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Review

# The von Hippel–Lindau protein, HIF hydroxylation, and oxygen sensing

William G. Kaelin Jr. *

*Howard Hughes Medical Institute, Dana-Farber Cancer Institute and Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA*

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**Abstract**

The heterodimeric transcription factor HIF (hypoxia-inducible factor), consisting of a labile α-subunit and a stable β-subunit, is a master regulator of genes involved in acute or chronic adaptation to low oxygen. Studies performed over the past 5 years revealed that HIFα-subunits are enzymatically hydroxylated in an oxygen-dependent manner. Hydroxylation of either of two conserved prolyl residues targets HIFα for destruction by a ubiquitin ligase containing the von Hippel–Lindau tumor suppressor protein whereas hydroxylation on a C-terminal asparagine affects HIF transactivation function. Pharmacological manipulation of HIF activity might be beneficial in diseases characterized by abnormal tissue oxygenation including myocardical infarction, cerebrovascular disease, and cancer.
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Oxygen plays a pivotal role in metazoan biology and alterations in tissue oxygenation are a hallmark of many human diseases including myocardial infarction, cerebrovascular accidents, peripheral vascular disease, and cancer. It is therefore important to understand how metazoan cells sense and respond to changes in oxygen availability. Two lines of experimentation, one related to the rare hereditary cancer syndrome called von Hippel–Lindau disease and one related to a hypoxia-inducible transcription factor called HIF (hypoxia-inducible factor), converged over the past 10 years to provide a more complete understanding of this process. One anticipated dividend of this knowledge will be the ability to manipulate the hypoxic response for therapeutic benefit in man with drug-like molecules.

## von Hippel–Lindau disease

Patients with what is now called von Hippel–Lindau (VHL) disease were first described in the medical literature ~100 years ago [1–3]. This disease is characterized by an increased risk of blood vessel tumors called hemangioblastomas of the central nervous system and retina, clear cell renal carcinoma, and pheochromocytoma, in addition to other tumors [4]. The von Hippel–Lindau susceptibility gene, *VHL*, resides on chromosome 3p25 and acts as a tumor suppressor [5,6]. Individuals with VHL disease are *VHL* heterozygotes, harboring one wild-type allele and one defective allele [6]. Pathological changes arise when the remaining wild-type *VHL* allele is inactivated or lost in susceptible cell. Biallelic *VHL* inactivation is also common in sporadic (non-hereditary) hemangioblastomas and clear cell renal cancers [7].

The *VHL* gene encodes two proteins due to alternative translation initiation [8–10]. For simplicity both proteins are generically referred to as 'pVHL' since their functions appear, at the present time, to be similar. Restoring wild-type pVHL function in $VHL^{-/-}$ human renal carcinoma cells is sufficient to suppress their ability to form tumors in nude mice assays [11,12]. This activity is tightly correlated with pVHL's ability to form stable complexes with elongin B, elongin C, Cul2, and Rbx1, which resemble so called SCF ubiquitin ligases (Skp1, Cdc53 or Cullin, F-box protein) [13–19]. The idea that pVHL might play a role in polyubiquitination was supported by the demonstration that anti-pVHL immunoprecipitates contain ubiquitin

* Fax: +1 617 632 4760.
*E-mail address:* William_kaelin@dfci.harvard.edu.

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ligase activity [20,21] and by the crystal structure of pVHL bound to elongin B and C [22]. This structure confirmed the predication of Elledge and co-workers [23] that elongin C would structurally resemble Skp1 and that pVHL would be similar to an F-box protein. Moreover, it made clear that VHL-associated mutations cluster in two discrete areas of the protein called the α-domain and the β-domain [22]. The former had been shown earlier to bind to the elongins (and hence nucleate the complex) [13,17,24] whereas the latter had features of a substrate docking site. Identification of a potential substrate was aided tremendously by consideration of the clinical features of VHL-associated neoplasms.

Hemangioblastomas, clear cell renal carcinomas, and pheochromocytomas sometimes secrete erythropoietin, leading to excessive production of red blood cells (polycythemia) [25]. In addition, hemangioblastomas and clear cell renal carcinomas are highly vascular tumors, due at least partly to VEGF overproduction [26–30]. Erythropoietin and VEGF are normally induced by hypoxia (the former to increase blood oxygen carrying capacity and the latter to increase blood delivery). In short, VHL-associated neoplasms behave as though they are constitutively hypoxic. This led to the hypothesis that the *VHL* gene product, pVHL, might be involved in the mammalian oxygen sensing pathway [11,31]. This idea was strengthened by the observation that pVHL-defective tumor cells, in contrast to their wild-type counterparts, exhibit hypoxia-inducible mRNA levels (such as for the mRNA encoding VEGF) that are appropriate for hypoxic cells irrespective of actual oxygen availability [12,31–33]. In short, the uncoupling of hypoxia-inducible gene expression from oxygen availability is a molecular signature of pVHL inactivation.

## HIF

Many such hypoxia-inducible mRNAs are under the control of a sequence-specific DNA-binding transcription factor that is generically referred to as HIF (hypoxia-inducible factor) [34,35]. HIF is a heterodimer consisting of a labile α-subunit and a stable β-subunit. There are three HIFα genes (HIF1α, HIF2α, and HIF3α) in the human genome. HIF1α and HIF2α have been more thoroughly investigated with respect to activation of HIF target genes. HIF3α is less well studied and encodes at least one splice variant that is dominant-inhibitory to HIF called IPAS [36,37]. HIF1α is ubiquituously expressed whereas HIF2α expression is more restricted (nonetheless, HIF2α is frequently detected in tumor cells along with HIF1α) [38,39]. There are three HIFβ genes (also called Aryl Hydrocarbon Nuclear Translocators or ARNTs). HIF1α bound to HIF1β represents the canonical form of HIF.

Both HIFα and HIFβ proteins belong to the basic helix–loop–helix PAS family of transcription factors. HIF1α and HIF2α contain, in addition to dedicated DNA and dimerization binding domains, two transactivation domains referred to as the NTAD (N-terminal transactivation domain) and CTAD (C-terminal transactivation domain). Consequently, these two proteins can activate transcription when bound to DNA in a complex with a HIFβ family member. HIF activates a battery of genes involved in acute or chronic adaptation to hypoxia including genes involved in glucose uptake and metabolism, extracellular pH control, angiogenesis, erythropoiesis, mitogenesis, and apoptosis [40]. A recently identified HIF target, Redd1 (also called RTP801), appears to shut down protein translation as a means of conserving ATP [41,42]. There is increasing evidence that the sets of genes regulated by HIF1α and HIF2α are overlapping but non-identical. For example, HIF1α appears to be particularly important for activating genes important for anaerobic glycolysis [43–45].

Earlier studies showed that the hypoxic induction of HIF was due primarily to stabilization of the HIFα moiety under low oxygen conditions and mapped this oxygen dependence to a region overlapping with the NTAD sometimes referred to as the ODD or ODDD (oxygen-dependent degradation domain) [46–50]. In 1999, Maxwell et al. [51] documented that HIFα was not degraded in $VHL^{-/-}$ tumor cells and that HIFα and pVHL could bind to one another. Subsequently, it was shown that pVHL binds directly to the ODD via its β-domain and directs the polyubiqutination of HIFα, which earmarks it for destruction by the proteasome (Fig. 1) [52–55]. These studies therefore provided a mechanistic explanation for the overproduction of hypoxia-inducible mRNAs in pVHL defective tumors.

## Oxygen-dependent prolyl hydroxylation of HIF

The interaction between pVHL and HIFα is oxygen dependent because the latter must be hydroxylated on one of two conserved prolyl residues within the ODDD in order to be recognized by pVHL (hydroxylation of either residue is sufficient to create a pVHL-binding site) [56–62] (Fig. 1). This reaction is intrinsically oxygen dependent (the oxygen atom of the hydroxyl group is derived from molecular oxygen) and is catalyzed by members of the EglN family (also called PHD or HPH family), which belong to a superfamily of iron and 2-oxoglutarate dependent dioxygenases [63–68]. Under normal circumstances EglN1 (also called PHD2) appears to be the primary HIFα hydroxylase, although other members of the family likely become important after prolonged periods of hypoxia [69–73]. The oxygen $K_m$ for the EglNs is approximately ~200 μM [68]. Accordingly, hydroxylation of HIFα by EglN1 is sensitive to changes in oxygen over a physiologically relevant range [63,68]. Therefore, EglN1 is poised to act as an oxygen sensor. Hydroxylation by EglN requires several cofactors including $Fe^{2+}$, 2-oxoglutarate, and ascorbate, in addition to oxygen. Recent studies suggest that hypoxia causes a paradoxical burst of reactive oxygen species (ROS), which inhibit EglN activity (possibly due to changes in iron redox status) [74–76]. This suggests that hypoxia has both direct and indirect effects on EglN function. Cer-

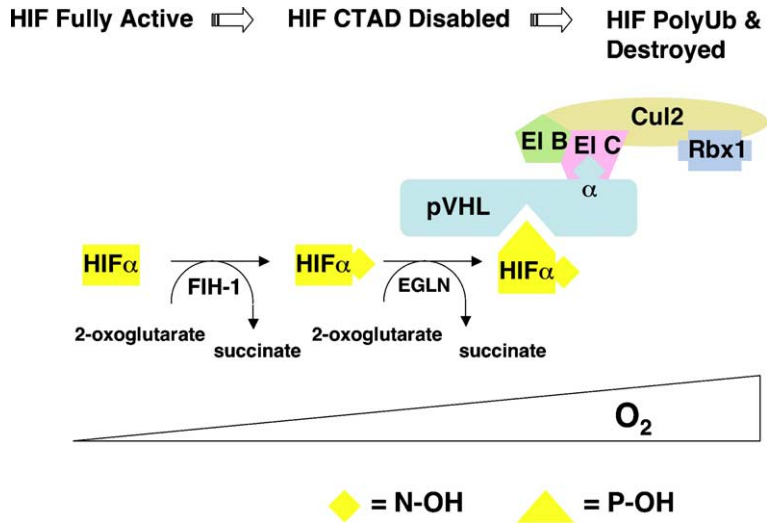


Fig. 1. Oxygen-dependent hydroxylation of HIF. In the presence of oxygen HIFα is hydroxylated on a conserved asparagine residue located with its C-terminal transactivation domain (CTAD) by FIH-1 and one (or both) of two prolyl residues located within the HIF N-terminal transactivation domain (NTAD) by EglN family members (especially EglN1). The former prevents the interaction of HIFα with the coactivators p300 and CBP (the HIFα NTAD is presumed to be remain active under these conditions). The latter targets HIFα for recognition and polyubiqutination by a complex containing pVHL. The oxygen $K_m$s for these enzymes suggest that FIH-1 is less dependent upon oxygen than EglN. El B, elongin B. El C, elongin C.

tain oncogenes also lead to increased ROS generation and HIF stabilization [77,78].

