Review

Patricia Mathieu, Pamela V. Martino Adami and Laura Morelli*

Notch signaling in the pathologic adult brain

Abstract: Along the entire lifetime, Notch is actively involved in dynamic changes in the cellular architecture and function of the nervous system. It controls neurogenesis, the growth of axons and dendrites, synaptic plasticity, and ultimately neuronal death. The specific roles of Notch in adult brain plasticity and neurological disorders have begun to be unraveled in recent years, and pieces of experimental evidence suggest that Notch is operative in diverse brain pathologies including tumorigenesis, stroke, and neurological disorders such as Alzheimer's disease, Down syndrome, and multiple sclerosis. In this review, we will cover the recent findings of Notch signaling and neural dysfunction in adult human brain and discuss its relevance in the pathogenesis of diseases of the central nervous system.

Keywords: Alzheimer; brain tumors; Down syndrome; ischemia; neurodegeneration.

***Corresponding author: Laura Morelli,** Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, Patricias Argentinas 435, Ciudad Autónoma de Buenos Aires, C1405BWE, Argentina, e-mail: lmorelli@leloir.org.ar
Patricia Mathieu and Pamela V. Martino Adami: Fundación Instituto Leloir, Instituto de Investigaciones Bioquímicas de Buenos Aires, Patricias Argentinas 435, Ciudad Autónoma de Buenos Aires, C1405BWE, Argentina

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; APH-1, anterior pharynx-defective 1; APP, amyloid precursor protein; DG, dentate gyrus; DS, Down syndrome; CNS, central nervous system; DLL, Delta-like ligands; Dab1, Disabled-1; DCX, double cortin; FAD, familial Alzheimer's disease; IP, intermediate progenitors; JAG, Jagged; MAMLs, Mastermind-like protein; MS, multiple sclerosis; Nct, nicastrin; NECD, Notch extracellular domain; NICD, Notch intracellular domain; NSCs, neural stem cells; OL, oligodendrocytes; OPCs, oligodendrocyte precursors cells; PEN2, presenilin enhancer protein 2; PS, Presenilin; Shh, Sonic hedgehog; SVZ, subventricular zone.

Introduction

Along the entire lifetime, Notch is actively involved in dynamic changes in the cellular architecture and function of the nervous system. Most of Notch target genes encode transcription regulators, many of which are critical in central nervous system (CNS) development. However, cell fate determination or other events influenced by Notch signaling can also result in the absence of transcriptional activation. In the CNS, Notch protein and ligands are not only present in the embryonic stages but also continuously in the adult nervous system. Recent studies have led to the recognition of the role of the Notch pathway in learning and memory, as well as late-life neurodegeneration. Notch controls neurogenesis, the growth of axons and dendrites, synaptic plasticity, and ultimately neuronal death. In the following, we review the cellular processes in the pathologic adult brain in which Notch signaling is involved and its impact on brain functionality.

Notch signal transduction

Notch signaling is unidirectional, with a 'signal-sending cell' that presents the Notch ligand to the 'signal-receiving cell', which expresses the Notch receptor. Notch receptors are single-pass transmembrane proteins with different domains that maintain the receptor as inactive in the absence of ligands (1, 2). These receptors are covered with a variety of glycans, and it is now clear that glycans modulate Notch signaling; however, it is not clearly known how this occurs (3). The Notch pathway in adult brain comprises multiple subtypes of ligands and receptors with differential expression patterns, which are summarized in Table 1. Mechanistically, Notch ligands are presented on the cell membrane and are subsequently endocytosed. These ligands can be 'activated' by an as-yet-unknown mechanism and re-presented to the membrane. Notch receptors are synthesized as a single peptide and then cleaved in the Golgi compartment to form a heterodimer that is presented on the cell membrane. Once on the membrane, the ligand can bind Notch. It was proposed that

Table 1 Notch signaling in the adult brain: ligands, receptors, and expression patterns.

Receptor	Expression pattern (References)	Ligand	Expression pattern (References)
Notch 1	Post-mitotic neurons Astrocytes Precursor cells Endothelium (96–99)	DLL-1	Neuronal progenitors (100, 101) Postmitotic neurons (98)
Notch 2 Notch 3	Precursor cells (96, 100)	DLL-3 DLL-4	Neuronal progenitors (100, 101) Endothelium (102)
Notch 4	Endothelium (103)	JAG-1	Precursor cells (97, 98, 104)
DNER	Postmitotic neurons (105)	JAG-2	Postmitotic neurons (103)

DLL, δ-like ligand; DNER, δ and Notch-like epidermal growth factor-related receptor; JAG, Jagged (Serrate-like ligand).

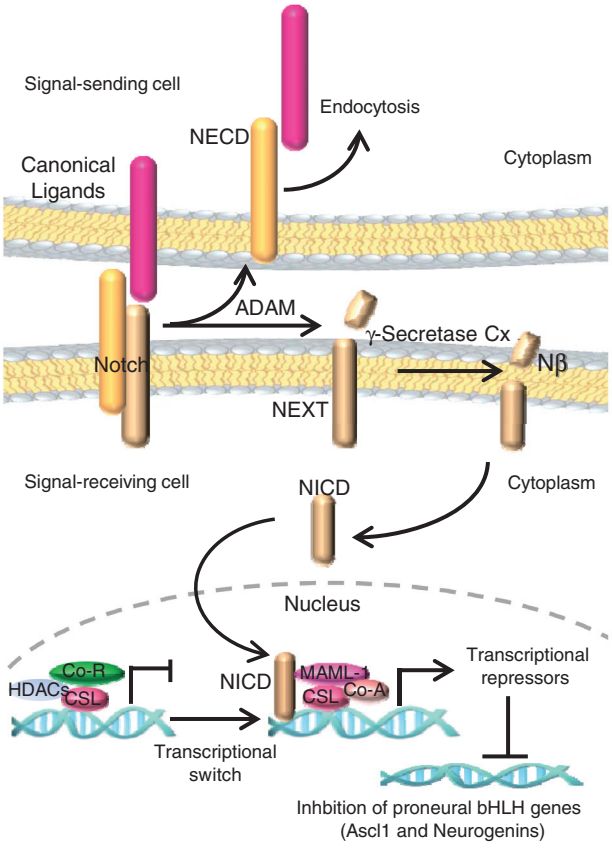

Figure 1 Canonical Notch signal transduction.The Notch receptor in the 'signal-receiving cell' is activated by binding to canonical ligands (DSL/DOS) presented by a 'signal-sending cell'. Endocytosis and membrane trafficking regulate ligand and receptor availability at the cell surface. Ligand endocytosis is also thought to generate sufficient force to promote a conformational change that exposes Notch to cleavage by ADAM metalloproteases. Juxtamembrane cleavage generates the membrane-anchored Notch extracellular truncation (NEXT) fragment, which is a substrate for the γ-secretase complex. γ-Secretase complex cleaves the Notch transmembrane domain to release NICD and Nβ peptides. In the absence of NICD, the DNA-binding protein CSL associates with ubiquitous corepressor (Co-R) proteins and histone deacetylases (HDACs) to repress transcription of target genes. When NICD enters the nucleus, its binding to CSL may trigger an allosteric change that facilitates displacement of transcriptional repressors. Mastermind (MAM) then recognizes the NICD/CSL interface, and this tri-protein complex recruits coactivators (Co-A) to activate transcription.

