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The Role of a Neurovascular Signaling Pathway Involving Hypoxia-Inducible Factor and Notch in the Function of the Central Nervous System

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Abstract

In the neurovascular unit, the neuronal and vascular systems communicate with each other. O_2 and nutrients, reaching endothelial cells (ECs) through the blood stream, spread into neighboring cells, such as neural stem cells, and neurons. The proper function of neural circuits in adults requires sufficient O_2 and glucose for their metabolic demands through angiogenesis. In a central nervous system (CNS) injury, such as glioma, Parkinson's disease, and Alzheimer's disease, damaged ECs can contribute to tissue hypoxia and to the consequent disruption of neuronal functions and accelerated neurodegeneration. This review discusses the current evidence regarding the contribution of oxygen deprivation to CNS injury, with an emphasis on hypoxia-inducible factor (HIF)-mediated pathways and Notch signaling. Additionally, it focuses on adult neurological functions and angiogenesis, as well as pathological conditions in the CNS. Furthermore, the functional interplay between HIFs and Notch is demonstrated in pathophysiological conditions.

Key Words: Hypoxia-inducible factor, Notch signaling, Oxygen, Central nervous system

INTRODUCTION

The brain hyperemia is accomplished by a group of cells, closely related to each other which refer to neurovascular unit. The neurovascular unit can be composed of neurons, neural stem cells (NSCs), astrocytes, microglia, endothelial cells (ECs) of the blood-brain barrier (BBB), pericytes, oligodendrocyte precursor cells, and extracellular matrix components (Lo and Rosenberg, 2009; Muoio *et al*., 2014; Maki *et al*., 2015). These cells detect the need for neuronal supply and trigger the necessary responses for sufficient O_2 and nutrient supply. Reduced O_2 availability, occurring due to various reasons including aging, inflammation, and mitochondrial dysfunction in the central nervous system (CNS), can regulate hypoxia-mediated gene expression in various CNS cells.

Hypoxia-inducible factors (HIFs) control transcriptional responses to reduced O_2 availability (Semenza, 2007). HIFs (HIF1-3) are heterodimeric proteins composed of an O_2-regulated HIF-α subunit and a constitutively expressed HIF-1β subunit. HIF-α subunits are subject to prolyl hydroxylation, which targets proteins for degradation under normoxic conditions (Epstein *et al*., 2001; Jaakkola *et al*., 2001). Two HIF-α proteins, HIF-1α and HIF-2α, are stabilized at low oxygen tension and dimerize with HIF-1β. Heterodimeric proteins bind to hypoxia-responsive elements (HREs) on multiple target genes and control their transcription (Semenza, 2003).

The Notch pathway is highly conserved throughout the animal kingdom; additionally, it orchestrates organogenesis and tissue homeostasis during development and in the adult brain (Bray, 2006; Ables *et al*., 2011). Notch ligands are transmembrane proteins; therefore, signaling is restricted to neighboring cells, enabling a functional interplay between HIF-1 and Notch signaling in the CNS. The Notch pathway may be involved in HIF-mediated CNS homeostasis through neuronal function and angiogenesis in the adult brain. Understanding the diverse functions of HIF-Notch signaling in the CNS, as well as of HIF-Notch dysfunction in neurodegenerative diseases and malignancy, is crucial to the development of new therapeutics that are centered around this pathway. In the current review, we will discuss the neurovascular functions of the HIF and Notch signaling pathways in the CNS.

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HIF-NOTCH SIGNALING IN NEUROLOGICAL FUNCTIONS AND ANGIOGENESIS

Adult neurogenesis occurs in two restricted regions of the brain, namely the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle. Moreover, newly generated neurons have been implicated in hippocampus-mediated learning and memory. Angiogenesis is the growth of blood vessels from the existing vasculature. It occurs throughout life in both health and disease, beginning *in utero* and continuing through old age. Metabolic activity changes lead to proportional alterations in angiogenesis. In this section, we will discuss the role of the HIF and Notch pathways in the CNS. The status of these signaling pathways in NSCs and ECs may contribute to alterations in neurological functions and angiogenesis.

Hypoxia-inducible factor (HIF)

HIF in neurological functions: Adult hippocampal neurogenesis in humans may be influenced by many factors including age, environment, genetics, drugs, and behavior (Spalding *et al*., 2013; Christian *et al*., 2014), and neurogenesis has been implicated as a potential therapeutic target for mitigating cognitive decline and behavioral dysregulation in a growing number of brain pathologies (Braun and Jessberger, 2014). The role of HIF-1α in human adult neurogenesis has not been thoroughly investigated; however, HIF-1α expression in rodent NSCs can be involved in adult neurogenesis. Some adult NSCs are located in the perivascular region, whereas others may occupy hypoxic niches and be regulated by O_2 gradients. Therefore, HIF-1α acts as a principal mediator of hypoxic adaptation to improve glucose metabolism and neurogenesis. Both nestin- and SOX2-positive NSCs in the adult mouse SVZ and SGZ express HIF-1α under non-pathological conditions (Roitbak *et al*., 2011). Genetic inactivation of NSC-encoded HIF-1α in the adult mouse hippocampus results in impaired neurogenesis (Carrica *et al*., 2019). In the adult SVZ region, HIF-1α inactivation in NSCs is associated with gradual NSC loss, concomitant with decreased vascular endothelial growth factor (VEGF) expression, and preceded by significant regression of the SVZ vasculature (Li *et al*., 2014). Therefore, NSC-encoded HIF-1α is required for the maintenance of hippocampal neurogenesis in the adult mouse brain.

HIF-1α deletion impairs hippocampal Wnt-dependent processes, including NSC proliferation, differentiation, and neuronal maturation (Mazumdar *et al*., 2010). Thus, HIF-1α deficient mice demonstrated reduced neural cell count, hydrocephalus, and impaired spatial memory (Mazumdar *et al*., 2010). Moreover, oligodendrocyte precursor cell (OPC)-encoded HIF function is involved in postnatal myelination. Constitutive HIF-1α/HIF-2α stabilization resulted in OPC maturation arrest through autocrine canonical Wnt7a/7b activation. Such OPCs also show paracrine activity inducing excessive postnatal white matter angiogenesis *in vivo*, and directly stimulating EC proliferation *in vitro* (Yuen *et al*., 2014). Oligodendrocyte HIF-1α/HIF-2α function is essential for angiogenesis and corpus callosum integrity. OPC-specific HIF-1α/HIF-2α loss-of-function leads to insufficient angiogenesis in the corpus callosum and to catastrophic axon loss. Furthermore, OPC-encoded HIF-1α/HIF-2α function mediates hypoxia-induced hypomyelination in postnatal brain (Yuen *et al*., 2014).