Nitric oxide can also affect prolyl hydroxylation at multiple levels. In particular, NO can affect intracellular oxygen concentrations (through changes in mitochondrial oxygen utilization), alter intracellular ROS, and directly interact with dioxgenases [79]. NO has been reported to stabilize HIF under normoxic conditions while blunting the induction of HIF under hypoxic conditions [80–88].

## Other HIF modifications

Several other posttranslational modifications have been reported to alter HIF function. For example, the ability of the HIFα CTAD to activate transcription is intrinsically oxygen-dependent over and above the effects of oxygen on HIF stability [89–93]. In the presence of oxygen, a conserved asparagine residue within the CTAD is hydroxylated by another iron and 2-oxoglutarate-dependent dioxygenase called FIH1 (factor inhibiting HIF) [94–99] (Fig. 1). Hydroxylation of this residue prevents the recruitment of the coactivator proteins p300 and CBP, thereby attenuating HIF-dependent transcription [100,101]. The oxygen $K_m$ for FIH1 is ~100 μM [98], suggesting that HIF might accumulate in an asparagine hydroxylated form at intermediate levels of hypoxia. Improved oxygenation would lead to HIF destruction whereas worsening hypoxia would enhance HIF function by blunting the action of FIH1.

A recent report suggested that acetylation of HIFα lysine 532 by ARD1 (arrest defective 1) enhances the interaction of pVHL with HIFα and thereby promotes HIFα polyubiquitination [102]. However, this conclusion has now been challenged by two other groups [103,104]. It has also recently been suggested that sumoylation of HIFα prevents its degradation and enhances HIF-dependent transcription [105,106].

## Development of HIF antagonists

HIF can have both protumorigenic or antitumorigenic in a context-dependent manner, although the weight of evidence suggests that HIF usually promotes tumor progression, presumably due to cell autonomous (for example, metabolic) and non-autonomous (for example, angiogenic) actions that facilitate the expansion of tumors in a hypoxic environment [107–110]. The case for a role for HIF is most compelling in tumors linked to pVHL inactivation. Hemangioblastomas are characterized by marked proliferation of blood vessels and hence endothelial cells and pericytes [111]. These two cell types are stimulated by the HIF-responsive growth factors VEGF and PDGF, respectively. Overproduction of specific VEGF isoforms is capable of inducing hemangioblastomas in models systems [112,113]. The hemangioblastoma tumor cells also overproduce HIF-responsive growth factors such as TGFα, erythropoietin, and SDF-1, which are suspected of establishing autocrine loops through their cognate receptors [114–117]. In addition, HIF regulates other genes suspected of playing a role in tumorigenesis including c-Met [118], TGFβ [119], CXCR4 (the receptor for SDF-1) [117,120], and matrix metalloproteinases [121,122] (Fig. 2).

HIF, especially HIF2α, also appears to play a critical role in clear cell renal carcinoma. These tumors typically overproduce both HIF1α and HIF2α or exclusively HIF2α [51]. The appearance of HIF2α in preneoplastic renal lesions in VHL kidneys coincides with histological features indicative of malignant progression (such as increased atypia) [123]. Elimination of HIF2α is sufficient to suppress

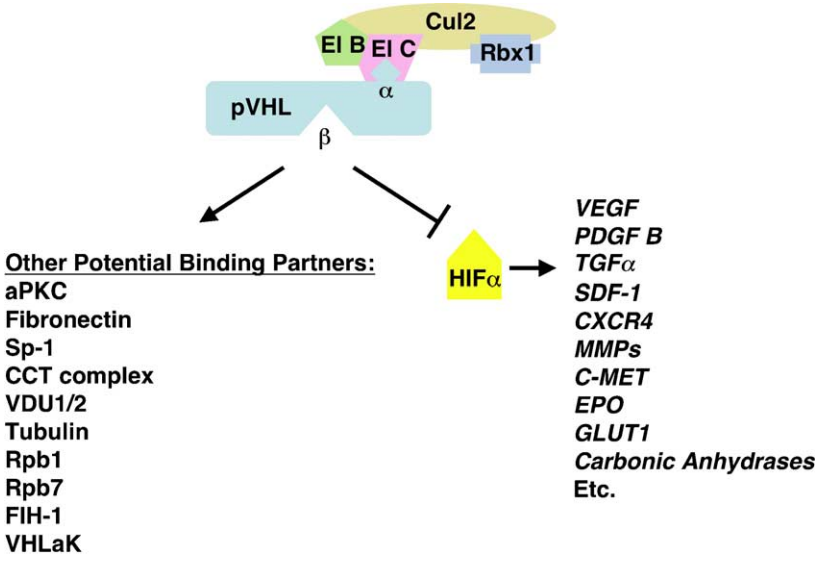


Fig. 2. pVHL targets. The best understood function of pVHL relates to its ability to polyubiquitinate HIFα-subunits. Loss of pVHL leads to overproduction of various HIF-responsive genes, examples of which are shown. Also shown are various other proteins that have been reported to interact with pVHL. The functional significance of many of these interactions is still being investigated.

tumor formation by $VHL^{-/-}$ renal carcinoma cells (at least those producing HIF2α alone) while a HIF2α variant that cannot be hydroxylated by EglN (but not the corresponding HIF1α variant) can override tumor suppression by pVHL [45,124–126]. Many of the HIF target genes listed above also likely play roles in renal carcinogenesis. For example, renal epithelial cells appear to be particularly sensitive to the mitogenic effects of TGFα [127,128]. Another HIF target implicated in renal carcinogenesis is cyclin D1 (whether the regulation of cyclin D1 by HIF is direct or indirect is still not clear) [129–132]. Induction of cyclin D1 by HIF appears to be a peculiar feature of renal epithelial cells, perhaps offering one insight into why inactivation of VHL, which is ubiquitously expressed, is tightly linked to renal cancer and not other epithelial malignancies.

These considerations suggest that drugs that inhibit HIF, or its downstream targets, might be useful for the treatment of pVHL-defective hemangioblastomas and renal cell carcinomas (recent studies suggest that HIF is not the driving force in pheochromocytomas-see below). Unfortunately, DNA-binding transcription factors have not proven to be highly tractable as drug targets with the exception of the steroid hormone receptors. Livingston and co-workers [133,134] did identify a small organic molecule that blocked the association of p300 with HIF and suppressed tumor growth. However, this compound was too toxic to allow further development. Dervan and co-workers [135] designed a polyamide that bound to the HRE in the VEGF promoter and blocked VEGF production in cell culture experiments. Whether such a compound would be efficacious in animals is not known. Attempts to disrupt the binding of HIFα to HIFβ would need to consider the size of the dimerization interface as well as the fact that HIFβ has HIF-independent functions related to its other dimerization partners (such as AhR and AhRR).

A number of drugs have, however, been identified that at least indirectly downregulate HIF including mTOR inhibitors [136–138], HSP90 inhibitors [139,140], HDAC inhibitors [141,142], thioredoxin-1 inhibitors [143,144], and some (but not all) agents that inhibit microtubules [145,146]. In addition, a number of HIF-responsive genes encode activities that are amenable to pharmacological attack. There is already anectodal evidence that drugs that inhibit the VEGF receptor KDR can alter the natural history of hemangioblastoma and multiple agents that inhibit VEGF or KDR are exhibiting activity against renal cell carcinoma (many of the newer KDR inhibitors currently being tested fortuitously inhibit the PDGF receptor as well) [147–151]. It is anticipated that at least two of these agents, SU11248 and BAY43-9006, will be approved by the FDA for the treatment of metastatic renal cancer this year.

## Development of HIF agonists

Metazoan genomes contain HIF as a failsafe mechanism against hypoxia, not as a means of inducing tumors. In theory, HIF stabilization might be useful in diseases characterized by acute or chronic hypoxia or as a means of increasing RBC production in erythropoietin-sensitive anemias. It has already been established that it is possible to inhibit HIF prolyl hydroxylation and activate HIF-dependent transcription with organic small molecules that interfere with the utilization of iron and/or 2-oxoglutarate by EglN [57,65,152–154]. It should be noted that loss of pVHL (or mutation of HIF's prolyl hydroxylation sites) is sufficient to activate HIF target genes, implying it is not necessary to simultaneously inhibit FIH1 to (at least partly) mimic a hypoxic response [31,44,45,51,124,125]. With time, however, it might be desirable to have agents

that, for example, specifically inhibit individual EglN family members or that do or do not inhibit FIH1 in addition to their actions on EglN. In this regard, Schofield and co-workers [155] recently reported a compound that specifically inhibits FIH1 while sparing the EglN family members.

There is evidence that prior exposure to hypoxia or hypoxia mimetics (ischemic preconditioning) can have beneficial effects mediated by HIF (and downstream HIF targets such as erythropoietin) in ischemic diseases such as myocardial infarction and stroke as well as certain forms of renal failure [156–161]. In addition, Nwogu et al. [162] showed that administration of FG0041, an orally available prolyl hydroxylase inhibitor, to rats for 4 weeks beginning 48 h after experimental myocardial infarction preserved left ventricular function. This agent, although originally developed as a collagen prolyl hydroxylase inhibitor, was later shown to inhibit the HIF prolyl hydroxylase [65]. The beneficial effects of FG0041 were too rapid to invoke an antifibrotic mechanism, raising the possibility that they were due instead to superinduction of HIF in the infarct and periinfarct zone. In another preclinical model, direct inoculation of a plasmid encoding a constitutively active version of HIF1α (HIF1α-VP16) into an evolving infarct led to reduction in infarct size and VEGF-dependent neoangiogenesis [163].

The safety of chronically administering HIF prolyl hydroxylase inhibitors to human remains an important question, especially given the potential links between HIF and cancer outlined above. Although caution seems warranted, there are a number of reasons why prolonged use of HIF agonists might not lead to an increase in cancer risk (for example, an increased risk of hemangioblastoma or renal carcinoma).