the Notch heterodimer is pulled apart through the force of endocytosis in the signal-sending cell, and then, the Notch extracellular domain (NECD) is endocytosed. The Notch domain that remains on the signal-receiving cell is sequentially cleaved by α-secretase, via A disintegrin and metalloproteinase (ADAM) 10 (4) and by the γ-secretase complex to release the Notch intracellular domain (NICD). Through a convergence of genetic, pharmacological, protein, and cell biology studies, it is now clear that γ-secretase is a multisubunit aspartyl protease that cleaves more than 70 type 1 transmembrane proteins within their transmembrane domains. Presenilin 1 and Presenilin 2 (PS1 and PS2) form the catalytic core of γ-secretase and three accessory proteins, anterior pharynx-defective 1 (APH-1), nicastrin (Nct), and presenilin enhancer protein 2 (PEN2), are required to complete the γ-secretase complex (5). The precise location of the γ-secretase cleavage is controversial, with some data indicating the endosome as the major site although there is some evidence suggesting that cleavage can also take place at the plasma membrane, leading to different NICD molecules. Nuclear responses due to Notch activation are tightly regulated by various posttranslational modifications that affect NICD trafficking, half-life, and transcriptional activity, thereby contributing to signaling diversity (6). Over the past decades, important progress has been made in deciphering Notch signal transduction and identifying processes that are influenced by Notch (1). The emerging picture posits that in the 'canonical' signaling pathway (Figure 1), most Notch-dependent physiological and pathological processes rely on the ability of nuclear NICD to convert the DNA-binding protein CSL from a transcriptional repressor into an activator. This regulation involves the formation of a stable ternary complex composed of CSL, NICD, and mastermind-like family of coactivators (MAML) (7, 8). Deciphering how this complex orchestrates transcriptional activation and how diversity in the transcriptional program is established depending on the cellular context is a major challenge in the field. The most studied Notch

targets are the hairy and enhancer of split-related genes, which are members of the bHLH family of transcriptional repressors (9). However, many additional genes have been recently identified as Notch targets (10). In addition, NICD can also signal in the absence of transcriptional activation presumably through protein-protein interactions, a pathway collectively known as 'noncanonical' signaling (11). Noncanonical Notch signaling can be either ligand-dependent or independent. The better-described function of the latter is the antagonism of Wnt/β-catenin pathway, an evolutionary conserved mechanism that defines the main body axis of vertebrates (12–16). Wnt signaling is activated by the binding of Wnt protein (the ligand) to a frizzled family receptor, which passes the biological signal to the protein dishevelled inside the cell. The three best-characterized Wnt signaling pathways are the canonical, the noncanonical planar cell polarity, and the noncanonical Wnt/calcium, respectively. The difference among them is that the canonical pathway involves the protein β-catenin. Stabilized β-catenin can enter the nucleus and activate several genes that induce dorsal cell characteristics (13). The link between Notch and β-catenin was confirmed in *Drosophila* and *Xenopus* (17–19) suggesting that Notch antagonizes Wnt signaling by promoting β-catenin degradation. Notch/β-catenin interaction is important in the development to control the size and anterior-posterior pattern of the brain (20) supporting the noncanonical function of Notch as a downregulator of constitutive Wnt activity (17, 19). Regarding noncanonical ligand-dependent Notch signaling, it was demonstrated that its major impact is on axon patterning through NICD interaction with the AbI cytoplasmic pathway (21–24). Molecularly, NICD binds to and suppresses the effects of two cofactors of Abl tyrosine kinase (23, 25, 26) controlling the actin structure and dynamics in the growth cone (22).

Notch activation and neurogenesis in adult brain

Notch pathway components are expressed in the neurogenic 'niches', specialized cellular microenvironments that modulate stem cell properties, including cell number, self-renewal, and fate decisions. In the adult brain, 'niches' that maintain a source of neural stem cells (NSCs) are the subventricular zone (SVZ) of the lateral ventricle and the dentate gyrus (DG) of the hippocampus. Persistence of neurogenesis in adulthood was identified more than a decade ago (32) and refers to the process by which new neurons are produced from NSCs in the adult brain. Physiologically, in the neurogenic 'niche', NSCs produce transient intermediate precursors (IP), which generate neuroblasts that exit the cell cycle and differentiate into neurons. The precise dynamics of neuron production from the NSCs remains controversial. Notch signaling is a key mediator of NSCs maintenance, suppressing the expression of proneural genes including Ascl1 and supporting the progenitor cell survival. The opposing states of quiescence vs. proliferation are controlled by Notch levels and seem to be cell-dependent: low levels of Notch lead to proliferation of NSCs and high levels, growth arrest (34). However, it was reported that neural progenitors are less responsive to Notch and more sensitive to environmental factors for the regulation of proliferation (27). If levels of Notch signaling remain low and growth factors are withdrawn, cells exit the cycle and differentiate into neurons. It was recently demonstrated that after the initiation of neurogenesis, cells remain at the immature neuron stage for weeks or months, allowing them to adapt to physiological or pathophysiological stimuli that may affect their maturation and differentiation (28). It has become increasingly evident that NSC self-renewal and differentiation in the SVZ and hippocampus are regulated by factors secreted by non-neuronal cell types, including microglia (35), endothelial cells (36, 37) or astrocytes (38–41) and that differences in the microenvironment and signaling pathways govern the two adult neurogenic niches (42–44). A schematic representation of NICD role in NSC renewal, cell cycle progression, and cell fate decision is depicted in Figure 2. In addition to neurogenic niches, Notch is also expressed throughout the adult brain, pointing at additional functions beyond its role in stem cell maintenance and differentiation. It was demonstrated that activation of the mammalian Notch pathway occurs reiteratively in migratory and postmitotic neurons, supporting that Notch acts as a master regulator of plasticity and neuronal migration.

Notch activation and neural dysfunction

The importance of Notch signaling for normal human adult brain function is demonstrated by its implications in neurological diseases as diverse as the Alagille syndrome, a developmental disorder associated with mental retardation (29), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (30), and certain types of schizophrenia (31). All of them show functional mutations in key

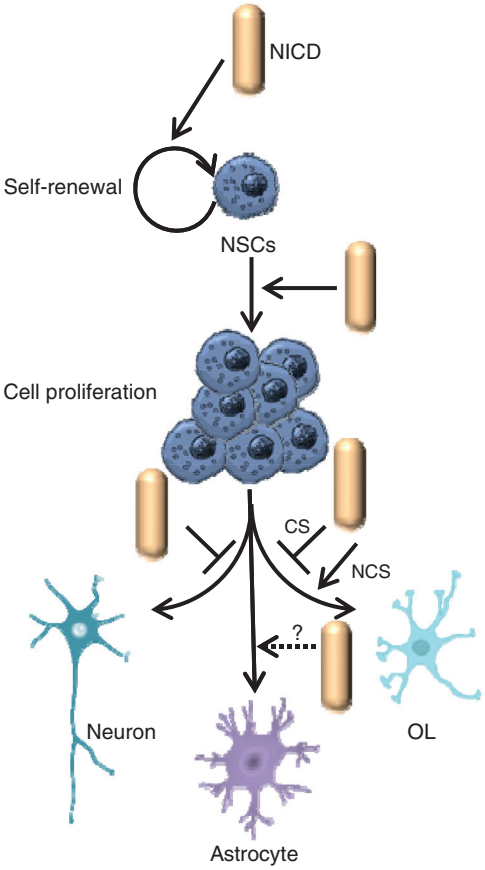

Figure 2 Role of NICD in NSCs renewal, proliferation and linage selection in adulthood. In the central nervous system, NICD mediates self-renewing divisions, and it has also been implicated in regulating quiescence of neural stem cells (NSCs). In progenitors, NICD is important for proliferation and de-differentiation. The most well-known role of Notch is the inhibition of neuronal differentiation of NSCs and progenitors. The activation of the canonical Notch signaling (CS) delays myelination, while the activation of the noncanonical pathway (NCS) leads to OPC maturation into oligodendrocytes (OL) and myelination of axons. By contrast, the role of NICD in the switch from neurogenesis to gliogenesis to generate astrocytes still presents controversies (dotted line).

