Neuronal apoptosis by prolyl hydroxylation: implication in nervous system tumours and the Warburg conundrum

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Abstract

Oxygen-sensing mechanisms are often dysfunctional in tumours. Oxygen sensing is mediated partly via prolyl hydroxylation. The EglN prolyl hydroxylases are well characterized in regulating the hypoxia inducible factor α (HIF-α) hypoxic response, but also are implicated in HIF-independent processes. EglN3 executes apoptosis in neural precursors during development and failure of EglN3-mediated apoptosis can lead to certain forms of sympathetic nervous system tumours. Mutations in metabolic/mitochondrial enzymes (SDH, FH, IDH) impair EglN activity and predisposes to certain cancers. This is because the EglNs not only require molecular oxygen to execute hydroxylation, but also equally require the electron donor L-ketoglutarate, a metabolite from the Krebs cycle. Therefore EglN enzymes are considered oxygen- and also metabolic sensors. L-Ketoglutarate is crucial for EglN hydroxylation activity, whereas the metabolites succinate and fumarate are inhibitors of the EglN enzymes. Since EglN activity is dependent upon metabolites that take part in the Krebs cycle, these enzymes are directly tied into the cellular metabolic network. Cancer cells tend to convert most glucose to lactate regardless of whether oxygen is present (aerobic glycolysis), an observation that was first made by Otto Warburg in 1924. Despite the striking difference in ATP production, cancer cells might favour aerobic glycolysis to escape from EglN hydroxylation, resulting in the accumulation of oncogenic HIF-α and/or resistance to EglN3-mediated apoptosis.

Keywords: prolyl hydroxylase EglN • PHD • neuronal apoptosis • phaeochromocytoma • neuroblastoma • gliomas • Otto Warburg • Krebs cycle TCA • succinate dehydrogenase SDH • isocitrate dehydrogenase IDH

Introduction

Oxygen-sensing mechanisms enable the cell to adapt to low oxygen environments and are also critical for normal development and apoptosis. Disruptions of oxygen-sensing pathways can lead to the development of certain forms of cancer [1–15]. Oxygen sensing is mediated partly via the EglN hydroxylases (EglN1/EglN2/EglN3) that are dependent upon molecular oxygen, iron (II) and L-ketoglutarate to perform proline hydroxylation on their target [16–20]. Therefore these enzymes are considered oxygen and also metabolic sensors. The availability of molecular oxygen is absolutely required for the hydroxylation reaction, because it donates the oxygen atom to the hydroxyl group [21]. But equally important for the hydroxylation reaction is the electron donor L-ketoglutarate, a metabolite from the Krebs cycle. Since EglN is dependent upon metabolites that take part in the Krebs cycle, they are directly tied into the cellular metabolic network (Fig. 1).

The first identified substrate for the EglN prolyl hydroxylases is the transcription factor hypoxia inducible factor α (HIF-α) [16, 17]. The identification of HIF-α as a direct hydroxylation substrate provided the first and direct link between tumour suppressor function and oxygen sensing. The tumour suppressor von Hippel
Lindau (VHL) acts as an ubiquitin ligase by targeting hydroxylated HIF-α for degradation when oxygen is available, as where under low oxygen environments, HIF-α escapes hydroxylation and subsequently escapes VHL recognition [3–5, 16, 22–27]. The escape from VHL recognition allows HIF-α to accumulate and to transactivate its target genes that are important for adaptation to low oxygen environment including energy metabolism and angiogenesis [28–30]. Both HIF-dependent and HIF-independent VHL functions contribute to VHL-defective tumorigenesis [31–34]. The VHL disease is caused by inactivating germline mutations of the VHL gene and predisposes to a variety of tumours including haemangioblastoma of the retina and nervous system, clear cell renal carcinomas (RCC, the most common form of kidney cancer) and pheochromocytomas/paragangliomas, tumours of the sympathoadrenal nervous system. HIF-α deregulation appears to have a causal role in VHL-defective clear cell RCC and in VHL-defective blood vessel tumours haemangioblastomas (HB) [33]. Although HIF-α regulation has been a major focus of VHL research, the genotype–phenotype correlation in the VHL disease gave insight into HIF-independent VHL function (Table 1). Specific VHL germline mutations corresponding to a specific subset of tumour phenotypes have a different relationship to HIF-α deregulation (categorized in type I/IIA/IIIB/IIIC disease) [35–37]. The type I disease associates with RCC and HB, and their tumours reflect deregulated high HIF-α expression, whereas a very different clinical outcome is observed in the type 2C-VHL disease. The type 2C disease does not develop RCC and HB instead develops pheochromocytomas only. More importantly, HIF-α is not deregulated and maintained in low levels in the type 2C tumours [31, 32]. These findings suggest that HIF-α deregulation is not necessary for pheochromocytoma development in VHL disease.