First of all, pVHL has functions in addition to its role in regulating HIF (Fig. 2). pVHL physically interacts with other proteins including aPKC family members (see also below), Sp1, fibronectin, certain RNA polymerase subunits, and VDU1 and 2 (reviewed in [164]). Although the functional significance of some of these interactions is unclear, there is strong evidence that pVHL plays HIF-independent roles in extracellular matrix control [165,166] and cell survival (see below). Genotype-phenotype correlations in VHL disease also suggest that HIF-independent pVHL functions modulate tumor risk [167]. For example, *VHL* alleles linked to Type 2A VHL disease (high risk of pheochromocytoma and hemangioblastoma, low risk of renal carcinoma) are, like Type 2B *VHL* alleles (high risk of all three tumors), defective with respect to HIF regulation [24,52,168]. Conceivably these alleles differentially alter another pVHL target that conspires with HIF to promote renal tumor formation. It is also worth noting that forced activation of HIF target genes in mice and rabbits has, at least in the tissues tested so far, induced normal appearing blood vessels without the development of hemangioblastomas or renal carcinomas [169,170] (W.R. Kim, W.G.K. unpublished data). Finally, *VHL* inactivation in the human kidney causes preneoplastic renal cysts [123,171,172] and it is assumed that mutations at other loci must occur to convert these lesions to frank carcinomas. Thus, there is presently no evidence that HIF dysregulation is sufficient to initiate tumors and some evidence that it is not. Whether a HIF agonist would have a tumor promoting effect on preexisting tumors is not known.

Studies of polycythemic syndromes also bear on the potential safety of HIF agonists. In particular, individuals with secondary (HIF-induced) polycythemia as a result of chronic hypoxemia do not phenocopy VHL disease with respect to tumor development. In this setting one can presume that the HIF prolyl hydroxylase is chronically inhibited. Similarly, individuals with Chuvash polycythemia do not appear to be tumor prone [173,174]. These patients are homozygous for a hypomorphic *VHL* allele that displays a quantitative defect in HIF regulation [173]. In this situation every cell in the body capable of producing erythropoietin is presumably characterized by a slight increase in HIF (Fig. 3). This is in contrast to the $VHL^{-/-}$ renal carcinoma situation wherein an isolated renal epithelial clone is characterized by a profound HIF defect as well other alterations in other pVHL functions. Therefore, quantitative differences with respect to HIF and qualitative differences with respect to HIF-independent pVHL functions probably account for the low renal cancer risk associated with Chuvash polycythemia and the high renal carcinoma risk associated with *VHL* nullizygous renal cells. One could argue that the systemic administration of a titratable HIF prolyl hydroxylase inhibitor would more closely mimic the former than the latter.

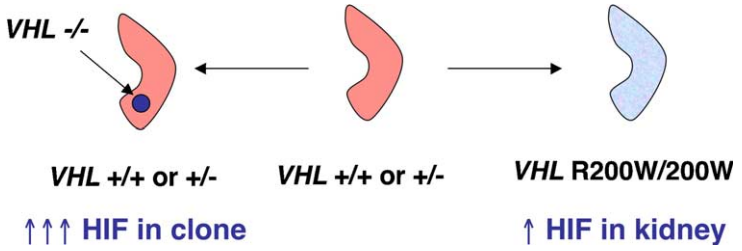


Fig. 3. HIF deregulation after pVHL inactivation. In renal carcinoma, a rare subclone (blue circle) is characterized by a profound defect in HIF regulation (left) compared to normal kidney (center) as a result of homozygous *VHL* mutations. In Chuvash polycythemia or with chronic hypoxia, many cells in the kidney (light blue) are characterized by a smaller increase in HIF levels (right). Carcinogenesis in the former might be due to a 'threshold' level of HIF being reached and/or to loss of HIF-independent pVHL functions, together with cooperating mutations at other genetic loci.

### HIF-independent role of EglN3 in pheochromocytoma and paraganglioma

Pheochromocytomas are neuroendocrine tumors of the adrenal medulla (similar tumors arising in extradrenal sites are called paragangliomas). Although typically benign, these tumors cause symptoms due to their ability to secrete catecholamines. Pheochromocytomas and paragangliomas are derived from the same embryological precursors that give rise to the sympathetic nervous system.

Some germline *VHL* mutations cause familial pheochromocytoma without the other stigmata of VHL disease (so called Type 2C VHL disease) [175]. The products of Type 2C *VHL* alleles are seemingly normal with respect to HIF polyubiquitination [166,168]. This, together with the fact that VHL-associated pheochromocytomas exhibit loss of heterozygosity at the VHL locus [176–179], implies that these tumors are caused by the loss of a HIF-independent pVHL function. Interestingly, virtually all germline *VHL* mutations linked to pheochromocytoma are missense mutations and individuals with truly null *VHL* alleles are at low risk of pheochromocytoma [175]. This raised the possibility (among several) that complete loss of pVHL function was incompatible with pheochromocytoma development.

Somatic *VHL* mutations are uncommon in sporadic pheochromocytomas in the absence of occult germline *VHL* mutations [7]. This is in contrast to sporadic hemangioblastoma and clear cell renal carcinomas, where *VHL* inactivation is common [7]. Familial pheochromocytoma has also been linked to germline mutations of *NF1*, *c-RET*, and the genes for succinate dehydrogenase subunits B, C, and D [180,181].

Sympathetic neuronal precursors compete during embryological development for growth factors such as NGF. Loss of NGF-dependent survival signals leads to c-Jun dependent apoptosis [182–185]. NF1 acts as a Ras-GAP for the NGF receptor TrkA and thereby attenuates its ability to promote survival. Parada and co-workers [186] showed earlier that inactivation of NF1 promotes neuronal survival after NGF withdrawal. It had also been shown previously that TrkA could activate c-RET, suggesting the possibility of crosstalk between these two receptors [187,188]. We recently found that pVHL mutants, including those linked to Type 2C disease, lead to increased levels of JunB [189]. This appears to be due at least partly to the previously described ability of pVHL to inhibit aPKC [190–192], which acts upstream of JunB [193]. JunB often antagonizes c-Jun and we found that pVHL loss (increased JunB), like NF1 loss, promotes survival after NGF withdrawal [189]. In addition, we found that activated version of c-RET, as suspected, could activate TrkA [189].

Another gene implicated in neuronal apoptosis is *SM-20*, which is now appreciated to be the rat ortholog of *EglN3* [194–196]. We found that EglN3 is unique among the EglN family members with respect to its ability to induce neuronal apotosis and acts downstream of c-Jun [189]. This activity is linked to EglN3 hydroxylase activity, which can be feedback inhibited by succinate [189]. Decreased SDH activity, such as might occur with disease-associated SDH mutations, blunts EglN3-induced neuronal apoptosis [189]. Collectively, these results place the familial pheochromocytoma/paraganglioma genes on a common pathway important for the normal culling of sympathogonia during development (Fig. 4). It is hypothesized that cells that have escaped this apoptotic program are at increased risk of malignant transformation.

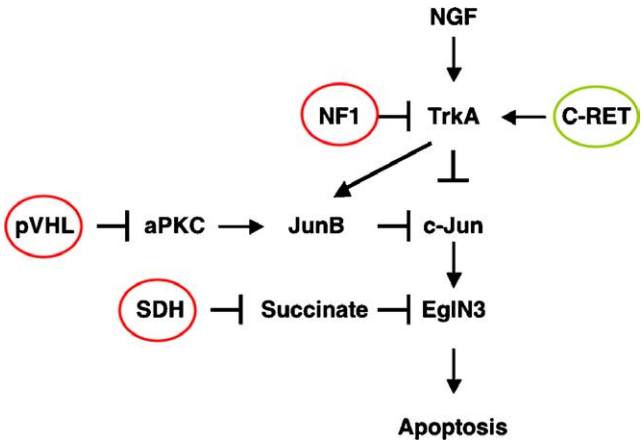


Fig. 4. EglN3 and neuronal apoptosis. See text for details.

### Conclusions

The VHL protein (pVHL), the heterodimeric transcription factor HIF, and the HIF prolyl hydroxylases (especially EglN1/PHD2) are central components of the pathway used by cells to sense and respond to changes in oxygen. Many other signals, including those generated by changes in ROS and NO, likely impinge upon this pathway as well. Increased HIF plays a pathogenic role in pVHL-defective hemangioblastomas and clear cell renal carcinomas. In addition, many solid tumors contain regions that are hypoxic. The accumulation of HIF in such tumors is likely to enhance survival and tumor growth. However, there is currently no evidence that activation of HIF per se is capable of inducing tumor growth and some evidence that it is not. It has already been established that HIF target genes can be induced with small organic molecules that inhibit HIF hydroxylation. Preclinical studies suggest that such agents might be useful in the treatment of ischemic diseases and anemia.

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### References

[1] E.T. Collins, Intra-ocular growths (two cases, brother and sister, with peculiar vascular new growth, probably retinal, affecting both eyes), Trans. Ophthal. Soc. UK 14 (1894) 141–149.