components of the Notch pathway. Late onset CADASIL and the Alagille syndrome are associated with mutations in, respectively, *NOTCH3* and *JAGGED1* genes, while a *NOTCH4* polymorphism is strongly associated with susceptibility to schizophrenia. In addition, gene expression and immunohistochemical studies show that Notch is overexpressed in neurogenic and non-neurogenic regions in sporadic Alzheimer's disease (AD) (32, 33) and adult Down syndrome (DS) brains (34, 35). Moreover, pieces of experimental evidence associate Notch signaling with the progression of brain injury after stroke (36) and prognosis of certain brain tumors (37). Regardless of the cause of these diseases, AD, DS, and multi-infarct dementia present cognitive impairments and/or a neurodegenerative phenotype, which highlight the importance of altered Notch signaling in the adult brain as a contributing factor to neuronal dysfunction. The roles of Notch signaling in CNS injury and disease are complex, involving multiple cell types (neurons, glial cells, vascular cells, and lymphocytes), and either detrimental or beneficial actions on the pathogenic process and functional outcome have been reported. In the following section, we will discuss the role of Notch in nongenetic neurological diseases.

Brain ischemia

In ischemia or stroke, there is an increased neurogenesis mediated by the Notch signal in the SVZ and CA1 region of the hippocampus (38), which is subsequently downregulated to promote neuronal differentiation. In addition, Notch signaling is also involved in the angiogenic process after ischemia as its activation is important in the reorganization of blood vessels branching during reperfusion (39). Specifically, Notch regulates angiogenesis by controlling endothelial cell proliferation, migration, and adhesion (40). It was demonstrated that after Notch interaction with δ and Jagged/Serrate (Table 1), these ligands are cleaved similarly to the Notch receptor producing an intracellular domain, which inhibits endothelial cell proliferation but do not affect endothelial migration, sprouting angiogenesis or cell adhesion as shown for NICD (40). The effect of NICD is regulated by its polyubiquitination and proteasomal degradation. This process involves Fbxw7, an F-box protein that acts as a substrate-recognizing component of a type E3 ubiquitin ligase (41). In Fbxw7 loss-of-function models, the lifetime of NICD is prolonged leading to a general slowdown in angiogenesis, sprouting, and proliferation of endothelial cells (42). Vascular endothelial growth factors (VEGFs) have important roles in the development and function of the circulatory and nervous systems, and their expression increases shortly after ischemia (43). It has been suggested that decrements of Notch signaling allows an upregulation of VEGFR3 activity, which is proangiogenic in endothelial cells and mimics aspects of cancer cells growth (44). Brain ischemia is also characterized by an inflammatory response, and it is known that the Notch pathway modulates the activation of inflammatory cells, such as lymphocytes and microglia. Increasing evidence indicates that Notch plays an important role in regulating the differentiation of activated T cells into distinct types of effector T cells (45). The trigger of Notch signaling induced by

cerebral ischemia produces the activation of microglia by increasing NF-κB activity (46). In summary, Notch signaling might be beneficial as a stimulator of proliferation of NSCs for repair, but might also impair the outcome by the inhibition of differentiation toward neurons and its participation in the inflammatory response (36). The roles of Notch during ischemia are complex, and the potential therapeutic benefits of inhibition or activation of the pathway require further studies.

Alzheimer's disease (AD) and Down syndrome (DS)

In postmitotic neurons of the adult brain, Notch molecules are coexpressed and interact physically and functionally with presenilins (PSs), the catalytic component of the γ-secretase complex to generate NICD (as described above). The same γ-secretase complex is also implicated in the proteolytic processing of the amyloid precursor protein (APP) to originate the amyloid β (Aβ) peptide (47), which accumulates in the brain of AD (48) and DS patients (49). Mutations in PSs genes are responsible for rare familial Alzheimer's disease (FAD) cases (50, 51) characterized by an increased proportion of the more amyloidogenic form of Aβ peptide (Aβ42) or increased levels of the less amyloidogenic form of Aβ peptide (Aβ40) in the brain. A very recent report showed that expression of several FAD PS1 mutants in NSCs leads to impaired NSC self-renewal due to a reduced γ-secretase cleavage of Notch (52) suggesting that inhibition of Notch signaling may have a direct impact on the neurodegenerative phenotype observed in FAD brain. It was recently reported that neurogenesis and NSC renewal are both γ-secretase activity-dependent processes; however, the data showed that NSC renewal relays not only in Notch signaling but also on other substrates of γ-secretases (33). *Post mortem* studies on the status of neurogenesis in human AD patients have been contradictory with reports showing an increase in hippocampal neurogenesis (53) and others finding no differences (54). Additionally, a potential decrease in progenitor cell numbers in AD (55) was associated with cortical cholinergic loss. Thus, AD may cause depletion of progenitor cells by pushing their cell fate to immature neurons or glial cells. It is plausible that decreased activity of NSCs in the SVZ of AD brains decreases the potential for spontaneous replacement of lost neurons in the cerebral cortex of AD brain. Neurogenesis has also been investigated in DS neurospheres isolated from post-abortion fetal tissue. Considerably fewer neurons emerged after *in vitro* differentiation of neurospheres derived from the brain of DS compared to controls, while the numbers of glial cells remained unchanged. It is unclear though whether the number of neurons generated from these neurospheres was decreased due to increased apoptosis or due to a preferential differentiation of neurosphere cells into glia (56). The lack of mutations and overexpression of APP in sporadic AD and in DS patients, respectively, suggests that disturbances in Aβ clearance may be relevant to cerebral amyloid deposition in both diseases. In this context, it was recently demonstrated that Notch activation represses the transcription of insulin-degrading enzyme (IDE), the major metalloprotease involved in the proteolysis of Aβ peptide in the brain (57) providing a novel functional link between Notch activation and Aβ accumulation in sporadic AD and DS cases (58). These results are in agreement with pieces of experimental evidence showing that γ-secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke (59), suggesting a potential role for Notch signaling in the pathogenesis of AD given that the incidence of AD and vascular dementia is greatly increased following cerebral ischemia and stroke. The opposite activity of Notch in FAD compared to sporadic AD reinforces the striking differences between both clinical entities as suggested (60).

