Hypoxia inducible factor and diffuse white matter injury in the premature brain: perspectives from genetic studies in mice

Fuzheng Guo*, Sheng Zhang

Hypoxia-inducible factors (HIFs) are transcriptional regulators playing important roles in adapting various types of cells to physiological and pathological hypoxia cues. Three structurally related, oxygen-sensitive HIFα proteins have been identified (HIF1α, HIF2α, and HIF3α), among which HIF3α has weak transcriptional capacity because of the absence of the C-terminal transactivation domain as present in HIF1α and HIF2α. The role of HIFα in regulating diverse biological processes is primarily through the actions of its downstream target genes and/or signaling pathways (**Figure 1**). The HIFα signaling is subjected to regulation at the multiple levels as detailed in **Figure 1**. Previous studies have shown that HIF1α and HIF2α activate both common (canonical) and distinct (non-canonical) sets of target genes in cell-type and context dependent manners. The importance of HIFα (HIF1α and HIF2α) in embryonic development is manifested by the lethality of early embryos or neonates of HIF1α$^{-/-}$ mice and HIF2α$^{-/-}$ mice due to the cardiovascular and lung malformation. In the developing central nervous system (CNS) where the maturation of the vascular network is still ongoing, the local oxygen concentration ranges from 0.5% to 7% (Ivanovic, 2009). The physiologically hypoxic environment in the CNS suggests that HIFα may play an important role in neural development.

HIFα regulates CNS neural development: The necessity of HIFα in neural development has been shown by neural cell-specific, *Cre-loxP*-based HIFα conditional knockout (cKO) mutant mice driven by Nestin-Cre, thus circumventing the early lethality of HIFα germline KO mutants. Nestin promoter is active in early neural progenitors that give rise to neurons and glial cells. *Nestin-Cre:Hif1a$^{fl/fl}$* (referred to as Nestin:HIF1α cKO) mice (Tomita et al., 2003) are viable; but they exhibit prominent loss of neural cells, and defective vascular formation in the brain starting from the late embryonic ages and progressively develop severe hydrocephalus and exhibit spatial memory impairment in the adult ages. Similarly, *Nestin-Cre:Hif2a$^{fl/fl}$* (Nestin:HIF2α cKO) mice are viable and fertile and show no obvious neurological deficits except for impaired learning and memory ability (Kleszka et al., 2020). The neural phenotypes of HIF2α cKO mice seem much milder than those in HIF1α cKO mutants. These observations clearly demonstrate an essential role of HIFα in neural development.

HIFα is a cellular rheostat for oligodendroglial development: In the human CNS, the differentiation of myelin-forming oligodendrocytes (OLs) from oligodendrocyte progenitor cells (OPCs) (referred to as OPC differentiation) initiates in the third trimester of gestational ages and peaks during the first several years after birth. In the murine CNS, OPC differentiation and myelination occur primarily during postnatal development (Semple et al., 2013) which is temporally concomitant with the maturation of the vasculature network (Harb et al., 2013). In a recent study, we have reported that HIF1α protein is stabilized in oligodendroglial lineage cells in the white matter (WM) tracts of early postnatal mice and reduced to the undetectable level by around postnatal day (P) 10 under normal conditions (Zhang et al., 2021). We hypothesize that HIFα may act as a cellular rheostat that controls OPC differentiation during CNS myelination; physiological HIFα activity may be beneficial for OPC differentiation and developmental myelination while pathological HIFα hyperactivity may be detrimental.