During an acute ischemic injury (associated with limited O_2 availability), the HIF pathway may exacerbate neuronal cell death. Genetic neuronal HIF-1α/HIF-2α deficiency improves neuronal survival and sensorimotor function in the early post-injury stages in an acute ischemic stroke model (Barteczek *et al*., 2017). During the chronic phase of ischemic stroke, the HIF pathway may be beneficial for regeneration through NSC survival, possibly through angiogenic niche. Reducing HIF-1α expression, using siRNA or Cre-mediated HIF-1α gene deletion, attenuates NSC ability to survive ischemic conditions (Li *et al*., 2014). Therefore, O_2 adaptive mechanisms activate HIFs, contributing to neuronal survival and NSCs-mediated regeneration.

HIF in angiogenesis: Oxygen represents a key cofactor for both prolyl-hydroxylases (PHDs) and the factor inhibiting HIF (FIH). Four isoforms of PHD have been identified (PHD1-4) and have been shown to hydroxylate HIF-1α (Bruick and McKnight, 2001; Epstein *et al*., 2001; Koivunen *et al*., 2007; Dengler *et al*., 2014). In addition, asparaginyl hydroxylation is mediated by FIH. The functional activity of both PHDs and FIH requires iron (Fe^{2+}), ascorbate, 2-oxoglutarate, and O_2 as co-factors, producing succinate and CO_2 (Hewitson *et al*., 2002; Berra *et al*., 2003). Whereas the FIH-dependent hydroxylation of HIF-α proteins (i.e., HIF-1α and HIF-2α) prevents their interaction with the coactivators cAMP response element-binding protein (CREB) binding protein (CBP) and p300, the PHD-dependent hydroxylation targets HIF-α for proteasomal degradation via von Hippel-Lindau (VHL)-dependent ubiquitination. Under hypoxic conditions, both FIH and PHDs are inactive, resulting in HIF-α protein stabilization, triggering the transcription of target genes involved in angiogenesis, such as VEGF, glucose transporter (GLUT), and erythropoietin (EPO) (Ferrara *et al*., 1996; Semenza, 2003).

This raises the question as to which cells trigger CNS angiogenesis. Previous studies have demonstrated that astrocyte VEGF secretion increases in response to hypoxia and decreases as a result of high oxygen tension (Stone *et al*., 1995; Choi *et al*., 2007). Mitochondria can sense the hypoxia producing ROS and can consume O_2 for ATP production, which is increased by a gaseous molecule, carbon monoxide (CO), a byproduct of heme oxygenase (HO) catabolism. Mitochondria-driven O_2 consumption and consequent hypoxia stimulate the astrocytic HIF-1α-VEGF axis in the preconditioning of CO (Choi *et al*., 2018). Interestingly, mitochondrial functions and angiogenesis can be synergistically upregulated by the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and estrogen-related receptor α (ERRα), whose effect can be abolished in HO-$1^{+/-}$ mice (Choi *et al*., 2016, 2017). The PGC-1α-ERRα axis enhances O_2 consumption, leading to HIF-1α stabilization in a PHD2-dependent manner (Choi *et al*., 2018). PHD2 may be the most important isoform for HIF regulation based on its ubiquitous expression and dominant function in HIF regulation during normoxia in various cell types (Berra *et al*., 2003). Thus, PHD2 homozygous knockout (KO) mice died *in utero* between E12.5 and 14.5, whereas PHD1 and PHD3 homozygous KO mice are viable (Takeda *et al*., 2006; Minamishima *et al*., 2008).

In the presence of sufficient O_2, HIF-1α can be stabilized through binding with heat shock protein 90 (Hsp90) (Liu *et al*., 2007). In the presence of a pharmacological Hsp90 inhibitor, the Hsp90 binding site on HIF-1α becomes occupied by RACK1 (receptor for activated C-kinase), which recruits ubiquitin ligase targeting HIF-1α for proteasomal degradation (Liu

and Semenza, 2007). CO can enhance the binding of HIF-1α-Hsp90 in astrocytes under normal O_2 concentration (Choi *et al*., 2010). Moreover, *Hsp90* knockdown or inhibition reduces CO-mediated HIF-1 stabilization and VEGF secretion (Choi *et al*., 2010). Recently, the physical association of intracellular Notch1 with Hsp90 has been reported (Wang *et al*., 2017), suggesting that Hsp90 may be important for the stabilization of both Notch1 and HIF-1α signaling pathways in an O_2-independent manner. Collectively, these data imply that gaseous molecules, such as O_2 and CO, act as bioactive signals for HIF-mediated angiogenesis.

Phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) activity regulates HIF-1α protein levels during normoxia and hypoxia. PI3K/Akt increases HIF-1α translation through mammalian target of rapamycin (mTOR) activation in an O_2-independent manner (Majmundar *et al*., 2010). Moreover, PI3K/Akt/glycogen synthase kinase 3β (GSK3β) regulates HIF-1α stability in an O_2-dependent manner (Mottet *et al*., 2003). GSK3β phosphorylates HIF-1α at several serine residues within the oxygen-dependent degradation domain (Ser551, 555, and 589), leading to decreased HIF-1α stability and degradation by the proteasome in a VHL-independent manner (Flugel *et al*., 2007). These results suggest that the angiogenic ability during neurological disorders such as Alzheimer's disease (AD) may be associated with HIF stability mediated by PI3K/Akt/GSK3β signaling.

Notch

Notch signaling role in neuronal functions in adults: In mammals, the Notch family consists of four transmembrane receptors (Notch1-Notch4) and five ligands (Jagged 1, Jagged 2, Delta 1, Delta 3, and Delta 4) (Kadesch, 2004). Notch activation is tightly regulated through post-translational modifications and a series of proteolytic cleavages. First, Notch is cleaved within the Golgi by Furin-like convertase (Logeat *et al*., 1998). Next, heterodimeric Notch traffics to the cell membrane, where it can interact with Delta/Serrate/Lag2 (DSL) family ligands on adjacent cells. Upon ligand binding, Notch undergoes two additional proteolytic processing events, an extracellular cleavage by ADAM (containing a disintegrin and metalloprotease) family proteases, and an intramembranous cleavage by the γ-secretase complex to release the Notch intracellular domain (NICD). The NICD translocates into the nucleus, where it binds to the DNA binding protein CSL (also known as recombination signal-binding protein-J (RBP-J)) and to the coactivator mastermind-like 1 (MAML1) to facilitate the transcription of target genes, including Hes/Hey family members [Reviewed in (Bray, 2006; Bazzoni and Bentivegna, 2019)].

Classically, Notch signaling has been implicated in neural progenitor identity maintenance and neuronal differentiation inhibition during early development (Louvi and Artavanis-Tsakonas, 2006). Notch1 KO mouse embryos died before 11.5 days of gestation (Swiatek *et al*., 1994), and histological analysis of mutant embryos revealed widespread cell death (Swiatek *et al*., 1994). Notch2 KO mutants, similar to Notch1 KO mutants, died around E11, whereas Notch 3 and Notch4 deletion does not appear to result in significant phenotypes (Hamada *et al*., 1999; Yoon and Gaiano, 2005). In the embryonic stage, Notch signaling can maintain neural progenitor cells in an undifferentiated state (Austin *et al*., 1995; Wakamatsu *et al*., 1999).