Multiple sclerosis

As previously described, Notch plays a critical role in the development of oligodendrocytes (OL) and has become a focus of attention in neurodegenerative diseases where oligodendrocytes are lost and axons demyelinated such as multiple sclerosis (MS). In the absence of pathology, Notch stimulates Schwann cell precursor differentiation and accelerates Schwann cell formation and proliferation in perinatal nerves during embryogenesis, but delays myelination in development and in adulthood (99). In the developing rat optic nerve, the differentiation of oligodendrocyte precursor cells (OPCs) is inhibited by Notch activation. Notch1 is expressed in OPCs and Jagged1 in axons, and it has been proposed that the expression of Jagged1 is downregulated to allow myelination (89). Thus, in this scenario, Notch seems to act as a restrictive rather than an instructive signal to keep the cells in their current developmental stage. On the contrary, the activation of the noncanonical Notch pathway by F3/contactin leads to OPC maturation and myelination via NICD/deltex interaction (61, 62, 100). MS is a neuroinflammatory disease associated with

demyelination that results in axonal degeneration. The process called remyelination can occur as a spontaneous regenerative process following demyelination. NSCs could be a potential source of OL, and it was demonstrated that Notch signaling maintains the pool of NSCs in their undifferentiated state enabling the formation of OL (63, 64). In demyelinating diseases, there is activation of astrocytes with increased JAG-1 expression, and this signal blocks OPC differentiation and myelination (65). Moreover, JAG-1 is absent in remyelinated lesion, suggesting that JAG 1-induced Notch signaling needs to be ended to allow myelin repair. Why the remyelinating processes fails in demyelinating diseases needs further study. However, Nakahara et al. (66) suggested that the problem in remyelination could be related to a failure of NICD nuclear transport after F3/contactin activation of Notch1 receptor in OPCs. In chronic demyelinated lesions, it was found that an increase in the expression of TAT-interacting protein 30 kDa (TIP30), a direct inhibitor of importin β, is the mediator of nuclear translocation of NICD (66). These dual roles of Notch signaling through canonical and/or noncanonical pathways in the demyelinating and remyelinating process needs to be fully elucidated to assess its real therapeutic value.

Brain tumors

Gliomas (ependymomas, oligodendrogliomas, and astrocytomas) and medulloblastoma (brain tumor of the cerebellum) are the most common primary brain tumors in adults and children, respectively. These tumors are thought to arise from glial cells in which Notch signaling plays a fundamental role during development. Recent findings have shown that deregulated Notch signaling contributes to the malignant potential of these tumors (67–69). Growing pieces of evidence point toward an important role for cancer stem cells in the initiation and maintenance of glioma and medulloblastoma. The study of human glioma specimens showed that Notch1 mRNA and protein expression were increased in the glioma cells compared with adjacent or normal tissue (67). Moreover, this upregulation correlates with the severity of the disease (67). Glioma cells share growth characteristics and gene expression patterns with normal NSCs. Several Notch ligands have been postulated to be responsible for the activation of Notch signaling within the glioma. A possible source of Notch ligands are endothelial cells adjacent to receptor-positive cancer cells (70, 71). The extracellular matrix might also provide ligands for Notch, such as Fibulin-3, a matrix protein that is absent in normal brain but upregulated in gliomas. Fibulin-3 expression correlated with expression levels of Notch-dependent genes (72). Notch signaling is also involved in tumor angiogenesis regulating VEGF actions (as described above). During development, VEGF stimulates angiogenesis and lymphangiogenesis through the VEGFR-2 and VEGFR-3 tyrosine kinases expressed in endothelial cells. However, VEGFR-3, whose expression is restricted to the lymphatic endothelium in adulthood, is upregulated in the microvasculature of tumor. It was suggested that VEGF induces the Notch ligand (DLL-4) in endothelial cells and that Notch signaling activation by DLL-4 reduces VEGF-3 gene expression and endothelial sprouting (73, 74). In addition to Notch signaling upregulation, increase in Sonic hedgehog (Shh) activity has been identified in medulloblastomas (75). As Notch signaling, Shh pathway has been implicated in a range of sporadic tumors in various organs and tissues (76). Shh has a widespread expression pattern, and similarly to Notch, it plays an important role in differentiation and proliferation during development (77). Shh signaling is primarily responsible for the dramatic expansion of the granule cell progenitor precursor pool in the cerebellum due to its mitogenic effect (78). Likewise, Notch signaling also stimulates granule cell proliferation through the upregulation of Hes1 (69). Moreover, Hes1 expression is also induced by Shh in a noncanonical signaling, suggesting a common downstream effector of these two pathways (79). Indeed, the inhibitions of Shh and Notch pathways showed a reduced tumor progression (80).

A summary of the consequences of Notch signaling activation in diseased adult brain is summarized in Table 2.

Inhibition of Notch signaling as a therapeutic target in neurological diseases

The Notch pathway is dysregulated in different neurological diseases, and its pharmacological modulation may be a potential therapeutic target. Compounds currently described as modulators of Notch signaling include inhibitors of ADAM10 or γ-secretase activity (which are necessary for Notch processing) and small molecules that act as posttranscriptional inhibitors of Notch. As ADAM10 is implicated in the shedding of dozens of substrates that drive cancer progression and inflammatory disease, including E-cadherin, EGF, ErbB2, and inflammatory

Table 2 Consequences of Notch activation in neurological diseases.

Disease	Result of Notch activation	Reference
Ischemia	Proliferation of progenitors	(106)
	Inhibition of terminal differentiation	(87, 106)
	Participation in microglial activation	(46, 88)
AD	Interference in APP and Aβ metabolism	(47, 58)
MS	Canonical pathway: proliferation of NSC and restrained differentiation	(63–65)
	Noncanonical pathway: OPC differentiation	(61, 62)
Brain tumor	Glial cell overproliferation	(67–69, 79) (82)

AD, Alzheimer disease; MS, multiple sclerosis.

cytokines, it has become the focus of intense interest as a potential drug target for disease treatment.

Different α-secretase inhibitors (ASI) and γ-secretase inhibitors (GSIs) were tested for the inhibition of glioblastoma growth, and pieces of experimental evidence showed that: 1) the treatment of cultures obtained from glioma samples with GSIs reduced the proliferation of the cells and induced their differentiation (81), 2) *In vitro* studies on medulloblastoma growth demonstrated that treatment with GSIs did not deplete the totality of the cells, but a population required for tumor xenograft formation (82). In contrast to these data, *in vivo* studies have pointed out that in Shh pathway-driven medulloblastomas, Notch signaling is not essential for the initiation, engraftment, or maintenance of the tumor, and that inhibition of the Notch pathway might not be the most suitable therapeutic approach (83); 3) Local nanoparticle delivery of ASIs, but not GSIs, increased the survival time significantly in a glioblastomas stem cell xenograft treatment model, decreased tumor size, and Notch activity (84). This work indicates ASIs as an alternative to GSIs for treatment of glioblastomas and possibly other cancers as well. The potential of inhibition of Notch activity for the therapy of different brain tumors is currently under analysis in different preclinical assays (85).