Interestingly, a second hydroxylation target of the prolyl hydroxylases EglN has recently been identified [38]. The β2-adrenergic receptor is a prototypic G protein-coupled receptor, which is hydroxylated by EglN3 and also oxygen dependent degraded by pVHL. The discovery of another hydroxylation substrate by the EglN enzymes not only broadens the functionality of prolyl hydroxylation, but also expands our understanding of cellular response to oxygen and its relationship to disease.

Therefore, the identification of other EglN substrates and the clues from the genotype–phenotype correlation that emerged in the VHL disease clearly indicates HIF-independent VHL functions. Of great insights are studies from EglN3-mediated neuronal apoptosis during sympathetic neuronal development, which shed light onto the genesis of pheochromocytoma by presumably HIF-independent pathways [9].

Lessons from a rare disease

Phaeochromocytomas are rare, with only five to eight cases diagnosed per million people a year [39]. They are neoplasias of neural crest origin arising from the adrenal medulla. Pheochromocytomas can also develop in extra-adrenal sympathetic ganglia 10% of the time. Extra-adrenal pheochromocytomas are sometimes referred to as paragangliomas [40]. In short, these tumours are sympathetic nervous system tumours. The predisposing germline mutations lend clues to the pathogenesis of this disease. The most frequent causes of pheochromocytoma susceptibility are VHL missense mutations, activating
mutations in c-RET, mutations in neurofibromatosis type 1 (NF1) and mutations in succinate dehydrogenase subunits of mitochondrial complex II (SDH B/C/D). Loss of NF1 has been reported to promote survival of embryonic sympathetic neurons in the absence of the nerve growth factor NGF [41]. Further, mutations in subunits of mitochondrial complex II suggest impairment of mitochondria-mediated apoptosis and have led to the early hypothesis that failure of apoptosis in neuroendocrine precursor cells could result in the development of pheochromocytoma and paraganglioma [40]. Given the evidence that NF1 promotes sympathetic neuronal survival and further that these neoplasias originate from the sympathetic nervous system, a closer look into the sympathetic neuronal development reveals important clues to the genesis of these tumours [9].

Since the discovery by Rita Levi-Montalcini and Viktor Hamburger of the neurotrophic factor NGF, our understanding of developmental apoptosis in the sympathetic nervous system has greatly increased [42]. During neuronal development, NGF is required for survival but is also limiting. Neurons that successfully compete for NGF during development survive whereas unsuccessful neurons die. Competition for NGF is an important developmental process for matching the size of a neuronal population, especially in the peripheral nervous system [43]. As much as 50% of neurons produced during embryogenesis die by apoptosis during neuronal development. Abnormal NGF signalling has been linked to paediatric nervous system tumours such as neuroblastoma [44, 45] and disease-associated mutations such as NF1 have been shown to enhance signalling by NGF receptors and promote neuronal survival in the absence of NGF [41]. In the last decades it became evident that JNK/c-Jun signalling is required for apoptosis when NGF is limiting in the developing nervous system [46–50]. Interestingly, the prolyl hydroxylase EglN3 has recently been implicated in this pathway. EglN3 is induced in sympathetic neurons deprived of NGF and further has pro-apoptotic activity when overexpressed [51]. Given that EglN3 is known to hydroxylate HIF-α and has been implicated in developmental apoptosis in sympathetic neurons, the following questions arise: (i) Does EglN3-mediated apoptosis depend upon its hydroxylation activity? (ii) Does it depend upon HIF hydroxylation or does it involve hydroxylation of unknown substrates? (iii) Is failure of EglN3-mediated apoptosis implicated in the genesis of pheochromocytomas and other tumours arising from the neural crest origin?

### Specificity of function within the EglN prolyl hydroxylases

The ability of EglN3 to induce neuronal apoptosis appears to be unique among the EglN family members [9]. Induction of apoptosis is dependent upon EglN3 hydroxylation activity, because catalytic impaired EglN3 fails to induce apoptosis [9, 52]. Importantly, EglN3-induced apoptosis is not diminished in the presence of stable HIF1α or HIF2α variants that cannot be hydroxylated on their prolines [9]. This suggests that hydroxylation targets of EglN3 other than HIF-α are crucial for apoptosis function.

EglN2 and EglN3 are induced by hypoxia and dampen the HIF-α response under chronic hypoxia [53–57]. However, EglN1 appears to be the primary HIF prolyl hydroxylase under normal conditions [58]. Consistent with this, mice lacking EglN2 or EglN3 are viable and grossly normal whereas mice lacking EglN1 are not viable [59]. Conditional inactivation of EglN1 in mice leads to polycythaemia due to HIF-α stabilization and increased transcription of HIF target genes including erythropoietin [60, 61]. Further, patients carrying EglN1 mutations have been reported to develop polycythaemia concluding that EglN1 couples HIF-α stability in vivo [13, 62, 63].

