FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Review

The multifaceted von Hippel–Lindau tumour suppressor protein

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ARTICLE INFO

Article history:
Received 16 January 2014
Revised 14 February 2014
Accepted 17 February 2014
Available online xxxx

Edited by Shairaz Baksh, Giovanni Blandino and Wilhelm Just

Keywords:
von Hippel–Lindau protein (pVHL)
Clear-cell renal cell carcinoma (ccRCC)
Hypoxia-inducible factor (HIF)
Epigenetics

ABSTRACT

Loss of von Hippel–Lindau protein (pVHL) is known to contribute to the initiation and progression of tumours associated with VHL disease as well as certain sporadic tumours including clear cell renal cell carcinoma (ccRCC). The *VHL* gene was first identified and cloned over 20 years ago and our understanding of its functions and effects has significantly increased since then. The best-known function of pVHL is its role in promoting the degradation of hypoxia-inducible factor α subunit (HIFα) as part of an E3 ubiquitin ligase complex. HIF stabilisation and transcriptional activation are also associated with various epigenetic alterations, indicating a potential role for VHL loss with changes in the epigenome. This review will highlight current knowledge regarding pVHL as well as discuss potentially novel roles of pVHL and how these may impact on cancer progression.

1. von Hippel–Lindau (VHL) disease and gene

The first indication of a tumour suppressor role for von Hippel–Lindau (*VHL*) gene was the segregation of the mutant or loss of *VHL* allele in kindreds with VHL disease. The disease was first described in the early 1900s and is named after Eugen von Hippel and Arvid Lindau. von Hippel described a family with highly vascularised tumours of the retina [1], whilst Lindau reported that these retinal tumours commonly occurred alongside lesions of the central nervous system [2]. The disease is now known to be a hereditary cancer syndrome that affects approximately 1 in 35,000 individuals. Patients with VHL disease are at a high risk of developing benign tumours most commonly found in the central nervous system (haemangioblastoma), retina (angioma) and adrenal glands (phaeochromocytoma), as well as malignant tumours of the kidney (clear-cell renal cell carcinoma; ccRCC). Although less frequent, a variety of other benign tumours are also associated with the disease including tumours of the pancreas, inner ear and bilateral papillary cystadenoma of the epidydimus in men or broad ligament in females. Despite a wide range of pathological outcomes, ccRCC is the most frequent cause of morbidity and mortality amongst these patients.

The disease is a result of germline mutations of *VHL*. Patients are heterozygotes for *VHL* with one wild-type and one defective allele. Somatic inactivation of the second functional allele in susceptible cells, and therefore loss of VHL function, leads to pathological features of disease. VHL can be described as a classic tumour suppressor gene as this outcome is in line with Knudson's two hit model of tumourgenesis, whereby tumour suppressors are recessive at the genetic level and require somatic inactivation of the remaining wild-type allele to achieve tumourgenesis [3].

2. VHL and sporadic cancer

While VHL is widely expressed in human tissue, its loss is not exclusive to VHL disease. Certain sporadic cancers are strongly associated with VHL mutation [4]. Biallelic inactivation of *VHL*, due to mutation, loss or hypermethylation, is the most frequent genetic mutation in sporadic ccRCC. ccRCC is the most common form of kidney cancer, accounting for more than 70% of all RCC cases and is often characterised by loss of chromosome 3p events [5]. The prevalence of mutated VHL is study dependent, but it is estimated that between 60–80% of sporadic ccRCC display VHL mutations [6–8]. Thus, the majority of sporadic and hereditary (VHL disease-associated) ccRCC lacks functional VHL due to loss or mutation of the *VHL* gene. VHL loss is also associated with sporadic cerebellar haemangioblastomas with prevalence between 25–50% [9,10].

$VHL^{-/-}$ mice die *in utero* due to defective placental dysgenesis [11], but targeted cell specific suppression of *VHL* is possible. In li-

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http://dx.doi.org/10.1016/j.febslet.2014.02.026
0014-5793/

ver cells of mice, *VHL* suppression forms benign tumours that are highly vascular in nature [12], while intriguingly targeted *VHL* inactivation in renal proximal tubule epithelial cells and pancreatic endocrine cells results in a polycystic, pre-cancerous, pathology in murine models [13,14]. In further support of its role as a tumour suppressor gene, reintroduction of wild-type VHL into $VHL^{-/-}$ ccRCC cell lines prevents RCC cells from forming tumours in nude mice [15].

3. VHL protein (pVHL)

Located on the short arm of chromosome 3 (3p25-p26), the VHL tumour suppressor gene was first identified and cloned in 1993 [16]. The gene, which contains 3 exons, encodes a protein of 213 amino acid residues, specifically known as $pVHL_{30}$. A second wild-type isoform of 160 amino acid residues is also expressed in human cells. $pVHL_{19}$ arises from alternate translation initiation at a second AUG codon (codon 54) within the VHL open reading frame [17]. Both forms are known to display tumour suppressor abilities. Reintroduction of RCC cells with either $pVHL_{19}$ or $pVHL_{30}$ inhibits tumour development in mouse xenograft models of the disease [18]. Although both are expressed in human cells, $pVHL_{19}$ is often the more prominent form in human tissue. Interestingly VHL isoforms are also located in different compartments of the cell. While VHL_{19} is equally distributed in the nucleus and cytoplasm, VHL_{30} is found predominantly in the cytoplasm [17], suggesting that under certain circumstances they display distinct roles. However, for the remainder of this review, unless otherwise stated, both protein forms of VHL will be referred to as pVHL.

