



Review Article

## Role of Von Hippel-Lindau gene mutation in Renal Cancer

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### Abstract

Renal cancer, specifically called renal cell carcinoma in the case of VHL disease, is a tumor of the renal cortex. It is the most common type of kidney cancer. The tumors associated with VHL inactivation are generally vastly vascular and are known to overproduce angiogenic factors such as vascular endothelial growth factor (VEGF). Besides, studies show that renal cell carcinoma is also linked with paraneoplasticerythrocytosis due to the overproduction of erythropoietin (Epo). Both VEGF and Epo are prototypical hypoxia-inducible mRNAs (i.e. their induction mostly occurs under the conditions illustrated by scarce oxygenation). The above contemplations have led to the sighting that the cells who lack pVHL (VHL protein) constitutively results in overproduction of hypoxia-inducible mRNAs and the restoration of pVHL function results in down-regulation of hypoxia-inducible mRNAs in the presence of oxygen. Thus, overproduction of hypoxia-inducible mRNAs is an attribute of pVHL-defective cells. Most genes, regulated by hypoxia, such as VEGF and Epo are under the control of a transcription factor called hypoxia-inducible factor (HIF). HIF is known to alter many target genes when it has been stabilized due to the dearth of pVHL. HIF plays an essential role in pVHL-defective tumor formation. This fact has led to an increase in the likelihood that the drugs which are intended against HIF or its downstream objectives might, in the near future, play a part in the management of renal cell carcinoma. Besides, many drugs have been developed that aim at HIF-responsive gene products. Many of these therapies have exhibited noteworthy doings in the clinical trials of renal cancer and indicate substantive progresses in the conduction, treatment and management of this disease.

**Keywords:** renal cell carcinoma. Von Hippel-Lindau gene, vascular endothelial growth factor.

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### 1. Introduction

Von Hippel-Lindau Syndrome (VHL) is a hereditary autosomal disorder characterized by its susceptibility to various forms of bilateral and multi-centric cancers, mainly including retinal

hemangioblastoma, CNS hemangioblastoma, renal cell carcinoma, pheochromocytoma, islet cell tumors of the pancreas, and endolymphatic sac tumors, as well as cysts (fluid-filled sacs) in the kidney, pancreas and epididymis [29].

The somatic mutation of the VHL gene has been associated with the development of renal cell carcinoma (RCC). The VHL gene is located (at chromosome 3p26-25, as mentioned later in the context) at a region of the genome which is frequently deleted or altered in RCC [26].

### Types of renal cell carcinoma

Pathologically, based on the genetic changes associated in kidney tumor formation, renal cell carcinoma is classified into 5 various types:

(1) **Clear cell carcinoma:** - It is the most common type of renal cell carcinoma accounting for upto 70% of the cases. It is also known as non-papillary carcinoma. Clear cell renal cell carcinoma is characterized by malignant epithelial cells having clear cytoplasm and a compact-alveolar or acinar pattern of growth. It originates from the proximal tubule of the nephrons. The cytogenetic abnormalities associated with it are the deletions of chromosome 3p and mutations of the VHL (tumor suppressor) gene.

(2) **Papillary cell carcinoma:** - It is also known as chromophil cell carcinoma. It accounts for 15% cases of renal cell carcinoma. It is associated with multi-focal papillary renal tumors. It originates from the proximal tubule of the nephrons. The cytogenetic abnormalities associated with it are trisomies of the chromosomes 3q, 7, 12, 16, 17 and 20 and loss of Y chromosome.

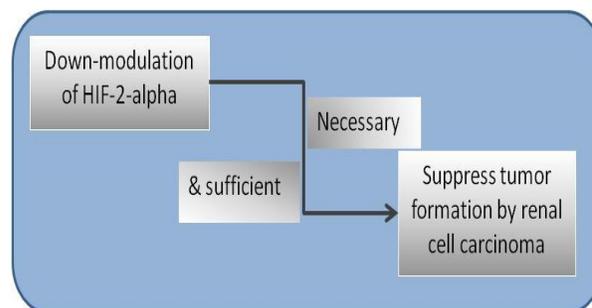
(3) **Chromophobe cell carcinoma:** - It accounts for 5% cases of renal cell carcinoma. It originates from the intercalated cells of the collecting ducts of the cortex. The cytogenetic abnormalities associated with it are the monosomies of chromosomes 1, 2, 6, 10, 13, 17 and 21 and hypodiploidy.

(4) **Collecting duct carcinoma:** - It accounts for less than 1% cases of renal cell carcinoma. It originates from the intercalated cells of the collecting ducts of the cortex. Demonstration of the origin of the tumor in the collecting ducts is the main reason of difficulty that arises while allotting any tumor to this subcategory.