GSIs have also been tested in animal models of AD (86) in an attempt to treat patients by blocking the production of Aβ peptides and subsequent plaque formation. Given that the potential patient population for the treatment of Alzheimer's disease is elderly, the profound effects of Notch disruption seen in embryonic and fetal development may not be of concern. However, it is now known that Notch signaling plays an important role in the ongoing differentiation processes of the immune system and the gastrointestinal tract. As a result, it may be necessary to separate γ-secretase activity on APP from Notch processing activity to achieve an adequate safety margin for clinical development of a GSI therapeutic agent for Alzheimer's disease. Additionally, GSIs were also tested for their potential utility in the treatment of ischemia. In this context, experiments in animal models showed that: 1) the administration of a GSI increased the number of newly generated hippocampal neurons in the CA1 region (87) reinforcing the concept that Notch signaling contributes to the regulation of neurogenesis in the adult brain after ischemia; 2) treatment of microglia with GSIs reduces NF-κB/p65 nuclear translocation, together with a decrease in microglia proliferation and expression of IL-1β and TNF-α, critical inflammatory cytokines in the damage after the ischemic insult (88).

While these studies highlight the significant progress in the development and potential efficacy of synthetic ASIs and GSIs, there are still many challenges to overcome. For example, some *in vitro* studies have shown stimulus-dependent redundancy for the ADAMs involved in specific shedding events, which if recapitulated *in vivo*, may limit the effectiveness of treatment with specific ADAM10 inhibitors (89). Regarding GSIs, it is important to note that γ-secretase is an unconventional aspartyl protease that resides and cleaves its substrates within the lipid bilayer. In fact, γ-secretase complex belongs to a group of proteases called intramembrane cleaving proteases (I-CLiPs) that are membrane-embedded enzymes. These enzymes hydrolyze transmembrane substrates, and the residues essential for catalysis reside within the boundaries of the lipid bilayer. γ-Secretase displays poor substrate specificity, but a functional γ-secretase cleavage has been clearly demonstrated for some substrates such as Notch, N-cadherin, and ErB4. Proteolysis of N-cadherin leads to degradation of the transcriptional factor CREB-binding protein (CBP), and cleavage of ErB4 inhibits astrocyte differentiation by interacting with repressors of astrocyte gene expression. The fact that GSIs block the processing of different proteins may be the main cause of toxicity in preclinical testing and represents a major source of concern in clinical trials. The discovery that some small organic molecules and some nonsteroidal anti-inflammatory drugs may modulate the cleavage

activity of γ-secretase on APP without interfering with the cleavage of other substrates has led to intensive efforts in designing γ-secretase modulators that appear much more attractive from a safety point of view than traditional GSIs in the treatment of Alzheimer disease (90).

Emerging evidence implicates microRNAs (miRNAs) as being intimately involved in the regulation of Notch signaling and different neurological disorders such as tumors (acting as either oncogenes or tumor suppressors), stroke, and hypoxia. MiRNAs are small noncoding RNA molecules that participate in all cellular processes of the organism, including development, differentiation, metabolism, and programmed cell death, among others (91, 92). Pieces of experimental evidence showed that: 1) a particular miRNA, miR-146a, acts as a tumor suppressor in gliomas through the inhibition of Notch1 posttranscriptionally. miR-146 seems to be able to integrate information from various pathways to detect whether they are moving in a protumorigenesis direction and then counteract that trend if necessary (93); 2) miR-124a is preferentially expressed in neurons and specifically binds to JAG-1 mRNA suppressing its expression. Following a stroke, this miRNA is downregulated allowing JAG1 expression in the NSCs and promoting NSC proliferation by the Notch pathway activation (94); and 3) upregulation of miR-210, a hypoxia-specific miRNA, may activate the Notch pathway and promote vessel formation (95). Knowledge acquired on miRNA function, expression, and deregulation has opened up new opportunities for therapeutic intervention. Emerging preclinical studies are demonstrating the feasibility of inhibiting overexpressed miRNAs or restoring the expression of lost miRNAs. The therapeutic use of miRNAs as single agents or in combination with current treatments may offer technical advantages over other approaches. However, in order to accelerate the translation of this knowledge into the clinics, several aspects must be improved and considered such as standardization of pharmacological preparations, pharmacokinetic and pharmacodynamic analysis to ensure that therapeutic doses of miRNAs are achieved in target tissues, the interaction with the host immune system, as it may improve or weaken the therapeutic effects of a particular miRNA and the consequences of long treatment periods in human clinical settings.

Conclusions

The first description by J.S. Dexter of a Notch gene mutation in *Drosophila* was made in March 1913 and now after nearly a century of research on Notch signaling, emerging concepts in the last years suggest that the developmental functions of the Notch signaling pathway may be reused throughout life to guarantee brain adaptation. However, we still have much to learn about the many roles of Notch in neural functionality and the structure of axons and dendrites in adulthood. There has been an impressive recent progress in the comprehension that this simple signaling mechanism produces such a bewildering array of downstream consequences, including completely different effects in the same cell at different times. However, further *in vivo* experiments are necessary to better understand the relevance of the two Notch signaling mechanisms (canonical and noncanonical), the dual roles of Notch signaling as a homeostatic factor both in health and disease. Conceptually, the identification of a link between two apparently distant physiopathological processes such as neurodevelopment and neurodegeneration provides new perspectives in the pathophysiology of Notch-related neurodegenerative diseases. However, due to the multiplicity of gene targets and cellular processes regulated by Notch, it is difficult to speculate about how its activation may impact upon the disease course. In summary, it is not inconceivable that future developments of genetic methods to manipulate Notch signaling in adult brain will provide pieces of novel evidence to encourage Notch modulation as an accepted target for medical treatment of human diseases.

Acknowledgments: We thank Dr. Eduardo M. Castaño for suggestions and critical reading of the manuscript that improved its quality. This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT2010 2715 (to PM) and from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET)-PIP693 and Florencio Fiorini Foundation (to LM).

Received March 5, 2013; accepted July 30, 2013

References

1. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 2009; 137: 216–33.

2. Kovall RA, Blacklow SC. Mechanistic insights into Notch receptor signaling from structural and biochemical studies. Curr Top Dev Biol 2010; 92: 31–71.