Oligodendroglial-specific genetic manipulation has shed light on the role of HIFα signaling pathway in oligodendroglial development (OPC differentiation and oligodendroglial myelination). Previous study showed that oligodendroglial HIFα is essential for murine WM integrity (Yuen et al., 2014). The necessity of HIFα in WM integrity is demonstrated by reduced corpus callosum volume, diminished OPC population, and presence of WM cysts in *Sox10-Cre:Hif1a$^{fl/fl}$:Hif2a$^{fl/fl}$* (referred to as Sox10:HIFα cKO) mice in which Sox10-Cre-medated HIFα depletion occurs in specifically in embryonic OPCs and oligodendrocytes. In addition, *Olig1-Cre:Hif1a$^{fl/fl}$:Hif2a$^{fl/fl}$* (Olig1:HIFα cKO) mice displays widespread WM axonal damage (Yuen et al., 2014). Since previous data show that *Olig1-Cre* mediates gene recombination in embryonic neural progenitors that give rise to OPCs and, to a lesser extent, astrocytes and interneurons (Yang et al., 2016), it is possible that HIFα may be also depleted in a subset of astrocytes and interneurons in Olig1:HIFα cKO mice.

The compromised WM integrity and axonal damage are proposed be stemmed from, at least in part, the defective vascular formation. Interestingly, these WM abnormalities are already observed by embryonic day 18 and apparent by P4 (Yuen et al., 2014) when OPC differentiation and oligodendroglial myelination barely occur in the murine brain. We employed *Pdgfra-CreER^{T2}:Hif1a$^{fl/fl}$:Hif2a$^{fl/fl}$* (Pdgfra:HIFα cKO) mice to study the role of HIFα in oligodendroglial development in the postnatal CNS. We conditionally depleted HIFα from PDGFRα^{+} OPCs by tamoxifen administration to neonatal mice at P1, P2, and P3 and analyze OPC differentiation and myelination at P8 and P14. We found that OPC differentiation and myelination were inhibited at P8 and appeared normal by P14, which is consistent with the dynamic HIFα stabilization during postnatal CNS development and suggests that HIFα is required for timely differentiation of OPCs into OLs and subsequent myelination. Unexpectedly, the density of oligodendroglial lineage cells (OPCs and OLs) and myelination appear normal in the CNS of *Cnp-Cre:Hif1a$^{fl/fl}$:Hif2a$^{fl/fl}$* (Cnp:HIFα cKO) mutants in which Cnp promoter is primarily active in immature and mature oligodendrocytes in the subcortical white matter (Zhang et al., 2018). In both Pdgfra:HIFα cKO and Cnp:HIFα cKO mutants, cell survival and axonal integrity are unaltered in the brain WM tract. Our genetic evidences, together with those from Yuen and colleagues, collectively suggest that HIFα in OPCs is essential in maintaining WM integrity during embryonic and neonatal ages and for timely OPC differentiation during development myelination.

Genetic gain-of-function studies suggest that chronic HIFα stabilization may be instead detrimental for OPC differentiation and myelination. The rapid turnover of HIFα requires the protein VHL (**Figure 1**); therefore, genetic depletion of VHL provides a valuable tool in probing the role of sustained HIFα activation in oligodendroglial development. *Sox10-Cre:Vhl$^{fl/fl}$* (Sox10:VHL cKO, rare survival by P7) mice exhibit severe disturbance in OPC differentiation and myelination in the WM tracts of the brain (Yuen et al., 2014) and spinal cord (Zhang et al., 2021). By leveraging tamoxifen-inducible *Pdgfra-CreER^{T2}:Vhl$^{fl/fl}$* (Pdgfra:VHL cKO) mutants, we demonstrated that sustained HIFα activation, starting in OPCs at P1-P3 neonatal ages, inhibited OPC differentiation and CNS myelination not only in early postnatal ages but also in adult ages. Very interestingly, sustained HIFα activation in OLs does not affect oligodendrocyte maturation nor myelination in the CNS of *Cnp-Cre:Vhl$^{fl/fl}$* (Cnp:VHL cKO) mice. The non-perturbation of oligodendroglial development in Cnp:HIFα