Consistent with the unique role of EglN3 in neuronal apoptosis are studies obtained from the EglN3-deficient mice that clearly demonstrate the requirement for EglN3-mediated apoptosis in the sympathetic neuronal development. EglN3-deficient sympathetic neurons are resistant to apoptosis after NGF withdrawal, as well as certain neurotoxins [64]. Consistently, EglN3−/− mice have an increased number of cells in the super cervical ganglia and in the adrenal medulla and show abnormalities in the sympathetic-adrenal system including systemic hypotension [65]. In fact, it appears that some of the adrenal abnormalities are caused through deregulation of the β2-adrenergic receptor (β2-AR) [38]. EglN3 specifically hydroxylates β2-AR, whereas EglN1 and EglN2 fail to do so. This leads to subsequent ubiquitination and degradation mediated by pVHL. Loss of EglN3 results in β2-AR up-regulation and accordingly, hypoxia stabilizes the β2-AR. This is consistent with the observations from the type 2C VHL patients that develop pheochromocytoma. Type 2C patients often show excessive secretion of catecholamines (endogenous β2-AR ligands) and increased sympathetic nerve activity [66].

In summary, this points towards distinct and unique functions within the family of EglN prolyl hydroxylases. Identification of
novel EglN hydroxylation targets will open new oxygen sensing pathways independent of what we have learned from HIF-α.

**Failure of EglN3-mediated apoptosis in the genesis of phaeochromocytoma**

Given the evidence that (i) phaeochromocytomas are sympathetic nervous system tumours and that (ii) EglN3 is critical for apoptosis during sympathetic neural development, an attractive hypothesis emerged in which failure of EglN3-mediated apoptosis during sympathetic development predisposes to the genesis of phaeochromocytomas and perhaps other neoplasia arising from neural crest origin [9]. Interestingly, apparently unlinked phaeochromocytoma lesions (VHL, NF1, c-RET and SDH) all appear to act on a single common pathway by decreasing EglN3-mediated apoptosis at the time during development when levels of NGF become limiting (Fig. 2) [9]. During this developmental window, the sympathetic precursors undergo c-Jun-dependent apoptosis [67–70]. EglN3 appears to act downstream of the c-Jun in the NGF signalling pathway [9]. Therefore, a single pathway was established in which the genetic phaeochromocytoma defects act either directly on EglN3 or upstream of EglN3 to impair apoptosis (Fig. 2). For instance, the genetic defect in SDH acts directly on EglN3-mediated apoptosis function. SDH inhibition results in accumulation of intracellular succinate, which in turn can product-inhibit the EglN prolyl hydroxylases [9, 71]. This succinate-mediated inhibition not only results in HIFα stabilization, but also importantly inhibits EglN3-mediated apoptosis. Further, SDH inhibition blunts neuronal apoptosis when NGF is limiting [9]. The succinate-mediated product-inhibition of EglN3 was overcome by re-addition of α-ketoglutarate restoring NGF-mediated apoptosis [9]. This suggests that SDH inhibition acts on the prolyl hydroxylases via succinate and not as alternatively suggested through the generation of reactive oxygen species.

In addition to the predisposing SDH genetic lesion, other phaeochromocytoma lesions have been implicated upstream of EglN3 to promote neuronal survival in the NGF signalling pathway. In the case of VHL predisposing lesion, loss of pVHL promotes survival through up-regulation of JunB. pVHL suppresses JunB and all VHL mutants tested (including type IIC VHL disease mutant) abrogate its ability to do so [9]. JunB acts as an antagonist of c-Jun and increased JunB levels attenuate the induction of c-Jun-mediated apoptosis in sympathetic neurons deprived from NGF.

Finally, previous evidence indicated that RET (the receptor for glial-derived neurotrophic factor) and NF1 could act through this pathway by modulating the action of the NGF receptor TrkA. Loss of NF1 promotes NGF-independent survival of embryonic peripheral neurons [41]. In addition, c-RET can crosstalk with the NGF receptor TrkA [72]. Activation of c-RET, like loss of pVHL, leads to the induction of JunB and attenuates apoptosis when NGF becomes limiting [9].

Thus, when mutated, all the genetic phaeochromocytoma lesions (SDH, VHL, c-RET and NF1) impair NGF-mediated apoptosis in neuroendocrine precursors during development. These findings provide an explanation as to why the mutations in tumour suppressors that are found in familial phaeochromocytoma are rare in sporadic phaeochromocytoma. This is because the pathway is no longer critical once development is completed.

In summary, all the genetic lesions associated with phaeochromocytoma act on a single common pathway that impinges upon EglN3 apoptotic activity during sympathetic neuronal development. Mutations in SDH, VHL, c-RET and NF1 would allow sympathetic neuronal precursors to escape from developmental apoptosis and set the stage for their neoplastic transformation. It will be interesting to determine not only if EglN3 is mutated in these neoplasias, but also, if failure of EglN3 developmental apoptosis plays a broader role in paediatric cancers and other forms of hereditary cancer.
Understanding the mechanistic basis of Egln3 killing

Egln3-mediated apoptosis is hydroxylation dependent, but independent of HIF-α hydroxylation [9]. An important challenge is to identify the link between this enzyme and apoptosis, which presumably involves hydroxylation of a protein other than HIF-α.