Notch signaling not only inhibits the neural fate during early development, but also plays a role in migration, morphology, synaptic plasticity, neuronal maturation, and long-term potentiation (LTP) regulation in the adult brain (Lutolf *et al*., 2002; Wang *et al*., 2004; Yoon and Gaiano, 2005; Mason *et al*., 2006; Ables *et al*., 2011). In addition, Notch signaling may be involved in the changes associated with the transition from multipotent CNS progenitors into adult NSCs (Yoon and Gaiano, 2005). Upregulated Notch1 signaling decreases the average dendritic length but increases dendritic branching (Franklin *et al*., 1999). Regulation of neurite plasticity by Notch signaling may be relevant for pruning development during critical periods, to selectively eliminate unneeded synapses, and thus generate highly refined mature neuronal circuits (Luo and O'Leary, 2005). Neurite morphology modification may provide a mechanism, by which Notch regulates synaptic plasticity, consequently influencing synaptic strength (e.g., LTP). *In vivo*, a Notch ligand, a peptide corresponding to the DSL domain of human Jagged 1, enhances LTP in normal mice and corrects LTP defects in Notch antisense transgenic (Tg) mice (Wang *et al*., 2004). Tg mice with reduced Notch1 levels show hippocampal LTP deficits, revealing that the Notch pathway is involved in long-term memory and cognitive functions.

Optimal Notch concentration may be important for the maintenance, differentiation, and neuronal survival of NSCs. In a mouse model of ischemia or in an oxygen-glucose deprivation (OGD)-exposed microglia cells, Notch1 and/or γ-secretase reduction decreases activated microglia and post-ischemic inflammation, and improves the functional outcome (Wei *et al*., 2011). Hyperactive Notch in oligodendrocytes is necessary and sufficient for myelin disruption (Lopez-Juarez *et al*., 2017), which is important for proper cognitive function. In the eye, elimination of mindbomb E3 ubiquitin protein ligase 1 (Mib1) fails to induce Notch activation in mouse retinal progenitor cells (RPCs), which have prematurely differentiated into neurons (Ha *et al*., 2017). Mib1 in the retinal pigment epithelium (RPE) contributes to Notch activation by supporting the localization of active Notch ligands at RPE-RPC contacts (Ha *et al*., 2017). Pigment epithelium-derived factor increases the self-renewal capability of stem cells in the adult SVZ by upregulating the Notch target gene epidermal growth factor receptor (Andreu-Agullo *et al*., 2009). These results suggest that the Notch pathway influences adult neuronal functions through cell-cell interactions in the eye and brain.

Notch signaling in angiogenesis: Notch signaling controls a variety of processes involving EC proliferation and survival. Delta-like ligand 4 (Dll4) is one of the Notch ligands in mammalian cells, and is expressed specifically in the physiological and pathological vasculature (Shutter *et al*., 2000). Further, Dll4 and its cognitive receptor Notch1 are expressed particularly at sites of vascular development and angiogenesis (Fig. 1A) (Claxton and Fruttiger, 2004; Benedito and Duarte, 2005). Dll4 can be detected in ECs of the arteries, arterioles, and capillaries, as well as in the brain, neural tube, retina, and in the olfactory epithelium (Benedito and Duarte, 2005). Moreover, Dll4 mRNA is observed in ECs at the very tips of growing vessels ('tip cells') and in arteries in developing retina (Claxton and Fruttiger, 2004).

VEGF-A is expressed by hypoxic astrocytes, resulting in paracrine EC activation (Fig. 1A) (Stone *et al*., 1995), but the growth factor is also produced by ECs to control their survival and angiogenesis in an autocrine manner (Lee *et al*., 2007;