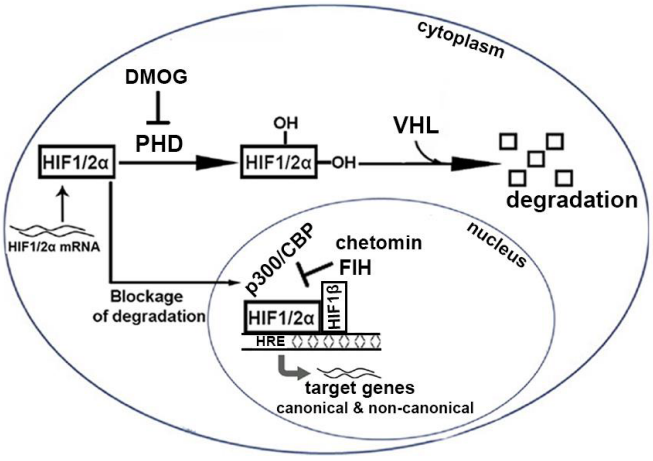

Figure 1 | **Schematic graph depicting the HIFα signaling pathway and its regulations at the different levels.**
The transcription factor family HIFα is a heterodimer consisting of an oxygen-sensitive HIF1α (or HIF2α) and a constitutively expressed HIF1β. Under normoxia, HIFα (HIF1α or HIF2α) is subjected to rapid proteasome-mediated degradation which requires the prolyl hydroxylase domain (PHD) proteins and the protein von Hippel-Lindau (VHL). Under hypoxia, PHD activity is inhibited, leading to HIF1/2α stabilization and subsequent accumulation in the nucleus where they dimerize with HIF1β at the hypoxia responsive elements (HRE) and recruit the co-activator p300/CBP to activate its target gene expression. Hydroxylation of the asparagine residue in the C-terminal activation domain of HIFα by the factor inhibiting HIF (FIH) prevents the recruitment of p300/CBP to HIFα at the HRE, thus negatively regulating HIFα-mediated transcriptional activity. DMOG, an inhibitor for PHD activity; chetomin, a compound blocking p300/CBP recruitment and subsequent signaling activation. Genetic or pharmacological manipulations of PHD, VHL, or FIH provides powerful avenues in activating or suppressing HIFα-mediated signaling pathway. The canonical HIFα target genes include those involved in angiogenesis, glucose metabolism, and glycolysis where the identities of the non-canonical HIFα target genes are cell type- and context-dependent, for example Ascl2, Dlx3, Wnt7a/b, and Sox9 (see the text for discussion). Ascl2: Achaete-scute like 2; CBP: CREB-binding protein; Dlx3: distal-less homeobox 3; DMOG: dimethyloxallyl glycine.

cKO and Cnp:VHL cKO mutants suggest that OLs are resistant to HIFα dysregulation, which is in line with previous concept that OLs are more resistant to hypoxia/ischemia injury than OPCs. Therefore, HIFα level has to be tightly controlled during normal developmental myelination and its dysregulation selectively impacts OPC differentiation but not subsequent oligodendrocyte maturation.

Molecular mechanisms underlying HIFα-regulated OPC differentiation: puzzles remain: It remains elusive how HIFα activation inhibits OPC differentiation. The HIFα family transcription factor exerts its biological effects through the action of its downstream target genes in a cell type- and context-dependent manner. Previous study by Yuen et al. (2014) reported that HIFα inhibited OPC differentiation by directly activating the ligands Wnt7a/7b and subsequent activation of autocrine Wnt/β-catenin signaling in OPCs. This hypothetic working model is consistent with the inhibitory role of Wnt/β-catenin signaling activation on OPC differentiation (Guo et al., 2015). However, whether HIFα regulates canonical Wnt signaling in oligodendroglial lineage cells is still controversial. We reported that oligodendroglial HIFα plays a dispensable role in regulating Wnt/β-catenin activity (Zhang et al., 2020, 2021). Recently, using *in vitro* mouse pluripotent stem cell-derived *Vhl*-deficient OPCs, Allan and colleagues (Allan et al., 2020) confirmed that HIFα did not regulate Wnt7a/Wnt7b nor the canonical Wnt signaling, instead, identified Ascl2 and Dlx3 as "non-canonical" HIFα target genes in OPCs. Both target genes are absent from neural cells including OPCs in the early postnatal murine CNS under physiological conditions and upregulated in the P11 forebrain of mice reared in 10% chronic hypoxic chamber (from P3 through P11) (Allan et al., 2020). Though ectopic expression of Ascl2 or Dlx3 inhibited the differentiation of pluripotent stem cell-derived OPCs, it is yet to be determined whether Ascl2 and/or Dlx3 functionally mediates HIFα stabilization-elicited inhibition of OPC differentiation *in vitro* and particularly *in vivo* under hypoxic conditions.