Early studies indicated that Egln3 mRNA and protein expression (at that time referred as SM-20) increases shortly after removal of NGF in primary sympathetic neurons and peaks between 10 and 15 hrs at a time when cells undergo apoptosis [51]. Overexpression of Egln3 is sufficient to promote cell death in NGF-maintained sympathetic neurons [51, 73]. Cell death is caspase dependent and accompanied by an increase of cytochrome c in cytosolic and mitochondria-enriched subcellular fractions [74]. The mechanism underlying Egln3-induced cytochrome c is not known although it appears to involve an increase in cytochrome c mRNA. Other studies have identified Egln3 as a growth factor inducible gene in vascular smooth muscle cells [75] and later proposed, that it might function in growth arrest, differentiation and cell death during muscle differentiation [76, 77]. Expression of Egln3 was also reported in fibroblasts upon activation of a temperature-sensitive form of p53 [78]. However, Egln3-induced apoptosis appears not to be impaired in cells lacking p53 or expressing a dominant negative p53 protein, indicating that p53 might not function downstream of Egln3 to induce apoptosis [9]. In addition, a recent study indicated that Egln3’s ability to induce apoptosis correlates with the formation of aggresome-like structures [52].

Recently, an unbiased genome-wide approach has been undertaken to understand how Egln3 causes neuronal cell death. A short interference RNA library was used to identify genes that suppress Egln3-induced apoptosis [64]. This led to the identification of the kinesin Kif1bβ as a downstream effector of Egln3. Kif1bβ, a member of its family, consists of two major splice variants α and β. Kif1bα and Kif1bβ are motor proteins implicated in anterograde transport of mitochondria and synaptic vesicle precursors, respectively [79, 80]. The recent study, which identified Kif1bβ as an Egln3 downstream target showed how Kif1bβ is both necessary and sufficient for neuronal apoptosis when NGF becomes limiting, but it remained unclear how Egln3 regulates Kif1bβ [64]. Interestingly, KIF1Bβ maps on to the chromosomal region 1p36.2, a region of the genome that is frequently deleted in neural crest-derived tumours including neuroblastomas [81]. The existence of one or more human tumour suppressor genes on chromosome 1p has been suspected for decades [82–84]. Further suggestion that KIF1Bβ acts as a 1p36 tumour suppressor comes from the current model for phaeochromocytoma development. Phaeochromocytomas develop when sympathetic neuronal precursors escape from Egln3-mediated developmental apoptosis, suggesting that mutations in KIF1Bβ may be relevant to phaeochromocytoma and other tumours of neuronal origin. Indeed, inherited loss-of-function KIF1Bβ missense mutations have been identified in phaeochromocytomas and neuroblastomas and an acquired loss-of-function mutation in a medullablastoma, arguing that KIF1Bβ is a pathogenic target of these deletions [64]. Nonetheless, it has been further reported that the remaining KIF1Bβ allele in 1p deleted tumours and cell lines is often wild-type, contrary to the Knudson TwoHit scenario [64, 85–88]. Perhaps KIF1Bβ haplinsufficiency is adequate for tumorgenesis in some contexts. Also, the existence of multiple neuroblastoma and phaeochromocytoma suppressor genes on 1p has been suggested [89, 90]. Additional studies are needed to address how often it is deregulated, epigenetically or genetically, in cancer. A spotlight is now on understanding the mechanistic basis of how Egln3 regulates KIF1Bβ and how this translates into cell death. KIF1Bβ appears not be a direct Egln3 hydroxylation target, but Egln3 hydroxylation activity is required for KIF1Bβ regulation. Therefore Egln3 presumably involves hydroxylation of a protein that in turn might regulate KIF1Bβ to induce apoptosis.