[2] A. Lindau, Zur frage der angiomatosis retinae und ihrer hirncomplikation, Acta Opthal. 4 (1927) 193–226.
[3] E. von Hippel, Ueber eine sehr seltene erkrankung der nethaut, Graefe Arch. Ophthal. 59 (1904) 83–106.
[4] E. Maher, W.G. Kaelin, von Hippel–Lindau disease, Medicine 76 (1997) 381–391.
[5] B.R. Seizinger, G.A. Rouleau, L.J. Ozelius, A.H. Lane, G.E. Farmer, J.M. Lamiell, J. Haines, J.W.M. Yuen, D. Collins, D. Majoor-Krakauer, T. Bonner, C. Mathew, A. Rubenstein, J. Halperin, A. McConkie-Rosell, J.S. Green, J.A. Trofatter, B.A. Ponder, L. Eierman, M.I. Bowmer, R. Schimke, B. Oostra, N. Aronin, D.I. Smith, H. Drabkin, M.H. Waziri, W.J. Hobbs, R.L. Martuza, P.M. Conneally, Y.E. Hsia, J.F. Gusella, Von Hippel–Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma, Nature 332 (1988) 268–269.
[6] F. Latif, K. Tory, J. Gnarra, M. Yao, F.-M. Duh, M.L. Orcutt, T. Stackhouse, I. Kuzmin, W. Modi, L. Geil, L. Schmidt, F. Zhou, H. Li, M.H. Wei, F. Chen, G. Glenn, P. Choyke, M.M. Walther, Y. Weng, D.-S.R. Duan, M. Dean, D. Glavac, F.M. Richards, P.A. Crossey, M.A. Ferguson-Smith, D.L. Pasiler, I. Chumakov, D. Cohen, A.C. Chinault, E.R. Maher, W.M. Linehan, B. Zbar, M.I. Lerman, Identification of the von Hippel–Lindau disease tumor suppressor gene, Science 260 (1993) 1317–1320.
[7] W.Y. Kim, W.G. Kaelin, Role of VHL gene mutation in human cancer, J. Clin. Oncol. 22 (2004) 4991–5004.
[8] A. Schoenfeld, E. Davidowitz, R. Burk, A second major native von Hippel–Lindau gene product, initiated from an internal translation start site, functions as a tumor suppressor, Proc. Natl. Acad. Sci. USA 195 (1998) 8817–8822.
[9] O. Iliopoulos, M. Ohh, W. Kaelin, pVHL19 is a biologically active product of the von Hippel–Lindau gene arising from internal translation initiation, Proc. Natl. Acad. Sci. USA 95 (1998) 11661–11666.
[10] C. Blankenship, J. Naglich, J. Whaley, B. Seizinger, N. Kley, Alternate choice of initiation codon produces a biologically active product of the von Hippel–Lindau gene with tumor suppressor activity, Oncogene 18 (1999) 1529–1535.
[11] O. Iliopoulos, A. Kibel, S. Gray, W.G. Kaelin, Tumor suppression by the human von Hippel–Lindau gene product, Nature Medicine 1 (1995) 822–826.
[12] J.R. Gnarra, S. Zhou, M.J. Merrill, J. Wagner, A. Krumm, E. Papavassiliou, E.H. Oldfield, R.D. Klausner, W.M. Linehan, Post-transcriptional regulation of vascular endothelial growth factor mRNA by the VHL tumor suppressor gene product, Proc. Natl. Acad. Sci. USA 93 (1996) 10589–10594.
[13] A. Kibel, O. Iliopoulos, J.D. DeCaprio, W.G. Kaelin, Binding of the von Hippel–Lindau tumor suppressor protein to elongin B and C, Science 269 (1995) 1444–1446.
[14] K.M. Lonergan, O. Iliopoulos, M. Ohh, T. Kamura, R.C. Conaway, J.W. Conaway, W.G. Kaelin, Regulation of hypoxia-inducible mRNAs by the von Hippel–Lindau protein requires binding to complexes containing elongins B/C and Cul2, Mol. Cell. Biol. 18 (1998) 732–741.
[15] D.R. Duan, J.S. Humphrey, D.Y.T. Chen, Y. Weng, J. Sukegawa, S. Lee, J.R. Gnarra, W.M. Linehan, R.D. Klausner, Characterization of the VHL tumor suppressor gene product: localization, complex formation, and the effect of natural inactivating mutations, Proc. Natl. Acad. Sci. USA 92 (1995) 6495–6499.
[16] D.R. Duan, A. Pause, W. Burgress, T. Aso, D.Y.T. Chen, K.P. Garrett, R.C. Conaway, J.W. Conaway, W.M. Linehan, R.D. Klausner, Inhibition of transcriptional elongation by the VHL tumor suppressor protein, Science 269 (1995) 1402–1406.
[17] T. Kishida, T.M. Stackhouse, F. Chen, M.I. Lerman, B. Zbar, Cellular proteins that bind the von Hippel–Lindau disease gene product: mapping of binding domains and the effect of missense mutations, Cancer Res. 55 (1995) 4544–4548.
[18] T. Kamura, D.M. Koepp, M.N. Conrad, D. Skowyra, R.J. Moreland, O. Iliopoulos, W.S. Lane, W.G.J. Kaelin, S.J. Elledge, R.C. Conaway, J.W. Harper, J.W. Conaway, Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase, Science 284 (1999) 657–661.
[19] A. Pause, S. Lee, R.A. Worrell, D.Y.T. Chen, W.H. Burgess, W.M. Linehan, R.D. Klausner, The von Hippel–Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins, Proc. Natl. Acad. Sci. USA 94 (1997) 2156–2161.
[20] J. Lisztwan, G. Imbert, C. Wirbelauer, M. Gstaiger, W. Krek, The von Hippel–Lindau tumor suppressor protein is a component of an E3 ubiquitin–protein ligase activity, Genes Dev. 13 (1999) 1822–1833.
[21] K. Iwai, K. Yamanaka, T. Kamura, N. Minato, R. Conaway, J. Conaway, R. Klausner, A. Pause, Identification of the von Hippel–Lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex, Proc. Natl. Acad. Sci. USA 96 (1999) 12436–12441.
[22] C.E. Stebbins, W.G. Kaelin, N.P. Pavletich, Structure of the VHL-elonginC–elonginB complex: implications for VHL tumor suppressor function, Science 284 (1999) 455–461.
[23] C. Bai, P. Sen, K. Hofmann, L. Ma, M. Goebl, J.W. Harper, S.J. Elledge, SKP1 connects cell cycle regulators to he ubiquitin proteolysis machinery through a novel motif, the F-box, Cell 86 (1996) 263–274.
[24] M. Ohh, Y. Takagi, T. Aso, C. Stebbins, N. Pavletich, B. Zbar, R. Conaway, J. Conaway, W.J. Kaelin, Synthetic peptides define critical contacts between elongin C, elongin B, and the von Hippel–Lindau protein, J. Clin. Invest. 104 (1999) 1583–1591.
[25] D.W. Golde, W.G. Hocking, Polycythemia: mechanisms and management, Ann. Intern. Med. 95 (1981) 71–87.
[26] L. Brown, B. Berse, R. Jackman, K. Tognazzi, E. Manseau, H. Dvorak, D. Senger, Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas, Am. J. Pathol. 143 (1993) 1255–1262.
[27] K. Sato, K. Terada, T. Sugiyama, S. Takahashi, M. Saito, M. Moriyama, H. Kakinuma, Y. Suzuki, M. Kato, T. Kato, Frequent overexpression of vascular endothelial growth factor gene in human renal cell carcinoma, Tohoku J. Exp. Med. 173 (1994) 355–360.
[28] A. Takahashi, H. Sasaki, S. Kim, K. Tobisu, T. Kakizoe, T. Tsukamoto, Y. Kumamoto, T. Sugimura, M. Terada, Markedly increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated with angiogenesis, Cancer Res. 54 (1994) 4233–4237.
[29] K. Morii, R. Tanaka, K. Washiyama, T. Kumanishi, R. Kuwano, Expression of vascular endothelial growth factor in capillary hemangioblastoma, Biochem. Biophys. Res. Commun. 194 (1993) 749–755.
[30] S. Wizigmann-Voos, G. Breier, W. Risau, K. Plate, Up-regulation of vascular endothelial growth factor and its receptors in von Hippel–Lindau disease-associated and sporadic hemangioblastomas, Cancer Res. 55 (1995) 1358–1364.
[31] O. Iliopoulos, C. Jiang, A.P. Levy, W.G. Kaelin, M.A. Goldberg, Negative regulation of hypoxia-inducible genes by the von Hippel–Lindau protein, Proc. Natl. Acad. Sci. USA 93 (1996) 10595–10599.
[32] G. Siemeister, K. Weindel, K. Mohrs, B. Barleon, G. Martiny-Baron, D. Marme, Reversion of deregulated expression of vascular endothelial growth factor in human renal carcinoma cells by von Hippel–Lindau tumor suppressor protein, Cancer Research 56 (1996) 2299–2301.
[33] R. Stratmann, M. Krieg, R. Haas, K. Plate, Putative control of angiogenesis in hemangioblastomas by the von Hippel–Lindau tumor suppressor gene, J. Neuropathol. Exp. Neurol. 56 (1997) 1242–1252.
[34] G. Semenza, HIF-1 and human disease: one highly involved factor, Genes Dev. 14 (2000) 1983–1991.
[35] C.J. Schofield, P.J. Ratcliffe, Oxygen sensing by HIF hydroxylases, Nat. Rev. Mol. Cell Biol. 5 (2004) 343–354.
[36] Y. Makino, R. Cao, K. Svensson, G. Bertilsson, M. Asman, H. Tanaka, Y. Cao, A. Berkenstam, L. Poellinger, Inhibitory PAS