3. Stanley P, Okajima T. Roles of glycosylation in Notch signaling. Curr Top Dev Biol 2010; 92: 131–64.
4. Crawford HC, Dempsey PJ, Brown G, Adam L, Moss ML. ADAM10 as a therapeutic target for cancer and inflammation. Curr Pharm Des 2009; 15: 2288–99.
5. Wolfe MS. Gamma-secretase: structure, function, and modulation for Alzheimer's disease. Curr Top Med Chem 2008; 8: 2–8.
6. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. Development 2011; 138: 3593–612.
7. Nam Y, Sliz P, Song L, Aster JC, Blacklow SC. Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. Cell 124: 973–83.
8. Wilson JJ, Kovall RA. Crystal structure of the CSL-Notch-Mastermind ternary complex bound to DNA. Cell 2006; 124: 985–96.
9. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. Dev Cell 2009; 16: 633–47.
10. Bray S, Bernard F. Notch targets and their regulation. Curr Top Dev Biol 2010; 92: 253–75.
11. Sanalkumar R, Dhanesh SB, James J. Non-canonical activation of Notch signaling/target genes in vertebrates. Cell Mol Life Sci 2010; 67: 2957–68.
12. Heasman J, Crawford A, Goldstone K, Garner-Hamrick P, Gumbiner B, McCrea P, Kintner C, Noro CY, Wylie C. Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. Cell 1994; 79: 791–803.
13. Schneider S, Steinbeisser H, Warga RM, Hausen P. Beta-catenin translocation into nuclei demarcates the dorsalizing centers in frog and fish embryos. Mech Dev 1996; 57: 191–8.
14. Kimelman D, Griffin KJ. Mesoderm induction: a postmodern view. Cell 1998; 94: 419–21.
15. Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. Curr Opin Genet Dev 2001; 11: 547–53.
16. Marikawa Y. Wnt/beta-catenin signaling and body plan formation in mouse embryos. Semin Cell Dev Biol 2006; 17: 175–84.
17. Hayward P, Brennan K, Sanders P, Balayo T, DasGupta R, Perrimon N, Martinez Arias A. Notch modulates Wnt signalling by associating with Armadillo/beta-catenin and regulating its transcriptional activity. Development 2005; 132: 1819–30.
18. Hayward P, Kalmar T, Arias AM. Wnt/Notch signalling and information processing during development. Development 2008; 135: 411–24.
19. Sanders PG, Munoz-Descalzo S, Balayo T, Wirtz-Peitz F, Hayward P, Arias AM. Ligand-independent traffic of Notch buffers activated Armadillo in Drosophila. PLoS Biol 2009; 7: e1000169.
20. Acosta H, Lopez SL, Revinski DR, Carrasco AE. Notch destabilises maternal beta-catenin and restricts dorsal-anterior development in Xenopus. Development 2011; 138: 2567–79.
21. Giniger E. A role for Abl in Notch signaling. Neuron 1998; 20: 667–81.
22. Kuzina I, Song JK, Giniger E. How Notch establishes longitudinal axon connections between successive segments of the Drosophila CNS. Development 2011; 138: 1839–49.
23. Crowner D, Le Gall M, Gates MA, Giniger E. Notch steers Drosophila ISNb motor axons by regulating the Abl signaling pathway. Curr Biol 2003; 13: 967–72.
24. Le Gall M, De Mattei C, Giniger E. Molecular separation of two signaling pathways for the receptor, Notch. Dev Biol 2008; 313: 556–67.
25. Song JK, Kannan R, Merdes G, Singh J, Mlodzik M, Giniger E. Disabled is a bona fide component of the Abl signaling network. Development 2010; 137: 3719–27.
26. Song JK, Giniger E. Noncanonical Notch function in motor axon guidance is mediated by Rac GTPase and the GEF1 domain of Trio. Dev Dyn 2011; 240: 324–32.
27. Ables JL, Breunig JJ, Eisch AJ, Rakic P. Not(ch) just development: Notch signalling in the adult brain. Nat Rev Neurosci 2011; 12: 269–83.
28. Minge D, Bahring R. Acute alterations of somatodendritic action potential dynamics in hippocampal CA1 pyramidal cells after kainate-induced status epilepticus in mice. PLoS One 2011; 6: e26664.
29. Li L, Krantz ID, Deng Y, Genin A, Banta AB, Collins CC, Qi M, Trask BJ, Kuo WL, Cochran J, Costa T, Pierpont ME, Rand EB, Piccoli DA, Hood L, Spinner NB. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat Genet 1997; 16: 243–51.
30. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cécillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996; 383: 707–10.
31. Wei J, Hemmings GP. The NOTCH4 locus is associated with susceptibility to schizophrenia. Nat Genet 2000; 25: 376–7.
32. Berezovska O, Xia MQ, Hyman BT. Notch is expressed in adult brain, is coexpressed with presenilin-1, and is altered in Alzheimer disease. J Neuropathol Exp Neurol 1998; 57: 738–45.
33. Nagarsheth MH, Viehman A, Lippa SM, Lippa CF. Notch-1 immunoexpression is increased in Alzheimer's and Pick's disease. J Neurol Sci 2006; 244: 111–6.
34. Fischer DF, van Dijk R, Sluijs JA, Nair SM, Racchi M, Levelt CN, van Leeuwen FW, Hol EM. Activation of the Notch pathway in Down syndrome: cross-talk of Notch and APP. FASEB J 2005; 19: 1451–8.
35. Lockstone HE, Harris LW, Swatton JE, Wayland MT, Holland AJ, Bahn S. Gene expression profiling in the adult Down syndrome brain. Genomics 2007; 90: 647–60.
36. Arumugam TV, Chan SL, Jo DG, Yilmaz G, Tang SC, Cheng A, Gleichmann M, Okun E, Dixit VD, Chigurupati S, Mughal MR, Ouyang X, Miele L, Magnus T, Poosala S, Granger DN, Mattson MP. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat Med 2006; 12: 621–3.
37. Stockhausen MT, Kristoffersen K, Poulsen HS. Notch signaling and brain tumors. Adv Exp Med Biol 2012; 727: 289–304.
38. Wang X, Mao X, Xie L, Greenberg DA, Jin K. Involvement of Notch1 signaling in neurogenesis in the subventricular zone of normal and ischemic rat brain in vivo. J Cereb Blood Flow Metab 2009; 29: 1644–54.
39. Cristofaro B, Shi Y, Faria M, Suchting S, Leroyer AS, Trindade A, Duarte A, Zovein AC, Iruela-Arispe ML, Nih LR, Kubis N, Henrion D, Loufrani L, Todiras M, Schleifenbaum J, Gollasch M,

