated with the log rank test and associations with CSS with uni- and multivariate Cox regression analyses. Recursive partition tree analysis was used to identify relevant cutoff values.
Results: High HIF-2α nuclear (N) (cutoff >32%) expression was associated with smaller tumour sizes ($p = 0.002$) and lower Fuhrman grades ($p = 0.044$), whereas tumours with high cytoplasmic (C) HIF-2α (>0%) more often had positive lymph nodes ($p = 0.004$), distant metastases

* *Corresponding author:* Address: Institute of Urologic Oncology, Department of Urology, David Geffen School of Medicine at UCLA, 924 Westwood Boulevard, Suite 1050, Los Angeles, CA 90095-7384, USA. Tel.: +1 310 206 2436; fax: +1 310 794 3513.
E-mail address: apantuck@mednet.ucla.edu (A.J. Pantuck).
[1] NK and DBS have contributed equally to this work.

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($p = 0.021$) and higher Fuhrman grades ($p < 0.0001$). After adjustment for TNM stage, Eastern Cooperative Oncology Group performance status (ECOG PS), and Fuhrman grade, both continuous ($p < 0.0001$) and dichotomised ($p < 0.0001$) HIF-2α C variables remained significant predictors of CSS, while neither HIF-2α N variable was retained.

Conclusion: Our investigation supports that HIF-2α may have a unique tumour promoter role in the cytoplasm. This preliminary finding justifies further experimental and mechanistic studies that examine the biological functions of HIF-2α when located in the cytoplasm.

1. Introduction

The hypoxia inducible factor (HIF) is a heterodimeric transcription factor consisting of an α- and a β-subunit. HIF-α is expressed and then degraded by the *von Hippel Lindau* (VHL)–ubiquitin ligase complex under physiological conditions. The complex of HIF-α and ARNT (HIF-1β) binds to co-activators (p300/CBP; PKM2) and the promoters of HIF-responsive genes leading to the transcription of proteins that are important for adaption to cellular hypoxia (e.g. VEGF, IGF, EPO, GLUT-1, and CAIX) [1].

The HIF-α family contains at least three different subunits with HIF-1α and HIF-2α being the best investigated members. Clear cell renal cell carcinomas (ccRCC) that have an inactivated *VHL gene* express either HIF-2α alone or both HIF-1α and HIF-2α [2]. In recent years, numerous pre-clinical investigations have suggested that HIF-2α rather than HIF-1α is the main tumour promoter in ccRCC [3–5]. Much effort has been spent to identify the key regulators that are responsible for the tumour promoter effects of HIF-2α. However, the full mode of action of HIF-2α has not yet been fully characterised.

In addition to serving as a well-characterised transcription factor, a recent study has implicated HIF-2α as a member of a protein initiation complex [6]. Authors have shown that protein expression under low oxygen conditions is initiated by a complex that contains HIF-2α, mRNA-binding protein 4 (RBM4) and eukaryotic translation ignition factor 4E type 2 (eIF4E2). This complex assembles on a hypoxia responsive element (rHRE) of messenger ribonucleic acid (mRNA) and targets mRNAs to polysomes for active translation. Yet, relatively few clinical studies have provided evidence for the crucial role of HIF-2α in ccRCC tumour progression, and therefore, the translational relevance of HIF-2α as a tumour promoter in ccRCC currently remains uncertain.

Protein translation is a biological process that occurs in the cytoplasm and transcription a process that occurs in the nucleus. Therefore, we hypothesised that both the absolute expression as well as the sub-cellular localisation of HIF-2α would predict clinicopathological features and cancer specific survival (CSS) in ccRCC.

2. Patients and methods

2.1. Patients

The study cohort was comprised of 308 ccRCC patients who were treated at the University of California Los Angeles (UCLA) between 1989 and 2000. Clinical and pathologic data were retrospectively gathered from the UCLA Institutional Review Board (IRB) approved kidney cancer database and electronic charts. Clinicopathological data included age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS) [7], TNM stages [8] and Fuhrman grades [9].

2.2. Immunohistochemistry

The sections were deparaffinised, rehydrated and heated in a pressure cooker to 125 °C for 30 s in EDTA for antigen retrieval. After cooling to room temperature, sections were incubated in 3% hydrogen peroxide (Dako, Carpinteria, CA) for 5 min to quench endogenous peroxidase. Sections were then incubated in avidin block for 15 min followed by incubation in biotin block for 15 min (Vector, Burlingame, CA). The sections were then incubated with serum-free protein block for 10 min (Dako, Carpinteria, CA). The primary antibody (clone UP15, kindly provided by Dr. William G. Kaelin, Dana-Farber Cancer Institute) was applied to sections for 1 h at 1:15,000 dilution. Detection was performed by incubation with Dako EnVision+ System HRP labelled polymer (Dako, Cat# K4003 and Cat# K4001) for 30 min followed by incubation with Biotin labelled tyramide (Perkin-Elmer, Cat #SAT700001EA) at a 1:50 dilution for 10 min. The slides were then incubated with LSAB2 Streptavidin-HRP (Dako, Cat # K1016) for 30 min. DAB chromogen (Dako, Cat # K3468) was then applied, and the slides were slightly counterstained with haematoxylin. Formalin-fixed paraffin-embedded cells with high (786-O-vector) or low (786-O-VHL) HIF2-alpha levels were utilised as positive and negative controls, respectively, to validate the specificity of the immunoassay. The immunohistochemical methods and the primary antibodies of other markers investigated in this study were described previously [10].