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EDITORIAL

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A triple sense of oxygen promotes neurovascular angiogenesis in NG2-derived cells

Expansion and remodelling of the cerebral vasculature via the induction of pro-angiogenic genes is a crucial issue in many pathologies. Urrutia et al[1] demonstrate how NG2-derived cells in the brain sense oxygen to promote neurovascular angiogenesis.

Master regulator of cellular adaptation to hypoxia, which includes angiogenesis, is the Hypoxia inducible factor (HIF). Three isoforms exist of the cellular oxygen sensor: HIF prolyl hydroxylases (PHD)-1,-2 and -3 (EGLN-2, -1, -3, respectively), enzymes that hydroxylate prolyl residues of the regulatory subunit HIF-α,[2-4] which, thus tagged, is easily captured by the von Hippel Lindau protein (VHL),[5] and subsequently degraded by the proteasome (review in Ref.6). PHDs are ubiquitously expressed, as is the HIF system. Highly specific, synthetic, orally available, small molecule PHD-inhibitors are being tested in large phase III clinical trials for treatment of renal anaemia (review in Ref.7). Historically, erythropoietin (EPO) led to the discovery of HIF[8] and PHD,[2,3] while in the future, PHD-inhibitors may complement or even replace EPO. PHD inhibitors are potent erythropoiesis stimulating agents, comparable to EPO and its related compounds.[9] Moreover, preclinical data suggest that PHD inhibition can improve tissue responses to hypoxia, and this may ameliorate highly relevant clinical conditions like ischemic stroke (review in Ref.10), myocardial infarction (review in Ref.11), acute kidney injury (review in Ref.12) etc Major outcome data from the above mentioned clinical trials are pending. In the meantime, it's worth taking a closer look at PHD inhibition in basic science, as it likely will help us understand upcoming clinical data. In this regard, Urrutia et al[1] present a meticulous assessment of brain angiogenesis, a highly relevant topic in ischemic and degenerative central nervous disorders.[13] With help of knockout technology ranging from single to quadruple, they dissect the contributions of PHD-1 to -3 and EPO in this process. To the best of our knowledge, this is the most thoroughly conducted study addressing this topic. But, before we review their results, we need a little closer look at the HIF-PHD system: The transcription factor HIF is a heterodimer of an oxygen-dependent, variable alpha subunit (HIF-1α, -2α or -3α) and a constitutive beta subunit (HIF-β, ARNT). HIF-1α and -2α, the most important and best studied alpha subunits, each have two oxygen dependent degradation domains (ODD), one at the C-terminus (CODD), and another at the N-terminus (NODD) (review in Ref.6). PHDs are dioxygenases using molecular O_2 as substrate, and 2-oxoglutarate as co-substrate. Their KmO_2 is above normal arterial O_2 concentration.[14] PHD-2 is considered the most important isoform, responsible for HIF-α degradation in normoxia. PHD-3 and -2 are HIF target genes, and hence, as a negative feedback loop, help terminate the HIF response engendered by hypoxia. The four clinically tested PHD inhibitors roxadustat, vadadustat, daprodustat and molidustat are highly specific, but differ in kinetics and their ability to target HIF-1α vs -2α, and the respective CODD vs NODD.[15] Hence, at least to a certain extent, the four compounds may exhibit different clinical effects. As a transcription factor, HIF acts through its target genes, the precise number of which remains unclear. Confusingly, the consensus DNA binding motif for HIF, the so-called hypoxia response element (HRE; RCGTG, with R = A or R = G) appears more than one million times in the genome.[16] Clearly, most of these potential HREs are not accessible to HIF, but covered by chromatin. To make things more complex, some HREs of proven relevance locate quite far from the transcription initiation site. Furthermore, for full gene transactivation, binding of HIF to a single HRE seems insufficient, but rather requires binding to multiple HREs, or contribution from additional co-factors. Chromatin binding assays suggest that both HIF-1α and HIF-2α regulate some hundreds of genes, with partial overlap.[17] Hypoxia response, thus, varies between underlying conditions, tissues and even individual cells. Altogether, interventions into the HIF-PHD system, whether pharmacological or genetical, are nearly unpredictable, which brings us back to the in vivo studies of Urrutia et al[1]: They chose NG2-Cre mice to specifically knockout genes of interest in central nervous cells with particular relevance to angiogenesis, namely pericytes and NG2 glia cells. VHL knockout, a robust model for combined HIF-1α and HIF-2α up-regulation, led to enhanced angiogenesis and EPO. In the past, the latter has been attributed angiogenic effects in the brain, but a plethora of clinical trials (review in Ref.18) proved that exogenous EPO was unable to ameliorate brain damage. By double knockout (VHL/EPO)

Urrutia et al[1] show that the angiogenic phenotype is independent of EPO. Interestingly, knockout of PHD-2, which carries the workload of HIF degradation in normoxia, did not lead to angiogenesis while double knockout (PHD-2/PHD-3) did. The authors speculate that PHD-3, as a HIF target gene, had compensated for the single PHD-2 knockout. Knockout of PHD-1 alone had no angiogenic effect, most likely since preserved PHD-2 activity was sufficient to degrade HIF. However, combined knockout of all three PHD isoforms further enhanced angiogenesis with respect to the PHD-2/PHD-3 knockout. Hence, as the authors put it, each PHD isoform has its contribution to neurovascular homeostasis. And, finally, quadruple knockouts (PHD-1/PHD-2/PHD-3/HIF-2α) proved that the angiogenic phenotype was fully dependent on HIF-2α.

Aside from sophisticated and refined methodology, the study by Urrutia et al[1] broadens our vision of the HIF-PHD components working together to shape a potentially relevant clinical effect. It seems that, indeed, cells make use of their triple oxygen sensor.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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