Connecting Egln activity to the Warburg conundrum

In 1924, Otto Warburg observed that cancer cells are highly glycolytic in the presence of oxygen and have reduced rates of oxidative phosphorylation [91]. Recent studies have argued that cancer cells might benefit from this persistence of high lactate production in the presence of oxygen, referred to as aerobic glycolysis or pseudohypoxia [92, 93]. From a bioenergetic perspective, it remains a conundrum how highly metabolic proliferative cancer cells undergo a pathway that results in 10 times less ATP production compared to their normal counterparts that oxidize their glycolysis endproduct within the mitochondria via the Krebs cycle (pyruvate conversion into Acetyl-CoA, Fig. 3). Interestingly, three major enzymes of the Krebs cycle (SDH, FH, IDH) have been recently identified as bona fide tumour suppressors, but more importantly, inactivation in either of them directly affects Egln activity [9, 10, 14, 71]. This is because the Egln activity is not only dependent upon molecular oxygen to perform their hydroxylation reaction, but also require the Krebs cycle metabolite α-ketoglutarate as electron donor, which ties them directly into this metabolic network. Therefore an attractive hypothesis arises: cancer cells favour aerobic glycolysis to inhibit Egln activity in order to escape either certain oncogenic apoptotic signals and/or activate oncogenic HIF-α. About 70% or more of low-grade gliomas bear loss of function mutation in IDH1 and IDH2 [94, 95]. Loss of function mutation in IDH1 results in a decrease of intracellular α-ketoglutarate. Subsequently Egln prolyl hydroxylases are inhibited in their hydroxylase activity with the consequences of HIF-α stabilization [14]. Presumably Egln3-mediated apoptosis might be impaired in these settings as well, but this has not been investigated yet. Further, these studies follow the discoveries that germline mutation in SDH are linked to phaeochromocytoma and that FH mutation lead to leiomysosarcoma and renal cell carcinoma, both of which affect also the Egln prolyl hydroxylase activity [9, 10, 71]. This is because loss of function mutation in SDH and
FH increases the accumulation of succinate and fumarate respectively. The excess of these metabolites inhibits the proline hydroxylases with the consequences in either accumulation of oncogenic HIF$\alpha$ and/or blunting EglN3-mediated apoptosis [9, 10, 71, 96].

**Future directions**

The recently identified mutations in the metabolic/mitochondrial enzymes (FH, SDHB/C/D, IDH1/2) provide convincing evidence that alteration in cellular metabolism contributes to the pathogenesis of cancer. Hence, 43 years later we are beginning to learn the depth of Otto Warburg’s foresight that ‘cancer cells should be interpreted as a mitochondrial dysfunction’ and that ‘the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar’ [97, 98]. Even if this is the prime cause for some cancers, the underlying mechanistic basis remains controversial. However, recently it has become more evident that the inactivation of the EglN prolyl hydroxylases is a downstream effector of these mitochondrial alterations.

Consequently, EglN inhibition results in activation of oncogenic HIF$\alpha$ and/or resistant to EglN3-mediated apoptosis. The identification of SDH and FH mutations has already led to the development of cell permeable $\alpha$-ketoglutarate derivates with the potential to suppress the transforming effects of these mutations through reactivation of the EglNs [99]. The exciting part of identifying metabolic-enzyme mutations in specific cancers is that they are ‘druggable’. They
might provide new opportunities as therapeutic targets that would be more susceptible and more effective than existing cancer therapies. Future work is needed to further elucidate the direct impact of cancer metabolism in prolyl hydroxylase functioning.