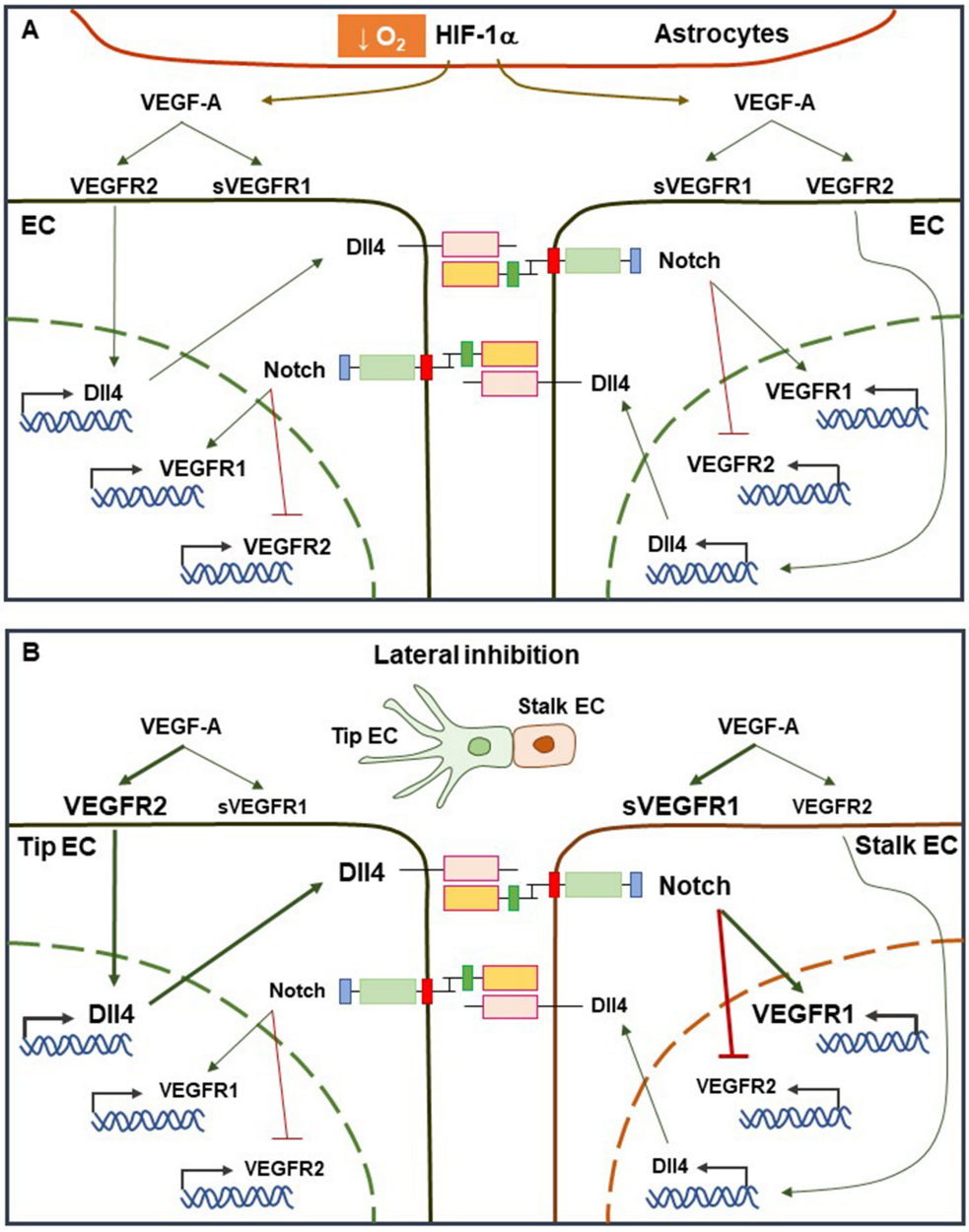

Fig. 1. HIF-VEGF axis and Notch signaling cooperatively regulate endothelial specification. (A) In hypoxia regions, HIF-1α can be upregulated in ECs and astrocytes, leading to enhanced VEGF-A secretion. All ECs become activated by VEGF-A stimulation to express Notch and Dll4. (B) Notch signaling induces lateral inhibition and gives rise to nonuniform population of ECs in the presence of VEGF-A stimulation. Stalk cells are subject to enhanced Notch signaling, which represses VEGFR2 transcription, while stimulating the expression of the decoy receptor, soluble VEGFR1 (sVEGFR1). Tip cells receive a low Notch signal, allowing for high VEGFR2 and low sVEGFR1.

Choi, 2017). The relationship between the Dll4-Notch and VEGF-A signaling pathways has been implicated in the specification of endothelial tip cells and in positional changes within sprouts (Lobov *et al*., 2007). In retina arterial endothelium, the VEGF and Notch pathways operate a negative-feedback loop. VEGF-A triggers endothelial Dll4 expression, and Dll4 activates Notch signaling in adjacent cells, leading to the downregulation of VEGFR-2 (Liu *et al*., 2003; Lobov *et al*., 2007), and the inhibition of tip cell formation (Fig. 1B) (Suchting *et al*., 2007). Adequate concentrations of VEGF-A stimulate ECs forming tip/stalk patterning (Bentley *et al*., 2008). Enhanced EC proliferation and sprouting after Notch inhibition involve the upregulation of endothelial VEGF-A together with a VEGF-A/VEGFR2 dependent increase in CXCR4 (Pitulescu *et al*., 2017). Endothelial tip cells lead sprouts and apparently suppress tip-like behavior in adjacent stalk endothelial cells by activating Notch activation. Notch directs tip-derived endothelial cells into developing arteries and thereby establishes Dll4–Notch signaling couples, sprouting angiogenesis and artery formation. VEGF-A triggers endothelial sprouting, migration, proliferation, cell survival, and consequently, vessel maintenance. When Notch signaling is absent or reduced,

no functional vessel patterning and perfusion can occur, and hypoxia/ischemia persists, consequently leading to neurodegeneration.

HIF-Notch interplay

Interplay during adult neuronal functions: Stem cells reside in specialized microenvironment or "niches", which regulate their function. Some stem cells are perivascular, whereas others may occupy hypoxic niches and be regulated by O_2 concentrations. Under hypoxic conditions, HIF-1α is stabilized and cooperates with Notch signaling to promote Notch target gene expression and NSC and progenitor proliferation in the adult brain (Bar *et al*., 2010; Pistollato *et al*., 2010). HIF-1α is recruited to Notch-responsive promoters on Notch in NSCs under hypoxia (Gustafsson *et al*., 2005). HIF-1α physically binds to NICD, which results in Notch signaling stabilization (Borggrefe *et al*., 2016), consequently maintaining stemness (Fig. 2). Furthermore, Notch signaling upregulates HIF-1α gene expression under hypoxic conditions by enhancing signal transducer and activator of transcription 3 (STAT3) phos-

