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Platinum Priority – Collaborative Review – Kidney Cancer

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Hypoxia, Hypoxia-inducible Transcription Factors, and Renal Cancer

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Abstract

Context: Renal cancer is a common urologic malignancy, and therapeutic options for metastatic disease are limited. Most clear cell renal cell carcinomas (ccRCC) are associated with loss of von Hippel-Lindau tumor suppressor (pVHL) function and deregulation of hypoxia pathways.
Objective: This review summarizes recent evidence from genetic and biological studies showing that hypoxia and hypoxia-related pathways play critical roles in the development and progress of renal cancer.
Evidence acquisition: We used a systematic search for articles using the keywords *hypoxia, HIF, renal cancer*, and *VHL*.
Evidence synthesis: Identification of the tumor suppressor pVHL has allowed the characterization of important ccRCC-associated pathways. pVHL targets α-subunits of hypoxia-inducible transcription factors (HIF) for proteasomal degradation. The two main HIF-α isoforms have opposing effects on RCC biology, possibly through distinct interactions with additional oncogenes. Furthermore, HIF-1α activity is commonly diminished by chromosomal deletion in ccRCCs, and increased HIF-1 activity reduces tumor burden in xenograft tumor models. Conversely, polymorphisms at the HIF-2α gene locus predispose to the development of ccRCCs, and HIF-2α promotes tumor growth. Genetic studies have revealed a prominent role for chromatin-modifying enzyme genes in ccRCC, and these may further modulate specific aspects of the HIF response. This suggests that, rather than global activation of HIF, specific components of the response are important in promoting kidney cancer. Some of these processes are already targets for current therapeutic strategies, and further dissection of this pathway might yield novel methods of treating RCC.
Conclusions: In contrast to many tumor types, HIF-1α and HIF-2α have opposing effects in ccRCC biology, with HIF-1α acting as a tumor suppressor and HIF-2α acting as an oncogene. The overall effect of VHL inactivation will depend on fine-tuning of the HIF response.
Patient summary: High levels of hypoxia-inducible transcription factors (HIF) are particularly important in the clear cell type of kidney cancer, in which they are no longer properly regulated by the von Hippel-Lindau protein. The two HIF-α proteins have opposing effects on tumor evolution.

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1. Introduction

Renal cell carcinoma (RCC) is the 14th most common malignancy and the third most common urologic cancer [1,2]. It has an age-standardized population incidence rate (per 100 000) of 15.8 in men and 7.1 in women in the European Union and mortality rates of 6.5 and 2.7, respectively [3]. Worldwide kidney cancer causes >100 000 deaths per year. A number of environmental risk factors for the development of renal cancer have been identified including smoking, obesity, hypertension, and diabetes [3]. Histopathologic classifications distinguish three major subtypes: clear cell RCC (ccRCC; 70–75%), papillary RCC (pRCC; 10–16%), and chromophobe RCC (chRCC; 5%) [4]. Each subtype is associated with a separate hereditary syndrome, and together they account for 2–3% of all RCC cases. ccRCC is associated with von Hippel-Lindau (VHL) disease, which also features development of hemangioblastomas and pheochromocytomas. Patients with familial mutations in the c-Met proto-oncogene have a high risk of developing type 1 pRCC, whereas patients with germline mutations in fumarate hydratase (FH) develop cutaneous and uterine leiomyomas and type 2 pRCC (hereditary leiomyomatosis and RCC [HLRCC]). chRCCs and other tumors of the kidney are seen in patients with the Birt-Hogg-Dubé syndrome, which is caused by mutations in the folliculin tumor suppressor gene. In addition, other genes such as tuberous sclerosis 1 or succinate dehydrogenase B are also associated with syndromes that predispose to the development of RCC [5]. In accordance with the Knudson hypothesis, persons with each syndrome have a hypomorphic germline mutation in one allele of the relevant tumor suppressor gene. Somatic inactivation of the remaining wild-type allele within the cancer cells then "exposes" this dysfunctional gene product.