By leveraging Cre-loxP genetic mouse models, we demonstrated that inhibiting autocrine Wnt/β-catenin signaling (by disrupting oligodendroglial WLS, an essential factor for Wnt secretion) did not affect the inhibitory degree of OPC differentiation elicited by HIFα stabilization, providing first functional evidence that the hypothetic autocrine HIFα-Wnt axis may play a minor role in HIFα-regulated OPC differentiation (Zhang et al., 2021). Interestingly, we identified the neural stem cell factor Sox9 as a novel "non-canonical" target gene in OPCs that is activated by HIFα stabilization (Zhang et al., 2021). In the CNS, Sox9 is highly expressed in early neural precursor and stem cells, rapidly downregulated in OPCs, and completely absent from differentiating OLs. We demonstrate that HIFα binds to the promoter region of the mouse Sox9 gene and HIFα stabilization activates Sox9 expression at both mRNA and protein levels. Our *in vitro* functional study performed in primary OPCs demonstrated that Sox9 knockdown rescued the inhibition of OPC differentiation elicited by HIFα hyperactivation, suggesting that hyperactive HIFα arrests OPC differentiation by sustaining Sox9 expression.

Implications and mechanisms of glial HIFα in diffuse WM injury of the premature brain: Diffuse WM injury is the major form of brain injury preferentially affecting the brain of preterm infants born before 37th gestational weeks. Due to the immaturity of the respiratory system and the vasculature of the brain WM, hypoxia/ischemia (H/I)-induced WM injury, formerly called periventricular leukomalacia (PVL), is commonly seen in preterm brain. Disturbed myelination (hypomyelination), resulted from arrested OPC differentiation, is one of the established pathological hallmarks in the preterm brain affected by diffuse WM injury. There are thus far no effective therapies for preventing hypomyelination in preterm infants.

HIFα is a master regulator adapting various cells to hypoxic environment. Previous data from genetic mouse models collectively suggest that HIFα acts as a cellular rheostat in controlling developmental myelination. Therefore, targeting HIFα and its downstream signaling pathways may represent potential therapeutic interventions in mitigating or preventing myelination disturbance observed in diffuse WM injury of premature brains. Our data showed that HIF1α protein is transiently stabilized in oligodendroglial lineage cells in early postnatal mice and downregulated to the undetectable level by around postnatal day 10 under physiological conditions. In contrast, HIF1α stabilization is sustained in glial cells including the oligodendroglial lineage cells in mice challenged by H/I injury, an animal model for diffuse WM injury in human preterm infants (Zhang et al., 2021). These data suggest that sustained HIFα stabilization may impair the timely differentiation of OPCs into OLs during diffuse WM injury. Previous study using *in vitro* cell and brain slice culture reported that depleting HIFα alleviated defective OPC differentiation and myelination elicited by hypoxia treatment (Yuen et al., 2014). Since diffuse WM injury involves multiple cell types and complex interaction between neural and vascular cells, *in vivo* genetic mouse models of HIFα gain- and loss-of function may help define whether sustained HIFα stabilization plays a pathogenic role in halting brain myelination or a beneficial role in protecting oligodendroglial lineage cells again ongoing H/I injury. Unfortunately, such