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References

1. Carmeliet P, Dor Y, Herbert JM, et al. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature. 1998; 394: 485–90.
2. Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J. 1998; 17: 3005–15.
3. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999; 399: 271–5.
4. Ivan M, Kondo K, Yang H, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science. 2001; 292: 464–8.
5. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitination complex by O2-regulated prolyl hydroxylation. Science. 2001; 292: 468–72.
6. Haase VH, Glickman JN, Socolovsky M, et al. Vascular tumors in livers with targeted inactivation of the von Hippel-Lindau tumor suppressor. Proc Natl Acad Sci USA. 2001; 98: 1583–8.
7. Kondo K, Kico J, Nakamura E, et al. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer Cell. 2002; 1: 237–46.
8. Rankin EB, Higgins DF, Walisser JA, et al. Inactivation of the arylhydrocarbon receptor nuclear translocator (Arnt) suppresses von Hippel-Lindau disease-associated vascular tumors in mice. Mol Cell Biol. 2005; 25: 3163–72.
9. Lee S, Nakamura E, Yang H, et al. Neuronal apoptosis linked to EgN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. Cancer Cell. 2005; 8: 155–67.
10. Isaacs JS, Jung YJ, Moie DR, et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell. 2005; 8: 143–53.
11. Erler JT, Bennenwith KL, Nicolau M, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature. 2006; 440: 1222–6.
12. Kim WY, Safran M, Buckley MR, et al. Failure to prolyl hydroxylate hypoxia-inducible factor alpha phenocopies VHL inactivation in vivo. EMBO J. 2006; 25: 4650–62.
13. Ladroue C, Carcenac R, Leporrier M, et al. PHD2 mutation and congenital erythrocytosis with paraganglioma. N Engl J Med. 2008; 359: 2685–92.
14. Zhao S, Lin Y, Xu W, et al. Gliona-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science. 2009; 324: 261–5.
15. Gordan JD, Simon MC. Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev. 2007; 17: 71–7.
16. Epstein AC, Gleade JM, McNeill LA, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell. 2001; 107: 43–54.
17. Ivan M, Haberberger T, Gervasi DC, et al. Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. Proc Natl Acad Sci USA. 2002; 99: 13459–64.
18. Kivirikko KI, Myllyharju J. A conserved prolyl 4-hydroxylase subunit. Matrix Biol. 1998; 16: 357–68.
19. Schofield CJ, Zhang Z. Structural and mechanistic studies on 2-oxoglutarate-dependent oxygenases and related enzymes. Curr Opin Struct Biol. 1999; 9: 722–31.
20. Bricuk RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science. 2001; 294: 1337–40.
21. McNeill LA, Hewitson KS, Gleade JM, et al. The use of dioxygen by HIF prolyl hydroxylase (PHD1). Bioorg Med Chem Lett. 2002; 12: 1547–50.
22. Ohh M, Park CW, Ivan M, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol. 2000; 2: 423–7.
23. Kamura T, Sato S, Iwai K, et al. Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci USA. 2000; 97: 10430–5.
24. Sutter CH, Laughner E, Semenza GL. 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. 2000; 97: 4748–53.
25. Kallio PJ, Wilson WJ, O’Brien S, et al. Regulation of the hypoxia-inducible transcription factor 1alpha by the ubiquitin-proteasome pathway. J Biol Chem. 1999; 274: 6519–25.
26. Tamimoto K, Makino Y, Pereira T, et al. Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. EMBO J. 2000; 19: 4298–309.
27. Iwai K, Yamanaka K, Kamura T, et al. Identification of the von Hippel-Lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. Proc Natl Acad Sci USA. 1999; 96: 12436–41.
28. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol. 2000; 88: 1474–80.
29. Wenger RH. Mammalian oxygen sensing, signalling and gene regulation. J Exp Biol. 2000; 203: 1253–63.
30. Hickey MM, Simon MC. Regulation of angiogenesis by hypoxia and hypoxia-inducible factors. Curr Top Dev Biol. 2006; 76: 217–57.
31. Hoffman MA, Ohh M, Yang H, et al. von Hippel-Lindau protein mutants linked to type 2C VHL disease preserve the ability to downregulate HIF. Hum Mol Genet. 2001; 10: 1019–27.
32. Clifford SC, Cockman ME, Smallwood AC, et al. Contrasting effects on HIF-1alpha regulation by disease-causing pVHL mutations correlate with patterns of tumourigenesis in von Hippel-Lindau disease. Hum Mol Genet. 2001; 10: 1029–38.
33. Kaelin WG Jr. The von Hippel-Lindau tumour suppressor protein: a O2 sensing and cancer. Nat Rev. 2008; 8: 865–73.
34. Young AP, Schlisio S, Minamishima YA, et al. VHL loss actuates a HIF-independent pathway.
senescence programme mediated by Rb and p400. Nat Cell Biol. 2008; 10: 361–9.
35. Neumann HP, Bender BU. Genotype-phenotype correlations in von Hippel-Lindau disease. J Intern Med. 1998; 243: 541–5.
36. Zbar B, Kishida T, Chen F, et al. Germline mutations in the von Hippel-Lindau disease (VHL) gene in families from North America, Europe, and Japan. Hum Mutat. 1996; 8: 348–57.
37. Chen F, Kishida T, Yao M, et al. Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat. 1995; 5: 66–75.
38. Xie L, Xiao K, Whalen EJ, et al. Oxygen-regulated beta(2)-adrenergic receptor hydroxylation by EGLN3 and ubiquitilation by pVHL. Sci Signal. 2009; 2: r23.
39. Kudva YC, Sawka AM, Young WF Jr. Clinical review 164: The laboratory diagnosis of adrenal phaeochromocytoma: the Mayo Clinic experience. J Clin Endocrin Metabol. 2003; 88: 4533–9.
40. Maher ER, Eng C. The pressure rises: update on the genetics of phaeochromocytoma. Hum Mol Genet. 2002; 11: 2347–54.
41. Vogel KS, Brannan CI, Jenkins NA, et al. Loss of neurofibromin results in neurofibromatosis type 2 associated with elevated hypoxia-inducible factor (HIF)-1alpha. J Cell Biol. 1999; 145: 491–501.