domain protein is a negative regulator of hypoxia-inducible gene expression, Nature 414 (2001) 550–554.
[37] Y. Makino, A. Kanopka, W.J. Wilson, H. Tanaka, L. Poellinger, Inhibitory PAS domain protein (IPAS) is a hypoxia-inducible splicing variant of the hypoxia-inducible factor-3alpha locus, J. Biol. Chem. 277 (2002) 32405–32408.
[38] K.L. Talks, H. Turley, K.C. Gatter, P.H. Maxwell, C.W. Pugh, P.J. Ratcliffe, A.L. Harris, The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages, Am. J. Pathol. 157 (2000) 411–421.
[39] M.S. Wiesener, J.S. Jurgensen, C. Rosenberger, C.K. Scholze, J.H. Horstrup, C. Warnecke, S. Mandriota, I. Bechmann, U.A. Frei, C.W. Pugh, P.J. Ratcliffe, S. Bachmann, P.H. Maxwell, K.U. Eckardt, Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs, FASEB J. 17 (2003) 271–273.
[40] G. Semenza, Regulation of mammalian $O_2$ homeostasis by hypoxia-inducible factor 1, Annu. Rev. Cell Dev. Biol. 15 (1999) 551–578.
[41] J. Brugarolas, K. Lei, R.L. Hurley, B.D. Manning, J.H. Reiling, E. Hafen, L.A. Witters, L.W. Ellisen, W.G. Kaelin Jr., Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex, Genes Dev. 18 (2004) 2893–2904.
[42] J.H. Reiling, E. Hafen, The hypoxia-induced paralogs Scylla and Charybdis inhibit growth by down-regulating S6K activity upstream of TSC in *Drosophila*, Genes Dev. 18 (2004) 2879–2892.
[43] V. Wang, D.A. Davis, M. Haque, L.E. Huang, R. Yarchoan, Differential gene up-regulation by hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2alpha in HEK293T cells, Cancer Res. 65 (2005) 3299–3306.
[44] C.J. Hu, L.Y. Wang, L.A. Chodosh, B. Keith, M.C. Simon, Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation, Mol. Cell. Biol. 23 (2003) 9361–9374.
[45] R.R. Raval, K.W. Lau, M.G. Tran, H.M. Sowter, S.J. Mandriota, J.L. Li, C.W. Pugh, P.H. Maxwell, A.L. Harris, P.J. Ratcliffe, Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel–Lindau-associated renal cell carcinoma, Mol. Cell. Biol. 25 (2005) 5675–5686.
[46] C. Pugh, J. O'Rourke, M. Nagao, J. Gleadle, P. Ratcliffe, Activation of hypoxia-inducible factor-1; definition of regulatory domains within the alpha subunit, J. Biol. Chem. 272 (1997) 11205–11214.
[47] L.E. Huang, J. Gu, M. Schau, H.F. Bunn, Regulation of hypoxia-inducible factor 1alpha is mediated by an $O_2$-dependent degradation domain via the ubiquitin–proteasome pathway, Proc. Natl. Acad. Sci. USA 95 (1998) 7987–7992.
[48] P. Kallio, W. Wilson, S. O'Brien, Y. Makino, L. Poellinger, Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin–proteasome pathway, J. Biol. Chem. 274 (1999) 6519–6525.
[49] S. Salceda, J. Caro, Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin–proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes, J. Biol. Chem. 272 (1997) 22642–22647.
[50] C.H. Sutter, E. Laughner, G.L. Semenza, Hypoxia-inducible factor 1alpha protein expression is controlled by oxygen-regulated ubiquitination that is disrupted by deletions and missense mutations, Proc. Natl. Acad. Sci. USA 97 (2000) 4748–4753.
[51] P. Maxwell, M. Weisner, G.-W. Chang, S. Clifford, E. Vaux, C. Pugh, E. Maher, P. Ratcliffe, The von Hippel–Lindau gene product is necessary for oxygen-dependent proteolysis of hypoxia-inducible factor $\alpha$ subunits, Nature 399 (1999) 271–275.
[52] M. Ohh, C.W. Park, M. Ivan, M.A. Hoffman, T.-Y. Kim, L.E. Huang, V. Chau, W.G. Kaelin, Ubiquitination of HIF requires direct binding to the von Hippel–Lindau protein beta domain, Nat. Cell Biol. 2 (2000) 423–427.
[53] M. Cockman, N. Masson, D. Mole, P. Jaakkola, G. Chang, S. Clifford, E. Maher, C. Pugh, P. Ratcliffe, P. Maxwell, Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel–Lindau tumor suppressor protein, J. Biol. Chem. 275 (2000) 25733–25741.
[54] T. Kamura, S. Sato, K. Iwain, M. Czyzyk-Krzeska, R.C. Conaway, J.W. Conaway, Activation of hif1a ubiquitination by a reconstituted von Hippel–Lindau tumor suppressor complex, Proc. Natl. Acad. Sci. USA 97 (2000) 10430–10435.
[55] K. Tanimoto, Y. Makino, T. Pereira, L. Poellinger, Mechanism of regulation of the hypoxia-inducible factor-1alpha by the von Hippel–Lindau tumor suppressor protein, EMBO J. 19 (2000) 4298–4309.
[56] M. Ivan, K. Kondo, H. Yang, W. Kim, J. Valiando, M. Ohh, A. Salic, J. Asara, W. Lane, W.J. Kaelin, HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for $O_2$ sensing, Science 292 (2001) 464–468.
[57] P. Jaakkola, D. Mole, Y. Tian, M. Wilson, J. Gielbert, S. Gaskell, A. Kriegsheim, H. Hebestreit, M. Mukherji, C. Schofield, P. Maxwell, C. Pugh, P. Ratcliffe, Targeting of hif-alpha to the von Hippel–Lindau ubiquitylation complex by $O_2$-regulated prolyl hydroxylation, Science 292 (2001) 468–472.
[58] N. Masson, C. Willam, P. Maxwell, C. Pugh, P. Ratcliffe, Independent function of two destruction domains in hypoxia-inducible factor-$\alpha$ chains activated by prolyl hydroylation, EMBO J. 20 (2001) 5197–5206.
[59] W.C. Hon, M.I. Wilson, K. Harlos, T.D. Claridge, C.J. Schofield, C.W. Pugh, P.H. Maxwell, P.J. Ratcliffe, D.I. Stuart, E.Y. Jones, Structural basis for the recognition of hydroxyproline in HIF-1alpha by pVHL, Nature (2002).
[60] J.H. Min, H. Yang, M. Ivan, F. Gertler, W.G. Kaelin Jr., N.P. Pavletich, Structure of an HIF-1alpha–pVHL complex: hydroxyproline recognition in signaling, Science 296 (2002) 1886–1889.
[61] F. Yu, S. White, Q. Zhao, F. Lee, HIF-1alpha binding to VHL is regulated by stimulus-sensitive proline hydroxylation, Proc. Natl. Acad. Sci. USA 98 (2001) 9630–9635.
[62] F. Yu, S. White, Q. Zhao, F. Lee, Dynamic, site-specific interaction of hypoxia-inducible factor-1alpha with the von Hippel–Lindau tumor suppressor protein, Cancer Res. 61 (2001) 4136–4142.
[63] A. Epstein, J. Gleadle, L. McNeill, K. Hewitson, J. O'Rourke, D. Mole, M. Mukherji, E. Metzen, M. Wilson, A. Dhanda, Y. Tian, N. Masson, D. Hamilton, P. Jaakkola, R. Barstead, J. Hodgkin, P. Maxwell, C. Pugh, C. Schofield, P. Ratcliffe, *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation, Cell 107 (2001) 43–54.
[64] R. Bruick, S. McKnight, A conserved family of prolyl-4-hydroxylases that modify HIF, Science 294 (2001) 1337–1340.
[65] M. Ivan, T. Haberberger, D.C. Gervasi, K.S. Michelson, V. Gunzler, K. Kondo, H. Yang, I. Sorokina, R.C. Conaway, J.W. Conaway, W.G. Kaelin Jr., Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor, Proc. Natl. Acad. Sci. USA 99 (2002) 13459–13464.
[66] M.S. Taylor, Characterization and comparative analysis of the EGLN gene family, Gene 275 (2001) 125–132.
[67] L. Aravind, E.V. Koonin, The DNA-repair protein AlkB, EGL-9, and leprecan define new families of 2-oxoglutarate- and iron-dependent dioxygenases, Genome Bio. 2 (2001), research0007.0001-0007.0008.
[68] M. Hirsila, P. Koivunen, V. Gunzler, K.I. Kivirikko, J. Myllyharju, Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor, J. Biol. Chem. 278 (2003) 30772–30780.
[69] E. Berra, E. Benizri, A. Ginouves, V. Volmat, D. Roux, J. Pouyssegur, HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia, EMBO J. 22 (2003) 4082–4090.
[70] L. del Peso, M.C. Castellanos, E. Temes, S. Martin-Puig, Y. Cuevas, G. Olmos, M.O. Landazuri, The von Hippel–Lindau/hypoxia-inducible factor (HIF) pathway regulates the transcription of the HIF-proline hydroxylase genes in response to low oxygen, J. Biol. Chem. 278 (2003) 48690–48695.