Zhuang ZW, Simons M, Eichmann A, le Noble F. Dll4-Notch signaling determines the formation of native arterial collateral networks and arterial function in mouse ischemia models. Development 2013; 140: 1720–9.
40. Liebler SS, Feldner A, Adam MG, Korff T, Augustin HG, Fischer A. No evidence for a functional role of bi-directional Notch signaling during angiogenesis. PLoS One 2012; 7: e53074.
41. Welcker M, Clurman BE. FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev Cancer 2008; 8: 83–93.
42. Izumi N, Helker C, Ehling M, Behrens A, Herzog W, Adams RH. Fbxw7 controls angiogenesis by regulating endothelial Notch activity. PLoS One 2012; 7: e41116.
43. Greenberg DA, Jin K. Vascular endothelial growth factors (VEGFs) and stroke. Cell Mol Life Sci 2013; 70: 1753–61.
44. Benedito R, Rocha SF, Woeste M, Zamykal M, Radtke F, Casanovas O, Duarte A, Pytowski B, Adams RH. Notch-dependent VEGFR3 upregulation allows angiogenesis without VEGF-VEGFR2 signalling. Nature 2012; 484: 110–4.
45. Talora C, Campese AF, Bellavia D, Felli MP, Vacca A, Gulino A, Screpanti I. Notch signaling and diseases: an evolutionary journey from a simple beginning to complex outcomes. Biochim Biophys Acta 2008; 1782: 489–97.
46. Li HY, Yuan ZY, Wang YG, Wan HJ, Hu J, Chai YS, Lei F, Xing DM, Du LJ. Role of baicalin in regulating Toll-like receptor 2/4 after ischemic neuronal injury. Chin Med J (Engl) 2012; 125: 1586–93.
47. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 2005; 120: 545–55.
48. Wisniewski T, Ghiso J, Frangione B. Alzheimer's disease and soluble A beta. Neurobiol Aging 1994; 15: 143–52.
49. Head E, Lott IT. Down syndrome and beta-amyloid deposition. Curr Opin Neurol 2004; 17: 95–100.
50. Lee FH, Fadel MP, Preston-Maher K, Cordes SP, Clapcote SJ, Price DJ, Roder JC, Wong AH. Disc1 point mutations in mice affect development of the cerebral cortex. J Neurosci 2011; 31: 3197–206.
51. Citron M, Eckman CB, Diehl TS, Corcoran C, Ostaszewski BL, Xia W, Levesque G, St George Hyslop P, Younkin SG, Selkoe DJ. Additive effects of PS1 and APP mutations on secretion of the 42-residue amyloid beta-protein. Neurobiol Dis 1998; 5: 107–16.
52. Veeraraghavalu K, Choi SH, Zhang X, Sisodia SS. Presenilin 1 mutants impair the self-renewal and differentiation of adult murine subventricular zone-neuronal progenitors via cell-autonomous mechanisms involving notch signaling. J Neurosci 2010; 30: 6903–15.
53. Jin K, Galvan V, Xie L, Mao XO, Gorostiza OF, Bredesen DE, Greenberg DA. Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APPSw,Ind) mice. Proc Natl Acad Sci USA 2004; 101: 13363–7.
54. Boekhoorn K, Joels M, Lucassen PJ. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol Dis 2006; 24: 1–14.
55. Ziabreva I, Perry E, Perry R, Minger SL, Ekonomou A, Przyborski S, Ballard C. Altered neurogenesis in Alzheimer's disease. J Psychosom Res 2006; 61: 311–6.
56. Bahn S, Mimmack M, Ryan M, Caldwell MA, Jauniaux E, Starkey M, Svendsen CN, Emson P. Neuronal target genes of the neuron-restrictive silencer factor in neurospheres derived from fetuses with Down's syndrome: a gene expression study. Lancet 2002; 359: 310–5.
57. Fernandez-Gamba A, Leal MC, Morelli L, Castano EM. Insulin-degrading enzyme: structure-function relationship and its possible roles in health and disease. Curr Pharm Des 2009; 15: 3644–55.
58. Leal MC, Surace EI, Holgado MP, Ferrari CC, Tarelli R, Pitossi F, Wisniewski T, Castaño EM, Morelli L. Notch signaling proteins HES-1 and Hey-1 bind to insulin degrading enzyme (IDE) proximal promoter and repress its transcription and activity: implications for cellular Abeta metabolism. Biochim Biophys Acta 2012; 1823: 227–35.
59. Arumugam TV, Cheng YL, Choi Y, Choi YH, Yang S, Yun YK, Park JS, Yang DK, Thundyil J, Gelderblom M, Karamyan VT, Tang SC, Chan SL, Magnus T, Sobey CG, Jo DG. Evidence that gamma-secretase-mediated Notch signaling induces neuronal cell death via the nuclear factor-kappaB-Bcl-2-interacting mediator of cell death pathway in ischemic stroke. Mol Pharmacol 2011; 80: 23–31.
60. Dorfman VB, Pasquini L, Riudavets M, López-Costa JJ, Villegas A, Troncoso JC, Lopera F, Castaño EM, Morelli L. Differential cerebral deposition of IDE and NEP in sporadic and familial Alzheimer's disease. Neurobiol Aging 2010; 31: 1743–57.
61. Cui XY, Hu QD, Tekaya M, Shimoda Y, Ang BT, Nie DY, Sun L, Hu WP, Karsak M, Duka T, Takeda Y, Ou LY, Dawe GS, Yu FG, Ahmed S, Jin LH, Schachner M, Watanabe K, Arsenijevic Y, Xiao ZC. NB-3/Notch1 pathway via Deltex1 promotes neural progenitor cell differentiation into oligodendrocytes. J Biol Chem 2004; 279: 25858–65.
62. Hu QD, Ang BT, Karsak M, Hu WP, Cui XY, Duka T, Takeda Y, Chia W, Sankar N, Ng YK, Ling EA, Maciag T, Small D, Trifonova R, Kopan R, Okano H, Nakafuku M, Chiba S, Hirai H, Aster JC, Schachner M, Pallen CJ, Watanabe K, Xiao ZC. F3/contactin acts as a functional ligand for Notch during oligodendrocyte maturation. Cell 2003; 115: 163–75.
63. Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA. Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 1998; 21: 63–75.
64. Jurynczyk M, Selmaj K. Notch: a new player in MS mechanisms. J Neuroimmunol 2010; 218: 3–11.
65. John GR, Shankar SL, Shafit-Zagardo B, Massimi A, Lee SC, Raine CS, Brosnan CF. Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med 2002; 8: 1115–21.
66. Nakahara J, Kanekura K, Nawa M, Aiso S, Suzuki N. Abnormal expression of TIP30 and arrested nucleocytoplasmic transport within oligodendrocyte precursor cells in multiple sclerosis. J Clin Invest 2009; 119: 169–81.
67. Jiang L, Wu J, Chen Q, Hu X, Li W, Hu G. Notch1 expression is upregulated in glioma and is associated with tumor progression. J Clin Neurosci 2011; 18: 387–90.
68. Purow BW, Sundaresan TK, Burdick MJ, Kefas BA, Comeau LD, Hawkinson MP, Su Q, Kotliarov Y, Lee J, Zhang W, Fine HA. Notch-1 regulates transcription of the epidermal growth factor receptor through p53. Carcinogenesis 2008; 29: 918–25.
69. Solecki DJ, Liu XL, Tomoda T, Fang Y, Hatten ME. Activated Notch2 signaling inhibits differentiation of cerebellar granule neuron precursors by maintaining proliferation. Neuron 2001; 31: 557–68.
70. Zhu TS, Costello MA, Talsma CE, Flack CG, Crowley JG, Hamm LL, He X, Hervey-Jumper SL, Heth JA, Muraszko KM, DiMeco F, Vescovi