42. Cowan WM. Viktor Hamburger and Rita Levi-Montalcini: the path to the discovery of nerve growth factor. Ann Rev Neurosci. 2001; 24: 551–600.
43. Oppenheim RW. Cell death during development of the nervous system. Ann Rev Neurosci. 1991; 14: 453–501.
44. Katsetos CD, Del Valle L, Legido A, et al. On the neuronal/neuroblastic nature of medulloblastomas: a tribute to Pio del Rio Hortega and Moises Polak. Acta Neuropathol. 2003; 105: 1–14.
45. Nakagawara A. Trk receptor tyrosine kinases: a bridge between cancer and neural development. Cancer Lett. 2001; 169: 107–14.
46. Maroney AC, Finn JP, Bozyczko-Cayne D, et al. CEP-1347 (KT7515), an inhibitor of JNK activation, rescues sympathetic neurons and neuronaically differentiated PC12 cells from death evoked by three distinct insults. J Neurochem. 1999; 73: 1901–12.
47. Eilers A, Whitfield J, Shah B, et al. Direct inhibition of c-Jun N-terminal kinase in sympathetic neurons prevents c-Jun promoter activation and NGF withdrawal-induced death. J Neurochem. 2001; 76: 1439–54.
48. Harding TC, Xue L, Bienemann A, et al. Inhibition of JNK by overexpression of the JNL binding domain of JIP-1 prevents apoptosis in sympathetic neurons. J Biol Chem. 2001; 276: 4531–4.
49. Harris CA, Deshmukh M, Tsui-Pierchala B, et al. Inhibition of the c-Jun N-terminal kinase signaling pathway by the mixed lineage kinase inhibitor CEP-1347 (KT7515) preserves metabolism and growth of trophic factor-deprived neurons. J Neurosci. 2002; 22: 103–13.
50. Palmada M, Kanwal S, Rutkoski NJ, et al. c-jun is essential for sympathetic neuronal death induced by NGF withdrawal but not by p75 activation. J Cell Biol. 2002; 158: 453–61.
51. Lipscomb EA, Sarmiere PD, Crowder RJ, et al. Expression of the SM-20 gene promotes death in nerve growth factor-dependant sympathetic neurons. J Neurochem. 1999; 73: 429–32.
52. Rantanen K, Pursiheimo J, Hogel H, et al. the HIF-1alpha prolyl hydroxylase domain proteins. Blood. 2007; 110: 2259–67.
53. Aprelikova O, Chandramouli GV, Wood WC, et al. Role of VHL gene mutations in families from North America, Europe, and Japan. Hum Mutat. 1995; 5: 37–38.
54. Kudva YC, Sawka AM, Young WF Jr. Clinical review 164: The laboratory diagnosis of adrenal phaeochromocytoma: the Mayo Clinic experience. J Clin Endocrin Metabol. 2003; 88: 4533–9.
55. Maher ER, Eng C. The pressure rises: update on the genetics of phaeochromocytoma. Hum Mol Genet. 2002; 11: 2347–54.
56. Vogel KS, Brannan CI, Jenkins NA, et al. Loss of neurofibromin results in neurofibromatosis type 2 associated with elevated hypoxia-inducible factor (HIF)-1alpha. J Cell Biol. 1999; 145: 491–501.
57. Apelhoff RJ, Tian YM, Raval RR, et al. Differential function of the prolyl hydroxylase genes by hypoxia-inducible factors. J Cell Biol. 2004; 19: 2231–40.
58. Appelhoff RJ, Tian YM, Raval RR, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of HIF-alpha prolyl-4-hydroxylases. Biochem J. 2004; 381: 761–7.
59. Appelhoff RJ, Tian YM, Raval RR, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of HIF-alpha-inducible factors. J Cell Biol. 2004; 92: 491–501.
60. Cioffi CL, Liu XQ, Kosinski PA, et al. Differential regulation of HIF-1 alpha prolyl-4-hydroxylase genes by hypoxia in human cardiovascular cells. Biochem Biophys Res Commun. 2003; 303: 947–53.
61. Apelhoff RJ, Tian YM, Raval RR, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem. 2004; 279: 38458–65.
62. Ginouves A, Ile K, Macias H, et al. PHDs overactivation during chronic hypoxia “desensitizes” HIFalpha and protects cells from necrosis. Proc Natl Acad Sci USA. 2008; 105: 4745–50.
63. Berra E, Benizri E, Ginouves A, et al. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003; 22: 4082–90.
64. Takeda K, Ho VC, Takeda H, et al. Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. Mol Cell Biol. 2006; 26: 8336–46.
65. Takeda K, Aguila HL, Parikh HS, et al. Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. Blood. 2008; 111: 3229–35.
66. Minamishima YA, Moslehi J, Bardeesy N, et al. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycthemia and congestive heart failure. Blood. 2008; 111: 3236–44.
67. Percy MJ, Zhao Q, Flores A, et al. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc Natl Acad Sci USA. 2006; 103: 654–9.
68. Eltzschig HK, Eckle T, Grenz A, PHD2 mutation and congenital erythrocytosis with paraganglioma. N Engl J Med. 2009; 360: 1361–2.
69. Schlisio S, Kenchappa RS, Vredeveld LC, et al. The kinesin KIF1Bbeta acts downstream from EglN3 to induce apoptosis and is a potential 1p36 tumor suppressor. Genes Dev. 2008; 22: 884–93.
70. Bishop T, Gallagher D, Pascual A, et al. Abnormal sympathoadrenal development and systemic hypotension in PHD3–/– mice. Mol Cell Biol. 2008; 28: 3386–400.
71. Schlingensiepen KH, Wolink F, Kunst M, et al. The role of Jun transcription factor expression and phosphorylation in neuronal differentiation, neuronal cell death, and plastic adaptations in vivo. Cell Mol Neurobiol. 1994; 14: 487–505.
72. Xia Z, Dickens M, Raingeaud J, et al. Opposing effects of ERK and JNK-938 MAP kinases on apoptosis. Science. 1995; 270: 1326–31.
73. Selak MA, Armour SM, MacKenzie ED, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell. 2005; 7: 77–85.
74. Dechant G. Chat in the trophic web: NGF activates Ret by inter-RTK signaling. Neuron. 2002; 33: 156–8.
73. Lipscomb EA, Sarmiere PD, Freeman RS. SM-20 is a novel mitochondrial protein that causes caspase-dependent cell death in nerve growth factor-dependent neurons. J Biol Chem. 2001; 276: 5085–92.