[71] R.J. Appelhoff, Y.M. Tian, R.R. Raval, H. Turley, A.L. Harris, C.W. Pugh, P.J. Ratcliffe, J.M. Gleadle, Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor, J. Biol. Chem. 279 (2004) 38458–38465.
[72] O. Aprelikova, G.V. Chandramouli, M. Wood, J.R. Vasselli, J. Riss, J.K. Maranchie, W.M. Linehan, J.C. Barrett, Regulation of HIF prolyl hydroxylases by hypoxia-inducible factors, J. Cell Biochem. 92 (2004) 491–501.
[73] C.L. Cioffi, X.Q. Liu, P.A. Kosinski, M. Garay, B.R. Bowen, Differential regulation of HIF-1 alpha prolyl-4-hydroxylase genes by hypoxia in human cardiovascular cells, Biochem. Biophys. Res. Commun. 303 (2003) 947–953.
[74] J.K. Brunelle, E.L. Bell, N.M. Quesada, K. Vercauteren, V. Tiranti, M. Zeviani, R.C. Scarpulla, N.S. Chandel, Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation, Cell Metab. 1 (2005) 409–414.
[75] K.D. Mansfield, R.D. Guzy, Y. Pan, R.M. Young, T.P. Cash, P.T. Schumacher, M.C. Simon, Loss of cytochrome *c* impairs cellular oxygen sensing and hypoxic HIF-a activation, Cell Metab. 1 (2005) 393–399.
[76] R.D. Guzy, B. Hoyos, E. Robin, H. Chen, L. Liu, K.D. Mansfield, M.C. Simon, U. Hammerling, P.T. Schumacker, Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing, Cell Metab. 1 (2005) 401–408.
[77] D.A. Chan, P.D. Sutphin, N.C. Denko, A.J. Giaccia, Role of prolyl hydroxylation in oncogenically stabilized hypoxia-inducible factor-1alpha, J. Biol. Chem. 277 (2002) 40112–40117.
[78] D. Gerald, E. Berra, Y.M. Frapart, D.A. Chan, A.J. Giaccia, D. Mansuy, J. Pouyssegur, M. Yaniv, F. Mechta-Grigoriou, JunD reduces tumor angiogenesis by protecting cells from oxidative stress, Cell 118 (2004) 781–794.
[79] W.G. Kaelin, Proline hydroxylation and gene expression, Annu. Rev. Biochem. 74 (2005) 115–128.
[80] Y. Liu, H. Christou, T. Morita, E. Laughner, G. Semenza, S. Kourembanas, Carbon monoxide and nitric oxide suppress the hypoxic induction of vascular endothelial growth factor gene via the 5′ enhancer, J. Biol. Chem. 273 (1998) 15257–15262.
[81] K. Sogawa, K. Numayama-Tsuruta, M. Ema, M. Abe, H. Abe, Y. Fujii-Kuriyama, Inhibition of hypoxia-inducible factor 1 activity by nitric oxide donors in hypoxia, Proc. Natl. Acad. Sci. USA 95 (1998) 7368–7373.
[82] L. Huang, W. Willmore, J. Gu, M. Goldberg, H. Bunn, Inhibition of hypoxia-inducible factor 1 activation by carbon monoxide and nitric oxide. Implications for oxygen sensing and signaling, J. Biol. Chem. 274 (1999) 9038–9044.
[83] L.A. Palmer, B. Gaston, R.A. Johns, Normoxic stabilization of hypoxia-inducible factor-1 expression and activity: redox-dependent effect of nitrogen oxides, Mol. Pharmacol. 58 (2000) 1197–1203.
[84] K.B. Sandau, J. Fandrey, B. Brune, Accumulation of HIF-1alpha under the influence of nitric oxide, Blood 97 (2001) 1009–1015.
[85] F.H. Agani, M. Puchowicz, J.C. Chavez, P. Pichiule, J. LaManna, Role of nitric oxide in the regulation of HIF-1alpha expression during hypoxia, Am. J. Physiol. Cell Physiol. 283 (2002) C178–C186.
[86] F. Wang, H. Sekine, Y. Kikuchi, C. Takasaki, C. Miura, O. Heiwa, T. Shuin, Y. Fujii-Kuriyama, K. Sogawa, HIF-1alpha-prolyl hydroxylase: molecular target of nitric oxide in the hypoxic signal transduction pathway, Biochem. Biophys. Res. Commun. 295 (2002) 657–662.
[87] T. Hagen, C.T. Taylor, F. Lam, S. Moncada, Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha, Science 302 (2003) 1975–1978.
[88] E. Metzen, J. Zhou, W. Jelkmann, J. Fandrey, B. Brune, Nitric oxide impairs normoxic degradation of HIF-1alpha by inhibition of prolyl hydroxylases, Mol. Biol. Cell 14 (2003) 3470–3481.
[89] B.H. Jiang, J.Z. Zheng, S.W. Leung, R. Roe, G.L. Semenza, Transactivation and inhibitory domains of hypoxia-inducible factor 1alpha. Modulation of transcriptional activity by oxygen tension, J. Biol. Chem. 272 (1997) 19253–19260.
[90] P.J. Kallio, K. Okamoto, S. O'Brien, P. Carrero, Y. Makino, H. Tanaka, L. Poellinger, Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1alpha, EMBO J. 17 (1998) 6573–6586.
[91] M. Ema, K. Hirota, J. Mimura, H. Abe, J. Yodoi, K. Sogawa, L. Poellinger, Y. Fujii-Kuriyama, Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300, EMBO J. 18 (1999) 1905–1914.
[92] J.F. O'Rourke, Y.M. Tian, P.J. Ratcliffe, C.W. Pugh, Oxygen-regulated and transactivating domains in endothelial PAS protein 1: comparison with hypoxia-inducible factor-1alpha, J. Biol. Chem. 274 (1999) 2060–2071.
[93] P. Carrero, K. Okamoto, P. Coumailleau, S. O'Brien, H. Tanaka, L. Poellinger, Redox-regulated recruitment of the transcriptional coactivators CREB-binding protein and SRC-1 to hypoxia-inducible factor 1alpha, Mol. Cell. Biol. 20 (2000) 402–415.
[94] P. Mahon, K. Hirota, G. Semenza, FIH-1: a novel protein that interacts with HIF-1α and VHL to mediate repression of HIF-1 transcriptional activity, Genes and Dev. 15 (2001) 2675–2686.
[95] K.S. Hewitson, L.A. McNeill, M.V. Riordan, Y.M. Tian, A.N. Bullock, R.W. Welford, J.M. Elkins, N.J. Oldham, S. Bhattacharya, J.M. Gleadle, P.J. Ratcliffe, C.W. Pugh, C.J. Schofield, Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family, J. Biol. Chem. 277 (2002) 26351–26355.
[96] D. Lando, D. Peet, J. Gorman, D. Whelan, M. Whitelaw, R. Bruick, FIH-1 is a an asparaginyl hydroxylase that regulates the transcriptional activity of hypoxia inducible factor, Genes Dev. (2002).
[97] J.M. Elkins, K.S. Hewitson, L.A. McNeill, J.F. Seibel, I. Schlemminger, C.W. Pugh, P.J. Ratcliffe, C.J. Schofield, Structure of factor-inhibiting hypoxia-inducible factor (HIF) reveals mechanism of oxidative modification of HIF-1 alpha, J. Biol. Chem. 278 (2003) 1802–1806.
[98] P. Koivunen, M. Hirsila, V. Gunzler, K.I. Kivirikko, J. Myllyharju, Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases, J. Biol. Chem. 279 (2004) 9899–9904.
[99] I.P. Stolze, Y.M. Tian, R.J. Appelhoff, H. Turley, C.C. Wykoff, J.M. Gleadle, P.J. Ratcliffe, Genetic analysis of the role of the asparaginyl hydroxylase FIH in regulating HIF transcriptional target genes, J. Biol. Chem. (2004).
[100] S. Dames, M. Martinez-Yamout, R. De Guzman, H. Dyson, P. Wright, From the cover: structural basis for Hif-1alpha/CBP recognition in the cellular hypoxic response, Proc. Natl. Acad. Sci. USA 99 (2002) 5271–5276.
[101] S. Freedman, Z. Sun, F. Poy, A. Kung, D. Livingston, G. Wagner, M. Eck, Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1alpha, Proc. Natl. Acad. Sci. USA 99 (2002) 5367–5372.
[102] J.W. Jeong, M.K. Bae, M.Y. Ahn, S.H. Kim, T.K. Sohn, M.H. Bae, M.A. Yoo, E.J. Song, K.J. Lee, K.W. Kim, Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation, Cell 111 (2002) 709–720.
[103] R. Bilton, N. Mazure, E. Trottier, M. Hattab, M.A. Dery, D.E. Richard, J. Pouyssegur, M.C. Brahimi-Horn, ARD1, an acetyltransferase, does not alter stability of hypoxia-inducible factor-1alpha and is not induced by hypoxia or HIF, J. Biol. Chem. (2005).
[104] T.S. Fisher, S.D. Etages, L. Hayes, K. Crimin, B. Li, Analysis of ARD1 function in hypoxia response using retroviral RNA interference, J. Biol. Chem. 280 (2005) 17749–17757.
[105] S.H. Bae, J.W. Jeong, J.A. Park, S.H. Kim, M.K. Bae, S.J. Choi, K.W. Kim, Sumoylation increases HIF-1alpha stability and its transcriptional activity, Biochem. Biophys. Res. Commun. 324 (2004) 394–400.
[106] R. Shao, F.P. Zhang, F. Tian, P. Anders Friberg, X. Wang, H. Sjoland, H. Billig, Increase of SUMO-1 expression in response to

hypoxia: direct interaction with HIF-1alpha in adult mouse brain and heart in vivo, FEBS Lett. 569 (2004) 293–300.
[107] H. Ryan, J. Lo, R. Johnson, HIF-1α is required for solid tumor formation and embryonic vascularization, EMBO J. 17 (1998) 3005–3015.
[108] P. Carmeliet, Y. Dor, J.M. Herbert, D. Fukumura, K. Brusselmans, M. Dewerchin, M. Neeman, F. Bono, R. Abramovitch, P. Maxwell, C.J. Koch, P. Ratcliffe, L. Moons, R.K. Jain, D. Collen, E. Keshert, E. Keshet, Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis, Nature 394 (1998) 485–490.
[109] P. Maxwell, G. Dachs, J. Gleadle, L. Nicholls, A. Harris, I. Stratford, O. Hankinson, C. Pugh, P. Ratcliffe, Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth, Proc. Natl. Acad. Sci. USA 94 (1997) 8104–8109.
[110] H. Zhong, A. De Marzo, E. Laughner, M. Lim, D. Hilton, D. Zagzag, P. Buechler, W. Isaacs, G. Semenza, J. Simons, Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases, Cancer Res. 59 (1999) 5830–5835.
[111] S. Richard, C. Campello, L. Taillandier, F. Parker, F. Resche, Haemangioblastoma of the central nervous system in von Hippel–Lindau disease, J. Intern. Med. 243 (1998) 547–553.
[112] L.E. Benjamin, E. Keshet, Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal, Proc. Natl. Acad. Sci. USA 94 (1997) 8761–8766.
[113] E.J. Rebar, Y. Huang, R. Hickey, A.K. Nath, D. Meoli, S. Nath, B. Chen, L. Xu, Y. Liang, A.C. Jamieson, L. Zhang, S.K. Spratt, C.C. Case, A. Wolffe, F.J. Giordano, Induction of angiogenesis in a mouse model using engineered transcription factors, Nat. Med. 8 (2002) 1427–1432.
[114] T. Bohling, E. Hatva, M. Kujala, L. Claesson-Welsh, K. Alitalo, M. Haltia, Expression of growth factors and growth factor receptors in capillary hemangioblastoma, J. Neuropathol. Exp. Neurol. 55 (1996) 522–527.
[115] B. Knebelmann, S. Ananth, H. Cohen, V. Sukhatme, Transforming growth factor alpha is a target for the von Hippel–Lindau tumor suppressor, Cancer Res. 58 (1998) 226–231.
[116] A.O. Vortmeyer, S. Frank, S.Y. Jeong, K. Yuan, B. Ikejiri, Y.S. Lee, D. Bhowmick, R.R. Lonser, R. Smith, G. Rodgers, E.H. Oldfield, Z. Zhuang, Developmental arrest of angioblastic lineage initiates tumorigenesis in von Hippel–Lindau disease, Cancer Res. 63 (2003) 7051–7055.
[117] D. Zagzag, B. Krishnamachary, H. Yee, H. Okuyama, L. Chiriboga, M.A. Ali, J. Melamed, G.L. Semenza, Stromal cell-derived factor-1alpha and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: von Hippel–Lindau loss-of-function induces expression of a ligand and its receptor, Cancer Res. 65 (2005) 6178–6188.
[118] S. Pennacchietti, P. Michieli, M. Galluzzo, M. Mazzone, S. Giordano, P.M. Comoglio, Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene, Cancer Cell 3 (2003) 347–361.
[119] S. Ananth, B. Knebelmann, W. Gruning, M. Dhanabal, G. Walz, I. Stillman, V. Sukhatme, Transforming growth factor beta1 is a target for the von Hippel–Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma, Cancer Res. 59 (1999) 2210–2216.
[120] P. Staller, J. Sulitkova, J. Lisztwan, H. Moch, E.J. Oakeley, W. Krek, Chemokine receptor CXCR4 downregulated by von Hippel–Lindau tumour suppressor pVHL, Nature 425 (2003) 307–311.
[121] S. Koochekpour, M. Jeffers, P. Wang, C. Gong, G. Taylor, L. Roessler, R. Stearman, J. Vasselli, W. Stetler-Stevenson, W.J. Kaelin, W. Linehan, R. Klausner, J. Gnarra, G. VandeWoude, The von Hippel–Lindau tumor suppressor gene inhibits hepatocyte growth factor/scatter factor-induced invasion and branching morphogenesis in renal carcinoma cells, Mol. Cell. Biol. 19 (1999) 5902–5912.
[122] B.L. Petrella, J. Lohi, C.E. Brinckerhoff, Identification of membrane type-1 matrix metalloproteinase as a target of hypoxia-inducible factor-2 alpha in von Hippel–Lindau renal cell carcinoma, Oncogene 24 (2005) 1043–1052.
[123] S.J. Mandriota, K.J. Turner, D.R. Davies, P.G. Murray, N.V. Morgan, H.M. Sowter, C.C. Wykoff, E.R. Maher, A.L. Harris, P.J. Ratcliffe, P.H. Maxwell, HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron, Cancer Cell 1 (2002) 459–468.
[124] K. Kondo, J. Klco, E. Nakamura, M. Lechpammer, W.G. Kaelin, Inhibition of HIF is necessary for tumor suppression by the von Hippel–Lindau protein, Cancer Cell 1 (2002) 237–246.
[125] K. Kondo, W.Y. Kim, M. Lechpammer, W.G. Kaelin Jr., Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth, PLoS Biol. 1 (2003) E83.
[126] M. Zimmer, D. Doucette, N. Siddiqui, O. Iliopoulos, Inhibition of hypoxia-inducible factor is sufficient for growth suppression of $VHL^{-/-}$ tumors, Mol. Cancer Res. 2 (2004) 89–95.
[127] N. de Paulsen, A. Brychzy, M.-C. Fournier, R.D. Klausner, J.R. Gnarra, A. Pause, S. Lee, Role of transforming growth factor-alpha in $VHL^{-/-}$ clear cell renal carcinoma cell proliferation: A possible mechanism coupling von Hippel–Lindau tumor suppressor inactivation and tumorigenesis, Proc. Natl. Acad. Sci. USA 13 (2001) 1387–1392.
[128] K. Smith, L. Gunaratnam, M. Morley, A. Franovic, K. Mekhail, S. Lee, Silencing of epidermal growth factor receptor suppresses hypoxia-inducible factor-2-driven $VHL^{-/-}$ renal cancer, Cancer Res. 65 (2005) 5221–5230.
[129] M. Zatyka, N.F. da Silva, S.C. Clifford, M.R. Morris, M.S. Wiesener, K.U. Eckardt, R.S. Houlston, F.M. Richards, F. Latif, E.R. Maher, Identification of cyclin D1 and other novel targets for the von Hippel–Lindau tumor suppressor gene by expression array analysis and investigation of cyclin D1 genotype as a modifier in von Hippel–Lindau disease, Cancer Res. 62 (2002) 3803–3811.
[130] M. Baba, S. Hirai, H. Yamada-Okabe, K. Hamada, H. Tabuchi, K. Kobayashi, K. Kondo, M. Yoshida, A. Yamashita, T. Kishida, N. Nakaigawa, Y. Nagashima, Y. Kubota, M. Yao, S. Ohno, Loss of von Hippel–Lindau protein causes cell density dependent deregulation of CyclinD1 expression through hypoxia-inducible factor, Oncogene 22 (2003) 2728–2738.
[131] R.S. Bindra, J.R. Vasselli, R. Stearman, W.M. Linehan, R.D. Klausner, VHL-mediated hypoxia regulation of cyclin D1 in renal carcinoma cells, Cancer Res. 62 (2002) 3014–3019.
[132] C. Wykoff, C. Pugh, P. Maxwell, A. Harris, P. Ratcliffe, Identification of novel hypoxia dependent and independent target genes of the von Hippel–Lindau (VHL) tumour suppressor by mRNA differential expression profiling, Oncogene 19 (2000) 6297–6305.
[133] A. Kung, S. Wang, J. Klco, W. Kaelin, D. Livingston, Suppression of tumor growth through disruption of hypoxia-inducible transcription, Nat. Med. 6 (2000) 1335–1340.
[134] A.L. Kung, S.D. Zabludoff, D.S. France, S.J. Freedman, E.A. Tanner, A. Vieira, S. Cornell-Kennon, J. Lee, B. Wang, J. Wang, K. Memmert, H.U. Naegeli, F. Petersen, M.J. Eck, K.W. Bair, A.W. Wood, D.M. Livingston, Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway, Cancer Cell 6 (2004) 33–43.
[135] B.Z. Olenyuk, G.J. Zhang, J.M. Klco, N.G. Nickols, W.G. Kaelin Jr., P.B. Dervan, Inhibition of vascular endothelial growth factor with a sequence-specific hypoxia response element antagonist, Proc. Natl. Acad. Sci. USA 101 (2004) 16768–16773.
[136] C.C. Hudson, M. Liu, G.G. Chiang, D.M. Otterness, D.C. Loomis, F. Kaper, A.J. Giaccia, R.T. Abraham, Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin, Mol. Cell. Biol. 22 (2002) 7004–7014.
[137] H. Zhong, K. Chiles, D. Feldser, E. Laughner, C. Hanrahan, M.M. Georgescu, J.W. Simons, G.L. Semenza, Modulation of hypoxia-

inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics, Cancer Res. 60 (2000) 1541–1545.
[138] J.B. Brugarolas, F. Vazquez, A. Reddy, W.R. Sellers, W.G. Kaelin Jr., TSC2 regulates VEGF through mTOR-dependent and -independent pathways, Cancer Cell 4 (2003) 147–158.
[139] J.S. Isaacs, Y.J. Jung, E.G. Mimnaugh, A. Martinez, F. Cuttitta, L.M. Neckers, Hsp90 regulates a von Hippel–Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway, J. Biol. Chem. 277 (2002) 29936–29944.
[140] N.J. Mabjeesh, D.E. Post, M.T. Willard, B. Kaur, E.G. Van Meir, J.W. Simons, H. Zhong, Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells, Cancer Res. 62 (2002) 2478–2482.
[141] M.S. Kim, H.J. Kwon, Y.M. Lee, J.H. Baek, J.E. Jang, S.W. Lee, E.J. Moon, H.S. Kim, S.K. Lee, H.Y. Chung, C.W. Kim, K.W. Kim, Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes, Nat. Med. 7 (2001) 437–443.
[142] P. Atadja, M. Hsu, P. Kwon, N. Trogani, K. Bhalla, S. Remiszewski, Molecular and cellular basis for the anti-proliferative effects of the HDAC inhibitor LAQ824, Novartis Found. Symp. 259 (2004) 249–266, discussion 266–248, 285–248.
[143] S.J. Welsh, R.R. Williams, A. Birmingham, D.J. Newman, D.L. Kirkpatrick, G. Powis, The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation, Mol. Cancer Ther. 2 (2003) 235–243.
[144] S. Welsh, R. Williams, L. Kirkpatrick, G. Paine-Murrieta, G. Powis, Antitumor activity and pharmacodynamic properties of PX-478, an inhibitor of hypoxia-inducible factor-1alpha, Mol. Cancer Ther. 3 (2004) 233–244.
[145] N.J. Mabjeesh, D. Escuin, T.M. LaVallee, V.S. Pribluda, G.M. Swartz, M.S. Johnson, M.T. Willard, H. Zhong, J.W. Simons, P. Giannakakou, 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF, Cancer Cell 3 (2003) 363–375.
[146] Y.J. Jung, J.S. Isaacs, S. Lee, J. Trepel, L. Neckers, Microtubule disruption utilizes an NFkappa B-dependent pathway to stabilize HIF-1alpha protein, J. Biol. Chem. 278 (2003) 7445–7452.
[147] W.G. Kaelin Jr., The von Hippel–Lindau tumor suppressor gene and kidney cancer, Clin. Cancer Res. 10 (2004) 6290S–6295S.
[148] L.P. Aiello, D.J. George, M.T. Cahill, J.S. Wong, J. Cavallerano, A.L. Hannah, W.G. Kaelin Jr., Rapid and durable recovery of visual function in a patient with von Hippel–Lindau syndrome after systemic therapy with vascular endothelial growth factor receptor inhibitor su5416, Ophthalmology 109 (2002) 1745–1751.
[149] S. Richard, L. Croisille, J. Yvart, N. Casadeval, P. Eschwege, N. Aghakhani, P. David, A. Gaudric, P. Scigalla, O. Hermine, Paradoxical secondary polycythemia in von Hippel–Lindau patients treated with anti-vascular endothelial growth factor receptor therapy, Blood 99 (2002) 3851–3853.
[150] J.F. Girmens, A. Erginay, P. Massin, P. Scigalla, A. Gaudric, S. Richard, Treatment of von Hippel–Lindau retinal hemangioblastoma by the vascular endothelial growth factor receptor inhibitor SU5416 is more effective for associated macular edema than for hemangioblastomas, Am. J. Ophthalmol. 136 (2003) 194–196.
[151] S. Madhusudan, G. Deplanque, J.P. Braybrooke, E. Cattell, M. Taylor, P. Price, M.D. Tsaloumas, N. Moore, S.M. Huson, C. Adams, P. Frith, P. Scigalla, A.L. Harris, Antiangiogenic therapy for von Hippel–Lindau disease, JAMA 291 (2004) 943–944.
[152] I. Schlemminger, D.R. Mole, L.A. McNeill, A. Dhanda, K.S. Hewitson, Y.M. Tian, P.J. Ratcliffe, C.W. Pugh, C.J. Schofield, Analogues of dealanylalahopcin are inhibitors of human HIF prolyl hydroxylases, Bioorg. Med. Chem. Lett. 13 (2003) 1451–1454.
[153] T.M. Asikainen, A. Ahmad, B.K. Schneider, W.B. Ho, M. Arend, M. Brenner, V. Gunzler, C.W. White, Stimulation of HIF-1alpha, HIF-2alpha, and VEGF by prolyl 4-hydroxylase inhibition in human lung endothelial and epithelial cells, Free Radic. Biol. Med. 38 (2005) 1002–1013.
[154] T.M. Asikainen, B.K. Schneider, N.S. Waleh, R.I. Clyman, W.B. Ho, L.A. Flippin, V. Gunzler, C.W. White, Activation of hypoxia-inducible factors in hyperoxia through prolyl 4-hydroxylase blockade in cells and explants of primate lung, Proc. Natl. Acad. Sci. USA 102 (2005) 10212–10217.
[155] M.A. McDonough, L.A. McNeill, M. Tilliet, C.A. Papamicael, Q.Y. Chen, B. Banerji, K.S. Hewitson, C.J. Schofield, Selective inhibition of factor inhibiting hypoxia-inducible factor, J. Am. Chem. Soc. 127 (2005) 7680–7681.
[156] T. Date, S. Mochizuki, A.J. Belanger, M. Yamakawa, Z. Luo, K.A. Vincent, S.H. Cheng, R.J. Gregory, C. Jiang, Expression of constitutively stable hybrid hypoxia-inducible factor-1alpha protects cultured rat cardiomyocytes against simulated ischemia–reperfusion injury, Am. J. Physiol. Cell Physiol. 288 (2005) C314–C320.
[157] R.R. Ratan, A. Siddiq, L. Aminova, P.S. Lange, B. Langley, I. Ayoub, J. Gensert, J. Chavez, Translation of ischemic preconditioning to the patient: prolyl hydroxylase inhibition and hypoxia inducible factor-1 as novel targets for stroke therapy, Stroke 35 (2004) 2687–2689.
[158] Z. Cai, D.J. Manalo, G. Wei, E.R. Rodriguez, K. Fox-Talbot, H. Lu, J.L. Zweier, G.L. Semenza, Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia–reperfusion injury, Circulation 108 (2003) 79–85.
[159] M. Bergeron, J.M. Gidday, A.Y. Yu, G.L. Semenza, D.M. Ferriero, F.R. Sharp, Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain, Ann. Neurol. 48 (2000) 285–296.
[160] M. Matsumoto, Y. Makino, T. Tanaka, H. Tanaka, N. Ishizaka, E. Noiri, T. Fujita, M. Nangaku, Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats, J. Am. Soc. Nephrol. 14 (2003) 1825–1832.
[161] C. Rosenberger, S.N. Heyman, S. Rosen, A. Shina, M. Goldfarb, W. Griethe, U. Frei, P. Reinke, S. Bachmann, K.U. Eckardt, Up-regulation of HIF in experimental acute renal failure: evidence for a protective transcriptional response to hypoxia, Kidney Int. 67 (2005) 531–542.
[162] J.I. Nwogu, D. Geenen, M. Bean, M.B. Brenner, X. Huang, P.M. Buttrick, Inhibition of collagen synthesis with prolyl 4-hydroxylase inhibitor improves left ventricular function and alters the pattern of left ventricular dilation after myocardial infarction, Circulation 104 (2001) 2216–2221.
[163] K.G. Shyu, M.T. Wang, B.W. Wang, C.C. Chang, J.G. Leu, P. Kuan, H. Chang, Intramyocardial injection of naked DNA encoding HIF-1alpha/VP16 hybrid to enhance angiogenesis in an acute myocardial infarction model in the rat, Cardiovasc. Res. 54 (2002) 576–583.
[164] M.F. Czyzyk-Krzeska, J. Meller, Von Hippel–Lindau tumor suppressor: not only HIF's executioner, Trends Mol. Med. 10 (2004) 146–149.
[165] T. Bishop, K.W. Lau, A.C. Epstein, S.K. Kim, M. Jiang, D. O'Rourke, C.W. Pugh, J.M. Gleadle, M.S. Taylor, J. Hodgkin, P.J. Ratcliffe, Genetic analysis of pathways regulated by the von Hippel–Lindau tumor suppressor in *Caenorhabditis elegans*, PLoS Biol. 2 (2004) e289.
[166] M. Hoffman, M. Ohh, H. Yang, J. Klco, M. Ivan, W.J. Kaelin, von Hippel–Lindau protein mutants linked to type 2C VHL disease preserve the ability to downregulate HIF, Hum. Mol. Genet. 10 (2001) 1019–1027.
[167] W.G. Kaelin, Molecular basis of the VHL hereditary cancer syndrome, Nat. Rev. Cancer 2 (2002) 673–682.
[168] S. Clifford, M. Cockman, A. Smallwood, D. Mole, E. Woodward, P. Maxwell, P. Ratcliffe, E. Maher, Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel–Lindau disease, Hum. Mol. Genet. 10 (2001) 1029–1038.
[169] D. Elson, G. Thurston, L. Huang, D. Ginzinger, D. McDonald, R. Johnson, J. Arbeit, Induction of hypervascularity without leakage or