genetic evidence has not yet been available to define the function of HIFα in H/I-induced oligodendroglial pathology in animal models. HIFα regulates a common set of canonical target genes that are crucial for energy metabolism and supply (such as glucose transport, glycolysis, and angiogenesis) (**Figure 1**) and participate in the adaptive reactions protecting the cells against hypoxia and other adverse cues. Theoretically, targeting HIFα itself may interfere with those key cellular processes and adaptive mechanisms. In this regard, it is important to discover novel "non-canonical" downstream targets or pathways of HIFα that could be intervened to alleviate arrested OPC differentiation in the context of hypoxia/ischemia-induced diffuse WM injury. The current popular hypothesis, derived from normal developmental study, proposes that HIFα controls OPC differentiation by activating autocrine Wnt/β-catenin signaling (Yuen et al., 2014). This is a very tempting working model as it may explain the hyperactivity of Wnt/β-catenin signaling observed in WM oligodendroglial lineage cells of premature infants affected by periventricular leukomalacia and mature infants affected by hypoxic-ischemic encephalopathy (Fancy et al., 2011). However, recent genetic evidences from our group (Zhang et al., 2020, 2021) and others (Allan et al., 2020) weakens this hypothesis at least in the context of normal developmental myelination in mice. Interestingly, our data obtained from astroglial-specific HIFα-stabilized mice (Zhang et al., 2020) suggest that astroglial HIFα may control OPC differentiation and myelination by activating Wnt/β-catenin signaling in the oligodendroglial lineage in a paracrine manner. Our hypothesis may provide novel insights into the importance of astroglial/oligodendroglial cross-talk in determining the outcome of myelination. Though it is known that hyperactivation of the intracellular Wnt/β-catenin signaling inhibits OPC differentiation (Guo et al., 2015), the cellular sources of Wnt ligands that initiates the intracellular Wnt/β-catenin activity in OPCs remains understudied. Furthermore, from a therapeutic perspective, it will be more feasible to manipulate intracellular Wnt/β-catenin signaling axis at the ligand level than the intracellular level. Future studies, particularly those employing cell-specific mouse genetics, are needed to test the hypothetic "paracrine" modulation of OPC differentiation by astroglia-derived/HIFα-regulated Wnt production.

There has been ongoing interest in studying the non-canonical HIFα target genes or signaling pathways that functionally mediate HIFα-regulated OPC differentiation. In our recent study, we identified Sox9 as a novel "non-canonical" target gene in oligodendroglial cells directly activated by HIFα stabilization (Zhang et al., 2021). However, it remains to be determined whether the connection between HIFα and Sox9 plays a cell-autonomous role in arresting OPC differentiation in the context of H/I-induced diffuse WM injury *in vivo*. To support the functional significance of the hypothetic HIFα-Sox9 axis in arresting OPC differentiation, we found in our unpublished study that Sox9, which is otherwise downregulated in differentiating OPCs under normal conditions, is sustained in OPCs in the WM of H/I-injury mouse brain. Therefore, we propose that prolonged HIFα activity in the subcortical WM, as observed in the brain of H/I-insulted neonatal mice, may impair OPC differentiation by sustaining chronic Sox9 expression. Compound transgenic mice of oligodendroglial-specific VHL/Sox9 double cKO will help define the role of the hypothetic HIFα-Sox9 axis in arresting OPC differentiation under H/I-induced WM injury conditions.

We thank my colleagues whose work lays the foundations for the article and apologize to those whose important work has not been cited here due to the space limitation.

The present work was supported by NIH/NINDS (R21NS109790 and R01NS094559) (to FG) and Shriners Hospitals for Children (85107-NCA-19 to FG, and 84307-NCAL to SZ).