74. Straub JA, Lipscomb EA, Yoshida ES, et al. 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. 2003; 85: 318–28.

75. Wax SD, Tsao L, Lieb ME, et al. SM-20 is a novel 40-kd protein whose expression in the arterial wall is restricted to smooth muscle. Lab Invest J Tech Methods Pathol. 1996; 74: 797–808.

76. Moschella MC, Menzies K, Tsao L, et al. SM-20 is a novel growth factor-responsive gene regulated during skeletal muscle development and differentiation. Gene Expr. 1999; 8: 59–66.

77. Fu J, Menzies K, Freeman RS, et al. EGLN3 prolyl hydroxylase regulates skeletal muscle differentiation and myogenin protein stability. J Biol Chem. 2007; 282: 12410–8.

78. Madden SL, Galella EA, Riley D, et al. Induction of cell growth regulatory genes by p53. Cancer Res. 1996; 56: 5384–90.

79. Nangaku M, Sato-Yoshitake R, Okada Y, et al. KIF1B, a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. Cell. 1994; 79: 1209–20.

80. Zhao C, Takita J, Tanaka Y, et al. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bbeta. Cell. 2001; 105: 587–97.

81. Schwab M, Praml C, Amler LC. Genomic instability in 1p and human malignancies. Genes Chromosomes Cancer. 1996; 16: 211–29.

82. Brodeur GM, Sekhon H, Goldstein MN. Chromosomal aberrations in human neuroblastomas. Cancer. 1977; 40: 2256–63.

83. Haag MM, Soukup SW, Neely JE. Chromosome analysis of a human neuroblastoma. Cancer Res. 1981; 41: 2995–9.

84. Stoler A, Bouck N. Identification of a single chromosome in the normal human genome essential for suppression of hamster cell transformation. Proc Natl Acad Sci USA. 1985; 82: 570–4.

85. Ohira M, Kageyama H, Mihara M, et al. Identification and characterization of a 500-kb homozgyously deleted region at 1p36.2-p36.3 in a neuroblastoma cell line. Oncogene. 2000; 19: 4302–7.

86. Yang HW, Chen YZ, Takita J, et al. Genomic structure and mutational analysis of the human KIF1B gene which is homozgyously deleted in neuroblastoma at chromosome 1p36.2. Oncogene. 2001; 20: 5075–83.

87. Munirajan AK, Ando K, Mukai A, et al. KIF1Bbeta functions as a haploinsufficient tumor suppressor gene mapped to chromosome 1p36.2 by inducing apoptotic cell death. J Biol Chem. 2008; 283: 24426–34.

88. Yeh IT, Lenci RE, Qin Y, et al. A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumors. Human genetics. 2008; 124: 279–85.

89. Takeda O, Homma C, Maseki N, et al. There may be two tumor suppressor genes on chromosome arm 1p closely associated with biologically distinct subtypes of neuroblastoma. Genes Chromosomes Cancer. 1994; 10: 30–9.

90. Bagchi A, Papazoglou C, Wu Y, et al. CHD5 is a tumor suppressor at human 1p36. Cell. 2007; 128: 459–75.

91. Warburg O, Posener, K, Negelein, E. Über den Stoffwechsel der Tumoren. Biochemische Zeitschrift. 1924; 152: 319–44.

92. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev. 2004; 4: 891–9.

93. Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell. 2006; 9: 425–34.

94. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009; 360: 765–73.

95. Balss J, Meyer J, Mueller W, et al. Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol. 2008; 116: 597–602.

96. Koivunen P, Hirsila M, Remes AM, et al. Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. J Biol Chem. 2007; 282: 4524–32.

97. Warburg O. The prime cause and prevention of cancer. Lindau lecture at the meeting of the Nobel-Laureates at Lindau, Lake Constance, Germany. 1966.

98. Warburg O. On the origin of cancer cells. Science. 1956; 123: 309–14.

99. MacKenzie ED, Selak MA, Tennant DA, et al. Cell-permeating alpha-ketoglutarate derivatives alleviate pseudo-hypoxia in succinate dehydrogenase-deficient cells. Mol Cell Biol. 2007; 27: 3282–9.