inflammation in transgenic mice overexpressing hypoxia-inducible factor-1α, Genes Dev. (2001) 2520–2532.
[170] K. Vincent, K. Shyu, Y. Luo, M. Magner, R. Tio, C. Jiang, M. Goldberg, G. Akita, R. Gregory, J. Isner, Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1alpha/VP16 hybrid transcription factor, Circulation 102 (2000) 2255–2261.
[171] I.A. Lubensky, J.R. Gnarra, P. Bertheau, M.M. Walther, W.M. Linehan, Z. Zhuang, Allelic deletions of the VHL gene detected in multiple microscopic clear cell renal lesions in von Hippel–Lindau disease patients, Am. J. Pathol. 149 (1996) 2089–2094.
[172] Z. Zhuang, P. Bertheau, M. Emmert-Buck, L. Liotta, J. Gnarra, W. Linehan, I. Lubensky, A microscopic dissection technique for archival DNA analysis of specific cell populations in lesions <1 mm in size, Am. J. Pathol. 146 (1995) 620–625.
[173] S.O. Ang, H. Chen, K. Hirota, V.R. Gordeuk, J. Jelinek, Y. Guan, E. Liu, A.I. Sergueeva, G.Y. Miasnikova, D. Mole, P.H. Maxwell, D.W. Stockton, G.L. Semenza, J.T. Prchal, Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia, Nat. Genet. 32 (2002) 614–621.
[174] V.R. Gordeuk, A.I. Sergueeva, G.Y. Miasnikova, D. Okhotin, Y. Voloshin, P.L. Choyke, J.A. Butman, K. Jedlickova, J.T. Prchal, L.A. Polyakova, Congenital disorder of oxygen sensing: association of the homozygous Chuvash polycythemia VHL mutation with thrombosis and vascular abnormalities but not tumors, Blood 103 (2004) 3924–3932.
[175] F. Chen, T. Kishida, M. Yao, T. Hustad, D. Glavac, M. Dean, J.R. Gnarra, M.L. Orcutt, F.M. Duh, G. Glenn, J. Green, Y.E. Hsia, J. Lamiell, H.W. Ming, L. Schmidt, T. Kalman, I. Kuzmin, T. Stackhouse, F. Latif, W.M. Linehan, M. Lerman, B. Zbar, Germline mutations in the von Hippel–Lindau disease tumor suppressor gene:correlations with phenotype, Hum. Mutat. 5 (1995) 66–75.
[176] P. Crossey, K. Foster, F. Richards, M. Phipps, F. Latif, K. Tory, M. Jones, E. Bentley, R. Kumar, M. Lerman, B. Zbar, N. Affara, M. Ferguson-Smith, E. Maher, Molecular genetic investigation of the mechanism of tumourigenesis in von Hippel–Lindau disease: analysis of allele loss in VHL tumours, Hum. Genet. 93 (1994) 53–58.
[177] M.A. Zeiger, B. Zbar, H. Keiser, W.M. Linehan, J.R. Gnarra, Loss of heterozygosity on the short arm of chromosome 3 in sporadic, von Hippel–Lindau disease-associated, and familial pheochromocytoma, Genes Chromosomes Cancer 13 (1995) 151–156.
[178] B. Bender, M. Gutsche, S. Glasker, B. Muller, G. Kirste, C. Eng, H. Neumann, Differential genetic alterations in von Hippel–Lindau syndrome-associated and sporadic pheochromocytomas, J. Clin. Endocrinol. Metab. 85 (2000) 4568–4574.
[179] A. Prowse, A. Webster, F. Richards, S. Richard, S. Olschwang, F. Resche, N. Affara, E. Maher, Somatic inactivation of the VHL gene in Von Hippel–Lindau disease tumors, Am. J. Hum. Genet. 60 (1997) 761–765.
[180] J. Bryant, J. Farmer, L.J. Kessler, R.R. Townsend, K.L. Nathanson, Pheochromocytoma: the expanding genetic differential diagnosis, J. Natl. Cancer Inst. 95 (2003) 1196–1204.
[181] E.R. Maher, C. Eng, The pressure rises: update on the genetics of phaeochromocytoma, Hum. Mol. Genet. 11 (2002) 2347–2354.
[182] S. Estus, W.J. Zaks, R.S. Freeman, M. Gruda, R. Bravo, E.M. Johnson Jr., Altered gene expression in neurons during programmed cell death: identification of c-jun as necessary for neuronal apoptosis, J. Cell Biol. 127 (1994) 1717–1727.
[183] K.H. Schlingensiepen, F. Wollnik, M. Kunst, R. Schlingensiepen, T. Herdegen, W. Brysch, The role of Jun transcription factor expression and phosphorylation in neuronal differentiation, neuronal cell death, and plastic adaptations in vivo, Cell Mol. Neurobiol. 14 (1994) 487–505.
[184] J. Ham, C. Babij, J. Whitfield, C.M. Pfarr, D. Lallemand, M. Yaniv, L.L. Rubin, A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death, Neuron 14 (1995) 927–939.
[185] Z. Xia, M. Dickens, J. Raingeaud, R.J. Davis, M.E. Greenberg, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, Science 270 (1995) 1326–1331.
[186] K.S. Vogel, C.I. Brannan, N.A. Jenkins, N.G. Copeland, L.F. Parada, Loss of neurofibromin results in neurotrophin-independent survival of embryonic sensory and sympathetic neurons, Cell 82 (1995) 733–742.
[187] B.A. Tsui-Pierchala, J. Milbrandt, E.M. Johnson Jr., NGF utilizes c-Ret via a novel GFL-independent, inter-RTK signaling mechanism to maintain the trophic status of mature sympathetic neurons, Neuron 33 (2002) 261–273.
[188] G. Dechant, Chat in the trophic web: NGF activates Ret by inter-RTK signaling, Neuron 33 (2002) 156–158.
[189] S. Lee, E. Nakamura, H. Yang, W. Wei, M. Linggi, M. Sajan, R. Farese, F. RS, B. Carter, W. Kaelin, S. Schlisio, Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: Developmental Culling and Cancer, Cancer Cell 8 (2005) 155–167.
[190] H. Okuda, K. Saitoh, S. Hirai, K. Iwai, Y. Takaki, M. Baba, N. Minato, S. Ohno, T. Shuin, The von Hippel–Lindau tumor suppressor protein mediates ubiquitination of activated atypical protein kinase C, J. Biol. Chem. 276 (2001) 43611–43617.
[191] S. Pal, K. Claffey, H. Dvorak, D. Mukhopadhyay, The von Hippel–Lindau gene product inhibits vascular permeability factor/vascular endothelial growth factor expression in renal cell carcinoma by blocking protein kinase C pathways, J. Biol. Chem. 272 (1997) 27509–27512.
[192] S. Pal, K. Claffey, H. Cohen, D. Mukhopadhyay, Activation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription requires specific interaction with protein kinase Cζ, J. Biol. Chem. 273 (1998) 26277–26280.
[193] A. Kieser, T. Seitz, H.S. Adler, P. Coffer, E. Kremmer, P. Crespo, J.S. Gutkind, D.W. Henderson, J.F. Mushinski, W. Kolch, H. Mischak, Protein kinase C-zeta reverts v-raf transformation of NIH-3T3 cells, Genes Dev. 10 (1996) 1455–1466.
[194] E. Lipscomb, P. Sarmiere, R. Crowder, R. Freeman, Expression of the SM-20 gene promotes death in nerve growth factor-dependent sympathetic neurons, J. Neurochem. 73 (1999) 429–432.
[195] E. Lipscomb, P. Sarmiere, R. Freeman, SM-20 is a novel mitochondrial protein that causes caspase-dependent cell death in nerve growth factor-dependent neurons, J. Biol. Chem. 276 (2001) 11775–11782.
[196] J.A. Straub, E.A. Lipscomb, E.S. Yoshida, R.S. Freeman, Induction of SM-20 in PC12 cells leads to increased cytochrome c levels, accumulation of cytochrome c in the cytosol, and caspase-dependent cell death, J. Neurochem. 85 (2003) 318–328.