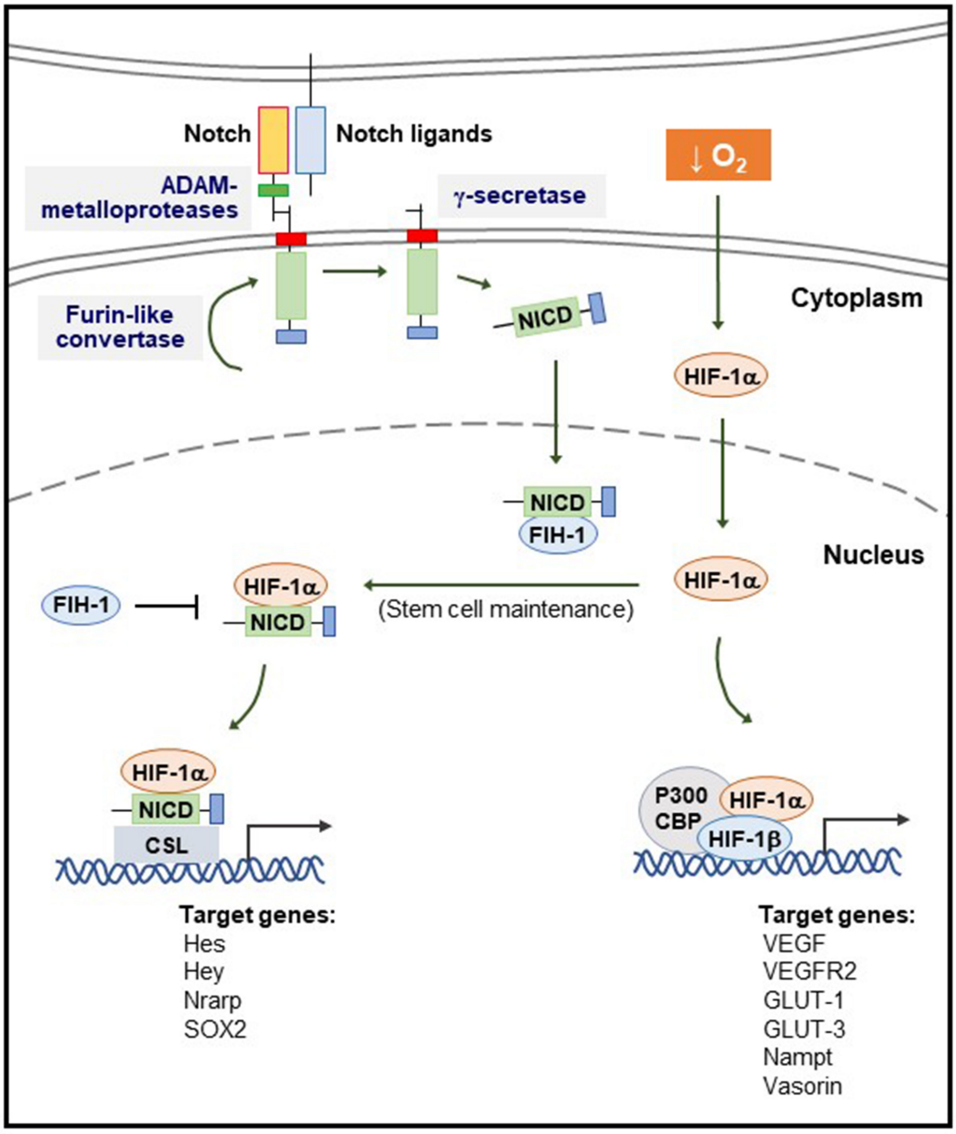

Fig. 2. Interplay between HIF-1α and Notch during hypoxia. The inactive Notch precursor is cleaved by a Furin-like convertase and translocates into the cell membrane. The binding to a Notch ligand induces a second cleavage by the ADAM (containing a disintegrin and metalloprotease) family, resulting in the formation of a membrane-tethered Notch truncated fragment, which is further processed at two sites by a presenilin-dependent γ-secretase complex; the Notch intracellular domain (NICD), the active form of the Notch receptor, is generated. In the absence of NICD, the CSL transcription factor represses Notch target gene transcription. Following NICD activation, CSL is converted into a transcriptional activator to stimulate transcription of the same genes. Upon hypoxia and activation of Notch, HIF-1α potentiates Notch signaling through interactions with the NICD and the CSL transcription factor, leading to maintenance of the stem cell state. In the presence of factor-inhibiting HIF-1α (FIH-1), HIF-1α becomes hydroxylated at asparagine. FIH-1 blocks the interaction of the HIF-1α with the transcriptional coactivator p300-CBP (CREB binding protein). NICD can sequester FIH-1, thereby preventing hydroxylation of HIF-1α and consequently enhancing HIF-1α recruitment to hypoxia-response element sites.

phorylation (Xu *et al*., 2005; Lee *et al*., 2009).

FIH-1 hydroxylates both HIF-1α and NICD at asparagine residues and negatively regulates HIF-1α and Notch activity [Reviewed in (Beaudry *et al*., 2016)]. During hypoxia, FIH-1-mediated hydroxylation is reduced. Moreover, FIH-1 hydroxylates ankyrin repeat domains in Notch receptors, thereby decreasing their activity and FIH ability to hydroxylate HIFs (Coleman *et al*., 2007). FIH-1 binds to NICD with a significantly higher affinity than to HIF-1α, thereby enhancing HIF-1α recruitment to HRE (Fig. 2) (Zheng *et al*., 2008).

HIF-1α is involved in stem cell survival and differentiation through Notch and Wnt/β-catenin signaling pathway modulation. *HIF-1α* gene knockdown in cultured postnatal neural stem/progenitor cells reduces NSC resistance to OGD and stimulates reciprocal changes in NICD (decreased) and β-catenin (increased) intracellular levels (Roitbak *et al*., 2011). Wnt/β-catenin signaling stimulates Notch ligand expression (i.e., Jagged 1) in progenitors (Estrach *et al*., 2006).

Interestingly, HIF-Notch signaling in NSC and progenitor cells may promote recovery. Adult NSCs undergo transition from proliferation to differentiation and may provide a reservoir of cells, capable of forming new neurons that may eventually integrate into neuronal circuits (Lathia *et al*., 2008). Various neurotrophic and growth factors (i.e., brain-derived neurotrophic factor (BDNF), EPO, VEGF, nitric oxide (NO), and osteopontin), regulated by the HIF-Notch pathway, may contribute to enhanced neurogenesis and functional recovery in rodents with ischemic brain damage (Shingo *et al*., 2001; Sun *et al*., 2003; Lee and Choi, 2018). Rapid activation of cytoplasmic signaling through PI3K/Akt, STAT3, and mTOR mediates HIF-Notch1 activation promoting NSC survival (Androutsellis-Theotokis *et al*., 2006).

Therefore, the HIF-Notch crosstalk can be harnessed in the adult CNS possessing the functions of regeneration, repair, or compensation of damaged or injured cells (Lathia *et al*., 2008; Baillieul *et al*., 2017). Adult stem cells express both HIF-1α and Notch protein (Cunningham *et al*., 2012; Wakabayashi *et al*., 2015), and the HIF-Notch interplay may be important for the maintenance of homeostasis of stem cell number and adult neurogenesis (Fig. 3) (Androutsellis-Theotokis *et al*., 2006; Li *et al*., 2018).

Interplay during angiogenesis: The HIF-1α-VEGF axis plays an important role in neural progenitor-mediated protection of both ECs and neurons following *in vitro* and *in vivo* hypoxia (Roitbak *et al*., 2008). The crosstalk between Notch signaling and the hypoxia response (i.e., HIF-1α and VEGF) is particularly important, as Notch signaling is critical in balancing sprouting angiogenesis (Phng and Gerhardt, 2009). Moreover, Delta-Notch signaling is essential for tip/stalk cell selection in ECs. The association of Dll4/Notch and HIF-1α-VEGF signaling pathways has been reported in embryonic and adult neovascularization (Dong *et al*., 2011; Fang *et al*., 2013). HIFs stimulate the transcription of Notch targets, such as Hey1, Hey2, and Dll4 (Diez *et al*., 2007). HIF-1α heterozygosity decreases the levels of activated Notch1 and its target genes Deltex and Notch-regulated ankyrin repeat protein (Nrarp) (Bertout *et al*., 2009). Loss of Nrarp leads to excessive endothelial junction segregation (i.e., Claudin 5) and vessel instability in postnatal day 5 mouse retinas (Phng and Gerhardt, 2009). Nrarp gene expression is directly induced by Notch signaling, but it may function as a negative regulator by promoting NICD degradation (Krebs *et al*., 2001; Lamar *et al*., 2001).

The link between HIF-1α and Notch signaling in ECs may be mediated by VEGFRs. HIF-1α enhances VEGF and VEGFR2 expression (Semenza, 2003), which induces Dll4 upregulation in tip cells (Gerhardt *et al*., 2003). In arterial endothelium, VEGF signaling activates Dll4 transcription through PI3K and extracellular-signal-regulated kinase (Erk) signaling in collaboration with FoxC transcription factors (Hayashi and Kume, 2008). In tip and stalk cells, Notch regulates VEGFR1 and VEGFR2 expression positively and negatively, respectively [Reviewed in (Phng and Gerhardt, 2009)]. In addition, the soluble splice variant of VEGFR1 (sVEGFR1) is upregulated upon Notch activation in ECs (Fig. 1) (Harrington *et al*., 2008), inducing a switch from the proliferative phase to the maturation and stabilization phase of angiogenesis by sVEGFR1. Moreover, HIF-1α can increase VEGFR3 expression in human lymphatic ECs (Han *et al*., 2019). Vegfr3 postnatal endothelial deletion leads to excessive angiogenic sprouting and branching and decreases the level of Notch signaling (Tammela *et al*., 2011). VEGFR3 reinforces Notch signaling through the FoxC2 transcription factor to control angiogenesis. Mice deficient in Notch signaling demonstrate severe vascular remodeling defects (Gridley, 2007) and reduced VEGFR3 expression (Benedito *et al*., 2012).