4. pVHL functions

pVHL displays no enzymatic activity, but it is known to have multiple binding partners. The protein comprises of an α and β subunit. The α-domain serves as a binding site, whereas the β-domain plays important roles in substrate recognition. Investigations into the binding partners of the protein reveal its vast array of functions, many of which are relevant to its role as a tumour suppressor protein. In addition, categorising VHL patients based on disease outcome supports the notion that the tumour suppressor roles of pVHL are diverse. Specific mutations place patients at a higher risk of developing specific tumours. Patients with type 1 VHL disease display haemangioblastoma with a low risk of phaeochromocytoma and ccRCC while type 2 patients, who also display haemangioblastoma, have a high risk of developing phaeochromocytoma. Type 2 is further subdivided into 2A (low risk of ccRCC), 2B (high risk of ccRCC) and 2C who develop phaeochromocytoma only. Taking these observations into account, research to date has led to multiple discoveries about the precise functions of pVHL. It can therefore be described as an adapter protein with both post-translational as well as transcriptional effects.

Ubiquitylation represents an efficient mechanism of tagging proteins for degradation by the 26S proteasome. Ubiquitylation of proteins is accomplished by the actions of a common ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin-ligating enzyme (E3 ligase). VHL forms a multi-protein complex with elongins B and C, cullin 2 and Rbx-1 [19–21]. This complex is structurally similar to the yeast multicomplex, SCF (Skp1/Cdc53/F-box protein). Like the SCF complex, the ECV (elongin/culin/VHL) complex displays E3 ubiquitin ligase activity, which acts to polyubiquitylate protein substrates. Within this complex, pVHL acts as a substrate recognition subunit [22]. The most extensively studied and arguably most important protein target of pVHL-mediated ubiquitylation is the hypoxia-inducible factor (HIF) family of transcription factors, which will be discussed in more detail in the following sections. However, other protein targets of the ECV have also been identified. These include certain isoforms of protein kinase C (PKC) [23], proposed to be of particular importance in the regulation of c-Jun dependent apoptosis of neurons that are potential precursors of phaechromocytoma [24]. The ECV also targets the hyperphosphorylated form of Rpb1, a subunit of RNA polymerase II, which is activated during UV radiation and associated with stress-induced transcription [25].

Not all proteins that pVHL binds to results in polyubiquitylation, indeed certain *VHL* mutations associated with cancer pathogenesis are known to display normal ubiquitylation function, including ubiquitylation of HIF. For example type 2C pVHL mutants appear to retain the ability to polyubiquitylate HIF, but still have a heightened likelihood of developing phaeochromocytoma [26]. Therefore, mutations such as these are likely to be promoting cancer progression independent of the VHL/HIF axis and the ECV complex. In support of this investigations into alternate functions of pVHL provide a diverse array of roles for this protein. pVHL assists in regulation of the extracellular matrix (ECM), where its loss in this context is proposed to promote angiogenesis by allowing vessels to easier infiltrate tumours. pVHL and fibronectin, a glycoprotein that interacts with integrin proteins to regulate the ECM, are known to bind [27]. $VHL^{-/-}$ cells secrete higher levels of fibronectin but the assembly of this fibronectin as part of the ECM is disorganised. Loss of the pVHL-fibronectin interaction is therefore associated with defective ECM formation. This is reversed upon reintroduction of wild type pVHL [27]. More recently this has been shown to be related to decreased RhoA GTPase signalling in $VHL^{-/-}$ renal cancer cells [28]. pVHL also binds to collagen IV alpha 2 (COL4α2) [29]. In this context *VHL* loss is associated with a loss of COL4α2 from the ECM [30], further deregulating the ECM architecture in the $VHL^{-/-}$ tumour environment.

Microtubules are crucial for the maintenance of cell shape and polarity, and in addition, form the mitotic spindle during cell division. pVHL associates and binds to microtubules and inhibits their depolymerisation [31]. In mammalian cells, Thoma et. al. demonstrated that pVHL localises to the mitotic spindle and that loss of this protein resulted in, amongst other things, chromosomal instability, a classic feature of cancer cells [32]. Microtubules are also essential for cilia maintenance and therefore linked to this function of pVHL is the role of the protein in cilia maintenance. Cilia are of great importance in renal epithelial cells where primary cilia play an important role in the development as well as maintaining the integrity of nephrons. pVHL loss disrupts cilia formation in mouse inner medullary collecting duct kidney cells where pVHL is needed to direct the growth of microtubules toward the cell periphery, a function that have been proven to be vital for cilia formation [33]. Likewise, human $VHL^{-/-}$ cells do not have cilia and reintroduction of pVHL into these cells results in cilia formation. Loss of cilia in adult kidney cells due to pVHL dysfunction promotes the development of renal cysts, indicative of a pre-cancerous pathology.

VHL loss in ccRCC is also associated with genomic instability, a prominent feature in multiple cancer cells. $pVHL_{19}$ is found in the nucleus [17], suggesting a nuclear relevant role for this isoform. Recently functions for pVHL in the DNA damage response have been reported. Upon DNA damage, $VHL^{-/-}$ cells display attenuated apoptosis or abnormal cell-cycle arrest, but when pVHL is restored this response is normal [34]. Roe et. al. reported that pVHL destabilises Skp2 protein, an integral component of the Skp, Cullin, F-box-containing complex that promotes DNA synthesis in the S phase [35]. The transcription factor E2F1 is also up-regulated as part of the DNA damage response. Wei et. al. reported a feedback loop wherein pVHL regulates E2F1 activity which in turn regulates pVHL expression [36]. Recently, a possible physiologic role for pVHL in the DNA damage response was revealed whereby suppres-

Please cite this article in press as: Robinson, C.M. and Ohh, M. The multifaceted von Hippel–Lindau tumour suppressor protein. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.02.026