(5) **Unclassified cell type:** - It accounts for upto 5% cases of renal cell carcinomas. The tumors which do not fall under any of the above mentioned categories are put under this category. Some of the characteristics of the cells which have been assigned to this category are: sarcomatoid cells having unrecognizable epithelium, mucin production, mixture of epithelial and stromal elements, etc [2].

The frequency of deaths attributable to renal cell carcinoma is quite large in number. Higher rates of epidemiology of RCC are reported for men as compared to women. The range of ratios of male-to-female varies from 1.5:1 to 2:1 [1].

At hand is an apparent data which implies that HIF is a significant downstream target with respect to the development of renal cell carcinoma. RCC is persuaded to produce HIF-2-alpha variants which escape recognition by pVHL (on account of the elimination of the related prolyl hydroxylation sites) and are accustomed to the tumor suppressor effects of pVHL. Similarly, the cells that generate a decoy protein that binds to the HIF-binding site within pVHL are prone to it. Conversely, elimination of HIF-2-alpha in VHL-/- renal cell carcinoma is sufficient to suppress their ability to form tumors within the cells [13]. Hence,



### VHL gene

Von Hippel-Lindau gene is a tumor suppressor gene (which means that it forbears cells from growing and dividing too quickly and also promotes cell death) and is E3 ubiquitin protein ligase. It is located on chromosome 3 at position

p26-p25 [25]. It is a protein-coding gene. Studies show that it also has an elemental role in the regulation of the biological pathways involved with normal growth of the blood vessels. Apart from the germ cells (sperm and egg cells), every cell of the body has two working copies of the VHL gene. In order to develop cancer, both copies of this gene must have to be mutated or altered. In Von Hippel-Lindau syndrome, the first mutation is a germline mutation (i.e., the first mutation is inherited from one of the parents and is thus present in all the cells of the body) while the second mutation occurs in the person's lifetime and location where the tumor or tumors will develop depends in the location of the cells in which the second mutation has occurred. So, if the second mutation in the VHL gene occurs in the kidneys, it would result in renal cell carcinoma [4, 26-29].

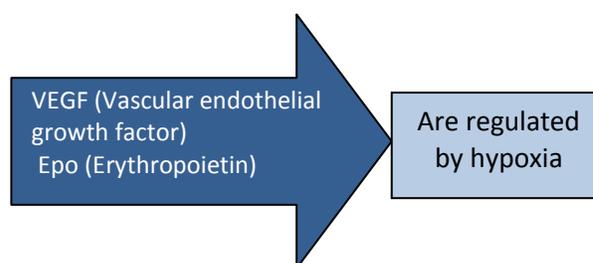
### The VHL protein: pVHL

Biochemical studies suggest that the protein encoded by the mRNA of the VHL gene is a constituent of a multi-protein complex which comprises of elongin B, elongin C and cullin – 2. Later, Rbx1 (also known as ROC1 or Hrt1) was also discovered to be a part of it. The VHL gene protein contains 213 amino acid residues and its perceptible molecular weight drifts from around 24 to 30 kDa [24]. It performs numerous functions associated with tumor suppression but the one which affects the development and growth of renal cell carcinoma is the ubiquitination and reticence (inhibition) of the hypoxia-inducible factor (HIF), which is a transcription factor that plays a vital role in the regulation of gene expression by oxygen [22]. The VHL protein (pVHL) is mostly found in two isoforms – VHL<sub>30</sub> and VHL<sub>19</sub>. – Both of which are capable of suppressing RCC growth in vivo. For the sake of easiness generically, both the isoforms are collectively known as pVHL. The pVHL shuttles back and forth between the nucleus and cytoplasm (but has also been found in association with mitochondria and endoplasmic reticulum). This act of shuttling by

the pVHL is vital in its tumor suppressing action. According to some researchers, VHL<sub>30</sub> chiefly inhabits the cytoplasm while VHL<sub>19</sub> is mostly found in the nucleus. This may be summed up to conclude that even if their functions seem to be similar, they are definitely not identical [23].

### HIF factor

Hypoxia-inducible factor (HIF) is made up of two non-identical subunits. One of these is known as an alpha subunit and the other one as beta subunit. HIF has the tendency to bind to certain DNA sequences and thereby activating the transcription. It controls many genes, such as



Further there are three alpha HIF genes and three beta HIF genes in the human genome. All the beta HIF family members are stable proteins whereas alpha HIF proteins are highly unstable (except in the absence of oxygen). Hence, the formation of an active HIF heterodimer is restricted to hypoxic conditions [3].