Endothelial angiogenesis can be mediated by nicotinamide phosphoribosyltransferase (Nampt) (Dietrich *et al*., 1966). It is also known as Visfatin (an adipokine) and as a pre-B cell colony-enhancing factor (PBEF) enhancing the effects of the stem cell factor on pre-B cells (Samal *et al*., 1994; Hug and Lodish, 2005). HIF-1α can enhance Nampt/Visfatin/PBEF gene expression to a higher extent than HIF-2α (Bae *et al*., 2006). EC treatment with Nampt/Visfatin/PBEF upregulates the Notch1-fibroblast growth factor (FGF)-2 axis and consequent angiogenesis (Bae *et al*., 2011). Overall, the HIF-Notch interplay may induce angiogenesis, vascular remodeling, and tip/stalk cell fate alterations through influencing various factors, such as VEGF, Nampt, and FGFs (Fig. 3).

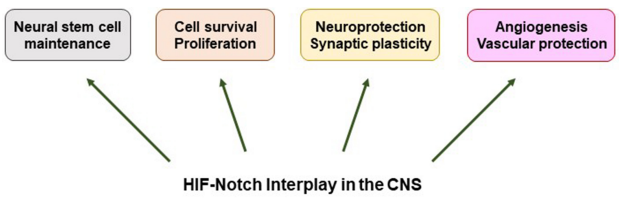

Fig. 3. HIF-Notch interplay may be involved in NSC maintenance, cell survival, proliferation, neuroprotection, synaptic plasticity, angiogenesis, and neurovascular protection in the CNS.

HIF AND NOTCH IN NEUROLOGICAL DISORDERS

HIFs are transcription factors involved in cellular adaptations to hypoxia optimizing the glucose metabolism and angiogenesis. Recent studies have demonstrated that HIF stabilization has neuroprotective effects in neurological disorders such as PD and AD (Ashok *et al*., 2017). Increased numbers of ECs and aberrant blood vessels have been detected in brains from patients with PD and AD (Vagnucci and Li, 2003; Janelidze *et al*., 2015). Notch signaling has also been implicated in many adult neurological disorders. The Notch pathway can commu-

nicate with HIF signaling to regulate cell survival in various cell types including cancer stem cells (Lathia *et al*., 2008). In this section, we will discuss the role of the HIF and Notch pathways in neurological diseases such as glioma, Parkinson's disease (PD), and Alzheimer's disease (AD).

Glioma

Glioblastoma is the most common and fatal primary brain tumor type (Seymour *et al*., 2015). Glioma stem-like cells (GSCs) exhibit an enhanced self-renewal capacity, tumor progression, therapy resistance, and tumor recapitulation post-treatment (Bradshaw *et al*., 2016). Under pathological conditions, HIF-1α regulates the maintenance and progression of several diseases by activating Notch1 signaling. Notch signaling is highly active in GSCs, leading to the maintenance of stem cell-like properties and suppression of differentiation (Bazzoni and Bentivegna, 2019). Notch1, Notch4, Dll1, Dll4, Jagged1, Hey1, Hey2, and Hes1, 2, and 4 protein levels are higher in brain tumor cells than in normal brain cells, correlating with elevated VEGF and phosphorylated Akt levels (Kanamori *et al*., 2007; Zhang *et al*., 2012; Hu *et al*., 2014; Bazzoni and Bentivegna, 2019). In glioma cells, NICD overexpression or Notch1 activation by Dll4 resulted in Akt induction; subsequently, β-catenin activity and nuclear factor κ light chain enhancer of activated B cells (NF-κB) signaling were upregulated (Zhang *et al*., 2012). During hypoxia, HIF-1α and HIF-2α bind to NICD and activate and repress Notch signaling activity in GSCs, respectively (Hu *et al*., 2014). HIF-1α and HIF-2α directly interact with NICD in a competitive manner, and differentially regulate NICD transactivation activity depending on oxygen levels (Hu *et al*., 2014). Another study suggests an indirect interaction between HIF-1α and NICD. HIF target genes, such as Vasorin, are upregulated in human glioblastoma, and the HIF-1α-Vasorin axis can activate the Notch pathway (Man *et al*., 2018). Vasorin binds to Notch1 to regulate its expression on the cell membrane and to calibrate Notch pathway activation, implying that Vasorin is a critical link between HIF-1α and Notch signaling in GSCs (Man *et al*., 2018). Additionally, Vasorin expression correlates with glioma aggressiveness. Vasorin is differentially induced in GSCs under hypoxic conditions by a HIF-1α/STAT3 co-activator complex. Moreover, it binds to and stabilizes Notch1 at the cell membrane by blocking Numb-mediated ubiquitination and lysosomal degradation (Man *et al*., 2018). Vasorin maintains the tumorigenic potential of GSCs, promotes tumor growth, and reduces survival in mouse models of glioblastoma through activation of the HIF-1α-Notch1 pathway.

The most employed and effective inhibitors in glioblastoma are γ-secretase inhibitors, which prevent the release of active NICD from the receptor by the γ-secretase complex. Notch signaling is at the center of a diverse signaling network, which includes pathways such as PI3K/Akt, NF-κB, STAT3, HIF-1α, Hedgehog, and Wnt/β-catenin (Borggrefe *et al*., 2016; Man *et al*., 2018; Bazzoni and Bentivegna, 2019).

A recent study shows that the CO/HO-1 pathway can activate Notch1 signaling in breast stem-like cells (Kim *et al*., 2018). Another study has demonstrated that cytoplasmic HIF-1α activates the γ-secretase complex, causing a strong increase in NICD release and in the target gene Hes1 expression in breast cancer (Villa *et al*., 2014). Therefore, in cancer cells, the Notch-mediated signaling network can regulate stem cell survival, stemness properties, and migration, partly through an interplay with HIF-1α.