AL, Fan X. Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. Cancer Res 2011; 71: 6061–72.
71. Jubb AM, Browning L, Campo L, Turley H, Steers G, Thurston G, Harris AL, Ansorge O. Expression of vascular Notch ligands Delta-like 4 and Jagged-1 in glioblastoma. Histopathology 2012; 60: 740–7.
72. Hu B, Nandhu MS, Sim H, Agudelo-Garcia PA, Saldivar JC, Dolan CE, Mora ME, Nuovo GJ, Cole SE, Viapiano MS. Fibulin-3 promotes glioma growth and resistance through a novel paracrine regulation of Notch signaling. Cancer Res 2012; 72: 3873–85.
73. Tammela T, Zarkada G, Wallgard E, Murtomäki A, Suchting S, Wirzenius M, Waltari M, Hellström M, Schomber T, Peltonen R, Freitas C, Duarte A, Isoniemi H, Laakkonen P, Christofori G, Ylä-Herttuala S, Shibuya M, Pytowski B, Eichmann A, Betsholtz C, Alitalo K. Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature 2008; 454: 656–60.
74. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009; 21: 154–65.
75. Roussel MF, Hatten ME. Cerebellum development and medulloblastoma. Curr Top Dev Biol 2011; 94: 235–82.
76. Borzillo GV, Lippa B. The Hedgehog signaling pathway as a target for anticancer drug discovery. Curr Top Med Chem 2005; 5: 147–57.
77. Jiang R, Bush JO, Lidral AC. Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn 2006; 235: 1152–66.
78. Kimura H, Stephen D, Joyner A, Curran T. Gli1 is important for medulloblastoma formation in Ptc1+/- mice. Oncogene 2005; 24: 4026–36.
79. Ingram WJ, McCue KI, Tran TH, Hallahan AR, Wainwright BJ. Sonic Hedgehog regulates Hes1 through a novel mechanism that is independent of canonical Notch pathway signalling. Oncogene 2008; 27: 1489–500.
80. Hallahan AR, Pritchard JI, Hansen S, Benson M, Stoeck J, Hatton BA, Russell TL, Ellenbogen RG, Bernstein ID, Beachy PA, Olson JM. The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. Cancer Res 2004; 64: 7794–800.
81. Hu YY, Zheng MH, Cheng G, Li L, Liang L, Gao F, Wei YN, Fu LA, Han H. Notch signaling contributes to the maintenance of both normal neural stem cells and patient-derived glioma stem cells. BMC Cancer 2011; 11: 1–13.
82. Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM, Eberhart CG. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 2006; 66: 7445–52.
83. Hatton BA, Villavicencio EH, Pritchard J, LeBlanc M, Hansen S, Ulrich M, Ditzler S, Pullar B, Stroud MR, Olson JM. Notch signaling is not essential in sonic hedgehog-activated medulloblastoma. Oncogene 2010; 29: 3865–72.
84. Floyd DH, Kefas B, Seleverstov O, Mykhaylyk O, Dominguez C, Comeau L, Plank C, Purow B. Alpha-secretase inhibition reduces human glioblastoma stem cell growth in vitro and in vivo by inhibiting Notch. Neuro Oncol 2012; 14: 1215–26.
85. Groth C, Fortini ME. Therapeutic approaches to modulating Notch signaling: current challenges and future prospects. Semin Cell Dev Biol 2012; 23: 465–72.
86. Kounnas MZ, Danks AM, Cheng S, Tyree C, Ackerman E, Zhang X, Ahn K, Nguyen P, Comer D, Mao L, Yu C, Pleynet D, Digregorio PJ, Velicelebi G, Stauderman KA, Comer WT, Mobley WC, Li YM, Sisodia SS, Tanzi RE, Wagner SL. Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. Neuron 2010; 67: 769–80.
87. Oya S, Yoshikawa G, Takai K, Tanaka JI, Higashiyama S, Saito N, Kirino T, Kawahara N. Attenuation of Notch signaling promotes the differentiation of neural progenitors into neurons in the hippocampal CA1 region after ischemic injury. Neuroscience 2009; 158: 683–92.
88. Wei Z, Chigurupati S, Arumugam TV, Jo DG, Li H, Chan SL. Notch activation enhances the microglia-mediated inflammatory response associated with focal cerebral ischemia. Stroke 2011; 42: 2589–94.
89. Fridman JS, Caulder E, Hansbury M, Liu X, Yang G, Wang Q, LoY, Zhou B-B, Pan M, Thomas SM, Grandis JR Zhuo J, Yao W, Newton RC, Friedman SM, Scherle PA, Vaddi K. Selective inhibition of ADAM metalloproteases as a novel approach for modulating ErbB pathways in cancer. Clin Cancer Res 2007; 13: 1892–902.
90. Panza F, Frisardi V, Solfrizzi V, Imbimbo BP, Logroscino G, Santamato A, Greco A, Seripa D, Pilotto A. Interacting with gamma-secretase for treating Alzheimer's disease: from inhibition to modulation. Curr Med Chem 2011; 18: 5430–47.
91. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 2006; 24: 857–64.
92. Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S. A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc Natl Acad Sci USA 2005; 102: 16426–31.
93. Mei J, Bachoo R, Zhang CL. MicroRNA-146a inhibits glioma development by targeting Notch1. Mol Cell Biol 2011; 31: 3584–92.
94. Liu XS, Chopp M, Zhang RL, Tao T, Wang XL, Wang XL, Kassis H, Hozeska-Solgot A, Zhang L, Chen C, Zhang ZG. MicroRNA profiling in subventricular zone after stroke: MiR-124a regulates proliferation of neural progenitor cells through Notch signaling pathway. PLoS One 2011; 6: e23461.
95. Lou YL, Guo F, Liu F, Gao FL, Zhang PQ, Niu X, Guo SC, Yin JH, Wang Y, Deng ZF. miR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. Mol Cell Biochem 2012; 370: 45–51.
96. Sestan N, Artavanis-Tsakonas S, Rakic P. Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. Science 1999; 286: 741–6.
97. Breunig JJ, Silbereis J, Vaccarino FM, Sestan N, Rakic P. Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus. Proc Natl Acad Sci USA 2007; 104: 20558–63.
98. Stump G, Durrer A, Klein AL, Lutolf S, Suter U, Taylor V. Notch1 and its ligands Delta-like and Jagged are expressed and active in distinct cell populations in the postnatal mouse brain. Mech Dev 2002; 114: 153–9.
99. Carlén M, Meletis K, Göritz C, Darsalia V, Evergren E, Tanigaki K, Amendola M, Barnabé-Heider F, Yeung MS, Naldini L, Honjo T, Kokaia Z, Shupliakov O, Cassidy RM, Lindvall O, Frisén J. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci 2009; 12: 259–67.

100. Irvin DK, Zurcher SD, Nguyen T, Weinmaster G, Kornblum HI. Expression patterns of Notch1, Notch2, and Notch3 suggest multiple functional roles for the Notch-DSL signaling system during brain development. J Comp Neurol 2001; 436: 167–81.
101. Yoon KJ, Koo BK, Im SK, Jeong HW, Ghim J, Kwon MC, Moon JS, Miyata T, Kong YY. Mind bomb 1-expressing intermediate progenitors generate notch signaling to maintain radial glial cells. Neuron 2008; 58: 519–31.
102. Shutter JR, Scully S, Fan W, Richards WG, Kitajewski J, Deblandre GA, Kintner CR, Stark KL. Dll4, a novel Notch ligand expressed in arterial endothelium. Genes Dev 2000; 14: 1313–8.
103. Murphy PA, Lam MT, Wu X, Kim TN, Vartanian SM, Bollen AW, Carlson TR, Wang RA. Endothelial Notch4 signaling induces hallmarks of brain arteriovenous malformations in mice. Proc Natl Acad Sci USA 2008; 105: 10901–6.
104. Alberi L, Liu S, Wang Y, Badie R, Smith-Hicks C, Wu J, Pierfelice TJ, Abazyan B, Mattson MP, Kuhl D, Pletnikov M, Worley PF, Gaiano N. Activity-induced Notch signaling in neurons requires Arc/Arg3.1 and is essential for synaptic plasticity in hippocampal networks. Neuron 2011; 69: 437–44.
105. Kurisu J, Fukuda T, Yokoyama S, Hirano T, Kengaku M. Polarized targeting of DNER into dendritic plasma membrane in hippocampal neurons depends on endocytosis. J Neurochem 2010; 113: 1598–610.
106. Liu ZQ, Zhang JT, Tang MK. [Regulation of microglia on neurogenesis in adult mammals]. Sheng Li Ke Xue Jin Zhan 2011; 42: 21–5.