Fuzheng Guo*, Sheng Zhang
Department of Neurology, School of Medicine, the University of California, Davis; Institute for Pediatric Regenerative Medicine (IPRM), Shriners Hospitals for Children, Northern California, Sacramento, CA, USA
***Correspondence to:** Fuzheng Guo, PhD, fzguo@ucdavis.edu.
https://orcid.org/0000-0003-3410-8389 (Fuzheng Guo)
Date of submission: January 14, 2021
Date of decision: February 2, 2021
Date of acceptance: March 12, 2021
Date of web publication: June 7, 2021

https://doi.org/10.4103/1673-5374.314301
How to cite this article: *Guo F, Zhang S (2022) Hypoxia inducible factor and diffuse white matter injury in the premature brain: perspectives from genetic studies in mice. Neural Regen Res 17(1):105-107.*

Open peer reviewers: *Sébastien Foulquie, Universiteit Maastricht, Netherlands; Joachim Fandrey, University of Duisburg-Essen, Germany.*
Additional file: *Open peer review reports 1 and 2.*

References

Allan KC, Hu LR, Scavuzzo MA, Morton AR, Gevorgyan AS, Cohn EF, Clayton BLL, Bederman IR, Hung S, Bartels CF, Madhavan M, Tesar PJ (2020) Non-canonical targets of HIF1a impair oligodendrocyte progenitor cell function. Cell Stem Cell 28:257-272.

Fancy SP, Harrington EP, Yuen TJ, Silbereis JC, Zhao C, Baranzini SE, Bruce CC, Otero JJ, Huang EJ, Nusse R, Franklin RJ, Rowitch DH (2011) Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. Nat Neurosci 14:1009-1016.

Guo F, Lang J, Sohn J, Hammond E, Chang M, Pleasure D (2015) Canonical Wnt signaling in the oligodendroglial lineage--puzzles remain. Glia 63:1671-1693.

Harb R, Whiteus C, Freitas C, Grutzendler J (2013) In vivo imaging of cerebral microvascular plasticity from birth to death. J Cereb Blood Flow Metab 33:146-156.

Ivanovic Z (2009) Hypoxia or in situ normoxia: the stem cell paradigm. J Cell Physiol 219:271-275.

Kleszka K, Leu T, Quinting T, Jastrow H, Pechlivanis S, Fandrey J, Schreiber T (2020) Hypoxia-inducible factor-2alpha is crucial for proper brain development. Sci Rep 10:19146.

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol 106-107:1-16.

Tomita S, Ueno M, Sakamoto M, Kitahama Y, Ueki M, Maekawa N, Sakamoto H, Gassmann M, Kageyama R, Ueda N, Gonzalez FJ, Takahama Y (2003) Defective brain development in mice lacking the Hif-1alpha gene in neural cells. Mol Cell Biol 23:6739-6749.

Yang J, Liu X, Zhang X, Zhao X, Pan Y, Qiu M, Wu S, Zhao G, Wang YZ (2016) Predominant neuronal differentiation of Olig1+ neural progenitors in forebrain cortex biased by beta-catenin over-expression. Neurosci Lett 622:19-23.

Yuen TJ, Silbereis JC, Griveau A, Chang SM, Daneman R, Fancy SP, Zahed H, Maltepe E, Rowitch DH (2014) Oligodendrocyte-encoded HIF function couples postnatal myelination and white matter angiogenesis. Cell 158:383-396.

Zhang S, Wang Y, Xu J, Kim B, Deng W, Guo F (2021) HIFalpha regulates developmental myelination independent of autocrine Wnt signaling. J Neurosci 41:251-268.

Zhang S, Zhu X, Gui X, Croteau C, Song L, Xu J, Wang A, Bannerman P, Guo F (2018) Sox2 is essential for oligodendroglial proliferation and differentiation during postnatal brain myelination and CNS remyelination. J Neurosci 38:1802-1820.

Zhang S, Kim B, Zhu X, Gui X, Wang Y, Lan Z, Prabhu P, Fond K, Wang A, Guo F (2020) Glial type specific regulation of CNS angiogenesis by HIFalpha-activated different signaling pathways. Nat Commun 11:2027.

P-Reviewers: Foulquie S, Fandrey J; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y