Parkinson's disease (PD)

Pathologically, PD is characterized by gradual attrition of dopaminergic neurons in the substantia nigra pars compacta, formation of α-synuclein-, ubiquitin-, and tau-containing fibrillar inclusions (Lewy bodies and Lewy neurites) in the affected dopaminergic neurons, and variable changes in other neurotransmitter systems (Lang, 2011). α-Synuclein has been implicated in modulating embryonic and adult neurogenesis and neuronal maturation. In addition, α-synuclein is a key protein in neuroinflammatory diseases, including Lewy body dementia. In the human hippocampus, endogenous α-synuclein levels were increased in Lewy body dementia and the number of cells positive for the stem cell marker SOX2 was decreased (Spillantini *et al*., 1997). Mice overexpressing human wild-type α-synuclein (WTS) under the PDGF promoter were compared to non-Tg littermate controls (Winner *et al*., 2012). In PDGF Tg mice, WTS was already expressed at the stem cell stage, as depicted by nuclear co-labeling with SOX2. In the PDGF WTS mouse model, α-synuclein was expressed from birth through maturation of adult-born neurons. Furthermore, human WTS overexpression decreased the survival and dendritic development of newborn neurons. In addition, α-synuclein accumulation in the limbic system may be involved in the neurodegenerative phenotype by interfering with adult neurogenesis (Winner and Winkler, 2015).

Five months old Tg mice, expressing human mutant α-synuclein under the control of the *PDGF*β promoter, demonstrate a significant decrease in the number of newly generated neural stem/progenitor cells in the SGZ of the dentate gyrus, accompanied by reduced Notch1 expression compared to non Tg mice (Crews *et al*., 2008). Notch1 and Hes5 mRNA and protein levels are significantly reduced in α-synuclein Tg mice compared to non Tg controls, whereas Notch4, Hes1, Jagged, and Delta expression are not changed (Crews *et al*., 2008). Notch1 expression alterations, associated with α-synuclein accumulation, play a detrimental role in the later stages of neurogenesis by interfering with the survival of neural progeny, resulting in the elimination of these cells via apoptosis.

The inhibition of Notch signaling in adult dopaminergic neurons impairs their functions and survival. Leucine-rich repeats kinase 2 (LRRK2), which encodes a ROCO protein with a Ras of complex (ROC) domain, has been identified as a causative gene for autosomal dominant familial PD. Loss of the LRRK2 gene in aged mice has been demonstrated to impair the autophagy-lysosome pathway, which leads to marked accumulation of α-synuclein and ubiquitinated proteins (Tong *et al*., 2010). Moreover, HIF-1α can bind to the *LRRK2* promoter region, leading to transcriptional induction of LRRK2 (Bae *et al*., 2018). Notch inhibition may be mediated by the LRRK2 complex, harboring *ROC* pathogenic mutations (Imai *et al*., 2015).

Mutations in *PARK2* (Parkin) and *PARK6* (PINK, PTEN-induced putative kinase 1) cause a familial form of PD, known as autosomal recessive PD (Lucking *et al*., 2000). The neuroprotective role of Parkin and PINK has been linked to mitochondrial homeostasis, antioxidative stress, and mitophagy. Mitophagy is a form of selective autophagy for damaged mitochondria removal. Mitophagy deficiencies can lead to oxidative damage and cell death (Winklhofer, 2014). Parkin can function as an E3 ubiquitin ligase to ubiquitinate and degrade substrate proteins involved in PD. Parkin also downregulates

HIF-1α stability by ubiquitination at lysine 477 (K477) (Liu *et al*., 2017). Upregulation of HIF-1α may protect against dopaminergic neuronal death via Erk activation in a PD mouse model (Guo *et al*., 2016). Therefore, an impaired HIF-Notch signaling pathway may be associated with mitochondrial dysfunction, α-synuclein accumulation, and adult neurogenesis interference in PD.

Alzheimer's disease (AD)

Pathologically, AD is characterized by an oligomeric form of the beta amyloid (Aβ) peptide in the extracellular senile plaques and excessive phosphorylated tau protein in the intracellular neurofibrillary tangles (Klein, 2013). Various pathogenetic mechanisms for AD have been considered, including age-related changes in the amyloid precursor protein (APP) expression, Aβ production, and inherent differences in the ability of different brain regions to clear or catabolize Aβ (Ashok *et al*., 2017). Ischemic vessels can decrease A clearance or catabolism in the adult brain. Individuals who have suffered severe ischemia or trauma are more susceptible to developing AD (Desmond *et al*., 2002; Ikonomovic *et al*., 2017). Cerebral hypoperfusion may serve as a basis for some cases of dementia after ischemic injury (Snowdon *et al*., 1997). Cerebrovascular disease not only hastens the pathological processes leading to dementia, but also increases the probability that individuals with Alzheimer lesions in their brains will express a dementia syndrome (Snowdon *et al*., 1997; Kalaria, 2000). Hypoxia facilitates plaque formation and consequent angiogenesis in an aged AD Tg mouse model, leading to memory deficits (Sun *et al*., 2006; Biron *et al*., 2011). Thus, impairment of O_2 supply can be involved in the pathogenesis of AD. VEGF-mediated aberrant angiogenesis and BBB dysfunction is described in AD (Kalaria *et al*., 1998; Tarkowski *et al*., 2002; Vagnucci and Li, 2003). However, recent finding shows that cerebrospinal fluid VEGF levels are significantly decreased in AD patients (n=69) as compared to the control group (n=92) (Guo *et al*., 2013). VEGF upregulation through VEGF loaded nanosphere or transplantation of encapsulated VEGF-secreting cells improves cognitive impairment of APP/presenilin-1 (PS-1) mouse model of AD, suggesting that VEGF has beneficial role in AD (Spuch *et al*., 2010; Herran *et al*., 2015; Shim and Madsen, 2018). However, the exact roles of the HIF and Notch pathways in AD pathology have not yet been completely elucidated.

Low levels of Aβ-mediated HIF-1α stabilization may be neuroprotective. Aβ directly induces HIF-1α expression and activity in astrocytes, neuronal cell lines, and cortical neurons *in vitro* (Soucek *et al*., 2003). Cortical extracts from 23-month-old Tg2576-AD mice demonstrated increased HIF-1α protein levels compared with those in control mice (Soucek *et al*., 2003). A viral vector, expressing the human HIF-1α gene, reduced Aβ protein-induced apoptosis in primary culture hippocampal neurons (Chai *et al*., 2014). Downregulated brain HIF-1α levels in AD patients were associated with the detrimental downregulation of important HIF target genes, such as GLUT-1 and GLUT-3, in comparison with age-matched controls; this may lead to reduced PI3K/Akt activation and accelerated GSK3β-mediated hyperphosphorylation of tau and HIF-1α (Fig. 4) (Liu *et al*., 2008; Ashok *et al*., 2017). HIF-α target genes, such as VEGF and EPO, have demonstrated neurovascular protective properties (Chong *et al*., 2005; Religa *et al*., 2013). HIF stabilization by PHD inhibition prevents oxidative glutamate toxicity in cortical neurons and is neuroprotective during OGD in hippocampal cultures (Batti *et al*., 2010). EPO inhibits apoptosis during Aβ exposure in primary hippocampal neurons by promoting NF-κB-mediated neuronal survival (Chong *et al*., 2005). VEGF neuronal overexpression in a Tg mouse AD model, harboring double mutations in the 695 amino acid isoform of *APP*, restored memory functions and EC survival (Religa *et al*., 2013). Therefore, reduction of the HIF-Notch interplay may lead to disrupted neurovascular functions (Fig. 4).