### Role of pVHL

The VHL tumor suppressor protein (pVHL) plays a pivotal role in the oxygen-sensing pathway which occurs in the mammals in the course of the oxygen-dependent polyubiquitylation (a post-translational modification) of hypoxia-inducible factor (HIF). In simpler words, it is explained as follows: -

pVHL has the ability to target specific proteins for their destruction. It forms stable complexes with other proteins (viz. elongin B and C, Cul2 and Rbx1). These complexes have the capability to direct the covalent connection of polyubiquitin tails to specific proteins which are

to be degraded by the proteasome. One such target of pVHL is hypoxia-inducible factor HIF) [5]. It post-transcriptionally controls the accretion (accumulation) of hypoxia-inducible mRNAs under normoxic conditions (i.e. when oxygen is available in its normal atmospheric concentration viz. 21%). This interface between pVHL and HIF is reigned and managed by post-translational prolyl hydroxylation of the hypoxia-inducible factor (in the presence of oxygen) by a conserved family of Egl-nine (EGLN) enzymes [17]. However, in the absence of pVHL, stabilization of HIF occurs and it is now eligible to persuade the expression of its target genes(out of which many are essential regulators of angiogenesis, cell growth or cell survival) because the cells lacking pVHL fail to degrade HIF-alpha subunits in the presence of oxygen [15-16]. The stabilization of HIF-alpha, usually occurring under hypoxic conditions, implies that the binding of pVHL to HIF-alpha is dependent on the availability of oxygen. HIF1-alpha (the oxygen dependent degradation domain-ODD) has to undergo an oxygen-dependent post-translational alteration in order to be recognized by the pVHL. This alteration is called polyubiquitylation of HIF [6-8].

**Renal cell carcinoma tumor formation**

Within the cells which lack pVHL, or in a condition of scarce availability of oxygen, HIF is free to accumulate. This activates the transcription of a cadre of genes involved in short-term and long-term adaptation to hypoxia. These genes include the ones which control various activities given below:-

These considerations explain the frequent overproduction of HIF, as well as its downstream targets, in renal cell carcinoma [9-11].Inspection and assessment of the kidneys of the patients of VHL disease have revealed the presence of both preneoplastic renal cysts and renal cell carcinomas. Immunohistochemical and genetic evaluation of the epithelial cells lining these renal cysts confirm early loss of VHL and over-expression of HIF and HIF-target genes such as

Action of genes	Examples
Control of uptake of glucose and its metabolism	Glut1 glucose transporter as well as some glycolytic enzymes
Control the extracellular pH	Carbonic anhydrase IX
Control angiogenesis	Vascular endothelial growth factor (VEGF)
Control mitogenesis	Transforming growth factor-alpha (TGF-alpha) and platelet-derived growth factor B (PDGF-B)

VEGF. The same (over-expression of HIF-responsive growth factors as well as their receptors) were found to be a feature of renal carcinomas as well [11].

Thus, in any case for renal carcinoma, VHL appears to be acritical gatekeeper tumor suppressor gene, and it is acknowledged that additional genetic modifications or alterations are needed for progression of pre-neoplastic renal cysts to renal carcinomas. Nonetheless, restoration of pVHL function in VHL-/renal carcinoma cells is sufficient to prevent them from growing as tumors within the cells [12]. This further suggests that genetic and epigenetic alterations successive to biallelic VHL inactivation do not leave renal carcinoma cells insensitive to pVHL function and establishes that anything that could imitate the effect of pVHL might be therapeutically useful in this disease [14].

For getting more details regarding the role of HIF in VHL-/tumors, two different studies separately swotted that whether HIF-alpha variants, that escape recognition by pVHL, could effectively neutralize the tumor suppressor activities of pVHL or not. Although HIF1-alpha was capable of affecting cell growth behavior in vitro, only HIF2-alpha and not HIF1-alpha was able to override tumor suppression by pVHL in vivo. Thus, down-

regulation of HIF2-alpha is necessary for tumor suppression by pVHL in kidney cancer [13]. Interestingly, examination of kidneys from VHL patients likewise suggests that HIF2-alpha is more oncogenic than HIF1-alpha [18].

Although it has not been formally proven that inhibition of HIF is sufficient to alter the growth of VHL-/-tumors, it seems reasonable (after the above considerations) to treat renal cell carcinoma with agents that block HIF or its downstream targets. As mentioned above, inhibition of HIF2-alpha is sufficient to suppress VHL-/-tumor growth within the cells [12-13]. A number of agents that inhibit VEGF are currently undergoing preclinical and clinical testing. Among these are drugs that prevent the receptors for the growth factors from transmitting signals following ligand binding [21].

## Conclusion

For the period of the last ten years, the various scientific studies regarding VHL has provided us with imminent and approaches hooked on with not only the molecular basis of the VHL disease but have also led to the detection of the decisive components of the oxygen-sensing pathways of the mammal. The significance of HIF concerning pVHL-defective tumor formation is already presenting a foundation for examining small molecule inhibitors of HIF-responsive growth factors as probable treatments for renal cell carcinoma [19-20].

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