The majority of sporadic ccRCCs have somatic inactivation of both *VHL* alleles with loss of function of the VHL tumor suppressor protein (pVHL). About 60–80% of ccRCC cases display loss-of-function coding mutations in the *VHL* gene, chromosomal aberrations on chromosome 3p25 that affect the *VHL* locus, or hypermethylation of the *VHL* promoter [6–8]. Re-expression of pVHL in VHL-defective RCC xenografts reduces tumor growth, confirming that *VHL* is a bona fide tumor suppressor gene [9].

The best-understood molecular function of pVHL is as the recognition component of an E3 ubiquitin ligase complex that targets proteins for proteasomal degradation by tagging them with ubiquitin [10]. Recently, ccRCC-associated mutations in *TCEB1*, which encodes for the elongin C component of the VHL E3 ligase complex, have also been described in ccRCC, suggesting that this function is important in its role as a tumor suppressor [11]. To date, the best-characterized targets of pVHL are the α-subunits of the hypoxia-inducible transcription factors (HIFs; HIF-1α and HIF-2α, also known as EPAS1). Oxygen-dependent hydroxylation of HIFs at specific proline residues by prolyl hydroxylase (PHD) enzymes triggers binding of pVHL, ubiquitination, and subsequent proteasomal degradation [12–15]. Consequently, when oxygen is abundant, HIF is rapidly degraded. In a hypoxic environment, hydroxylation is suppressed, and HIF-α escapes degradation to form dimers with the constitutively expressed HIF-1β isoform, also called the aryl hydrocarbon receptor nuclear translocator (ARNT) protein. This complex is imported into the nucleus and binds DNA at the hypoxia response elements to activate the transcription of a wide variety of genes [16,17]. Similarly, loss of function of pVHL also leads to stabilization of HIF-α and subsequent "pseudohypoxic" transcriptional responses; therefore, loss of pVHL in RCC is tightly associated with the activation of HIF and its transcriptional consequences.

Interestingly, activation of HIFs also has been described in the context of the other major RCC subtypes [18–20]. The loss of FH or succinate dehydrogenase B, for example, leads to increased levels of the Krebs cycle intermediates fumarate or succinate, respectively, which in turn inhibit PHD-mediated hydroxylation of HIF-α by competing with the cosubstrate 2-oxoglutarate [18,20,21]. In addition, many types of tumors frequently outgrow their blood supply, generating hypoxic regions that can activate HIF in both tumor and stromal cells, despite a functional degradation apparatus [22].

This review summarizes current knowledge of the effects of hypoxia and HIF in the context of RCC biology.

2. Evidence acquisition

A systematic literature search in PubMed was conducted using the keywords or phrases *hypoxia, HIF, VHL, renal cancer*, and *kidney cancer*. Articles were selected by relevance and the novelty of their findings. In addition, key publications in the field of hypoxia research were added.

3. Evidence synthesis

3.1. VHL and hypoxia-inducible transcription factors

Loss of function of VHL due to gene deletions, inactivating mutations, or epigenetic silencing is observed in the vast majority of both familial and sporadic ccRCCs [6,8,23]; however, the inherited RCC syndrome VHL disease may be subclassified according to the relative risk of different types of tumor (Fig. 1). Type 1 VHL syndrome is associated with ccRCC and hemangioblastoma, whereas patients with type 2 VHL syndrome develop pheochromocytomas. Type 2 VHL syndrome is subdivided into type 2a (pheochromocytomas and hemangioblastomas but low risk of ccRCC), type 2b (pheochromocytomas, hemangioblastomas and ccRCC), and type 2c (pheochromocytomas only).

These phenotypes were found to have distinct VHL genotypes that had disparate effects on the HIF pathway [24–26]. Specifically, all ccRCC-associated mutations caused complete HIF dysregulation, whereas those associated with pheochromocytoma alone regulated HIF normally (although pheochromocytoma and paraganglioma have since been associated with somatic activating mutations at the *EPAS1* locus). Although this correlation between ccRCC risk and HIF dysregulation is not absolute (some HIF dysregulation was seen with type 2a mutations at low risk

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