Conflicting evidence shows that HIF pathway induction as a consequence of AD could be detrimental to neuronal survival by inducing apoptotic and inflammatory responses (Ogunshola and Antoniou, 2009). HIF-1 can be linked to oxidative stress-induced Aβ accumulation and subsequent activation of the pro-death gene BNIP3 in primary cortical neurons (Zhang *et al*., 2007). Potential HIF binding sites, identified on the *PS* promoter region, imply that HIF-1 may be involved in oxidative stress-mediated PS-1 transcription regulation (Cui *et al*., 2004). Notch cleavage by PS-1 in the γ-secretase enzyme

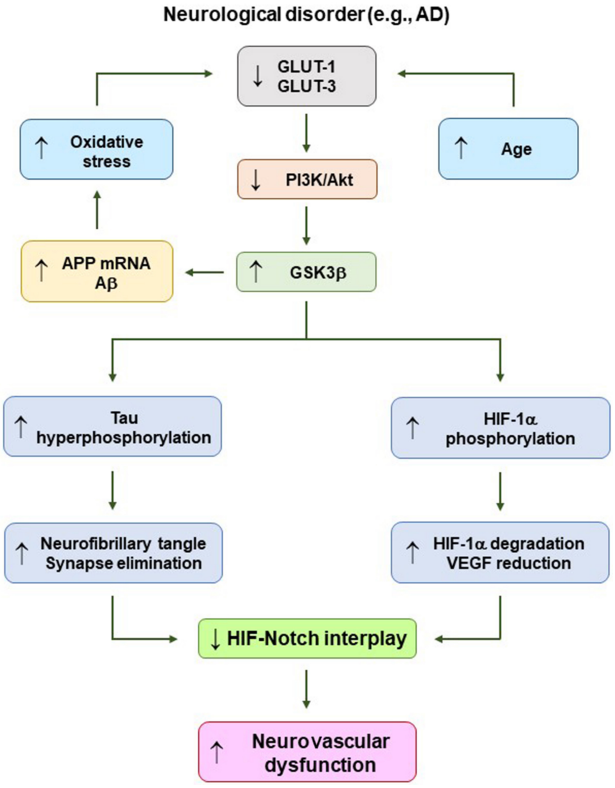

Fig. 4. Mechanism of the neurovascular dysfunction associated with neurological disorders such as Alzheimer's disease (AD). With increasing age, amyloid β (Aβ)-mediated oxidative stress may lead to reduction of the cerebral glucose uptake and metabolism through the decrease of the glucose transporter (GLUT)-1 and GLUT-3, which in turn reduces phosphoinositide 3-kinase/ protein kinase B (PI3K/Akt) activity. The enhanced activation of glycogen synthase kinase 3β (GSK3β) induces amyloid precursor protein (APP) expression and increases Aβ formation. GSK3β also induces tau hyperphosphorylation, neurofibrillary tangle formation, and synapse elimination. HIF-1α phosphorylation and proteasomal degradation by GSK3β can result in decreased VEGF expression. A depleted neural stem cell population and VEGF signaling by loss of the HIF-Notch interplay may lead to neurovascular dysfunction.

complex is essential for NICD generation and downstream signaling (De Strooper and Konig, 1999; Takasugi *et al*., 2003). The γ-secretase subunit gene (APH-1A) expression and subsequent γ-secretase-mediated Aβ and Notch generation are also regulated by HIF-1 binding to the *APH-1A* promoter during hypoxia (Wang *et al*., 2006).

The neurological phenotypes of *PS-1*-null and *Notch*-null mice are virtually indistinguishable and are characterized by premature differentiation of neural progenitor cells and dysgenesis of the brain during embryonic development (Handler *et al*., 2000). Notch downstream genes, such as Dll1 and Hes5, were mis-expressed in *PS-1* and *PS-2* double-null embryos, demonstrating that PS is essential for Notch signaling (Donoviel *et al*., 1999). In adult mice, forebrain-specific conditional KO of *Notch1* resulted in learning and memory impairments (Costa *et al*., 2003). Heterozygosity for the PS-1 knock-in mutation increased the $A\beta_{42}/A\beta_{40}$ ratio and exacerbated Aβ deposition (Xia *et al*., 2015). Conditional double *PS-1/PS-2* postnatal forebrain KO mice exhibit impairments in hippocampal memory and synaptic plasticity (Saura *et al*., 2004). Deficiency of PS-1/PS-2 causes reduced expression of CBP and of CREB/CBP target genes, such as c-fos and BDNF. With increasing age, the mutant mice develop striking neurodegeneration of the cerebral cortex and worsening memory and synaptic function impairments. Increased levels of the cyclin-dependent kinase 5 activator p25 and hyperphosphorylated tau can be observed in double *PS-1/PS-2* KO mice, implying that PS may play a role in adult synaptic plasticity, learning, and memory (Saura *et al*., 2004). In synaptic plasticity, Notch1 signaling reduction results in decreased LTP and learning and memory impairments. Null heterozygous mutations in *Notch1* and its downstream cofactor *CSL* result in spatial learning and memory deficits without affecting other forms of learning, motor control, or exploratory activity. These data indicate that abnormalities in Notch-dependent transcription may contribute to the cognitive deficits associated with AD (Costa *et al*., 2003).

POSSIBLE NEUROVASCULAR PROTECTION BY HIFS AND NOTCH

AD and PD pathology is characterized by progressive Aβ, α-synuclein, and neurofibrillary tangle accumulation, as well as cytoskeletal and synapse disruption; additionally, inflammation and deficits in the expression of neurotrophic factors, transcription factors, and antioxidant enzymes have been implicated in the pathophysiology of AD and PD (Lee and Choi, 2018; Kim *et al*., 2019). Altogether, these multi-faceted events underscore the complexity and therapeutic challenges associated with CNS diseases. Oxygen deprivation is apparently associated with most pathological processes and diseases, and in this regard, neurodegeneration is no exception. The mechanism through which oxygen deprivation affects disease progression remains unclear; however, it can likely exhaust the adaptive reserves of the brain. Thus, exploring the contribution of hypoxic-mediated pathways to age-related pathogenesis becomes increasingly important, to gain further insights into disease progression interventions. HIFs and Notch perform complex and dynamic functional roles in the CNS, and they have been implicated in these oxygen-mediated pathophysiological pathways. The proper manipulation of hypoxia and/or HIF-Notch-modulated signaling can aid the development of novel therapies urgently needed to combat neurodegenerative diseases.

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