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Notch Signaling & Micro RNAs - two tumblers of neurogenesis and Gliomas

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Abstract

Gliomas are one of the most annihilating types of brain tumors having a high rate of annual incidence worldwide. Notch signaling is an evolutionary conserved pathway that regulates differentiation and development. Aberrations in Notch signalling pathways lead to severe pathological state such as the Gliomas. MicroRNAs (miRNAs) are the tiny molecules less than 200 bps in length and regulate a myriad of cellular processes. Categorically, miRNAs are divided in to oncogenic and tumor suppressor miRNAs. Accumulating data have identified miRNAs, which positively or negatively regulate Notch signaling in Gliomas. Here, we have assessed status of our understanding of the interplay between miRNA-base regulation of Notch signaling in gliomas, interaction between Notch signaling and other signaling cascades and have also discussed use of natural compounds that will help us get closer to personalized medicine for gliomas.

Keywords: Notch signaling; MicroRNA; Therapeutic Targets; Gliomas.

Introduction

Gliomas are a type of brain tumors that are highly malignant and carry metastatic potential. One reason for this aggressive behaviour is the involvement of stem cells that elevate tumor potential and hamper the drug's efficacy (1). Glioma cells nurture along the neuronal cells due to their ability to take over normal cell growth regulatory mechanisms (2). Notch signaling has been reported to play a role in the development of glioma cells and is a major cause for tumorigenesis (3). Any mutation in notch receptor or associated machinery can trigger abrogated notch signaling that results in abnormal growth of glial cells (3). Furthermore, by taking possession of cell regulatory mechanism tumorous glial cells promote upregulation of notch receptor that in turn ensures active proliferation of glioblastoma cells (4). This preferable control also enables glioblastoma cells to hamper cell cycle progression, thus, making glial cells resistant to chemotherapy. Furthermore, studies have indicated that resistant glial cells, especially, primary glioblastoma cells have higher activity of the notch. Consequently such cells have more potential to become chemo-resistant. Studies conducted *in vitro* and on xenograft models have shown that abrogated notch signaling is the key element responsible for brain tumors and inhibition of notch signaling results in halted cell growth (5, 6). There are several studies, which have also documented the tumor suppressor role of notch signaling in some gliomas (7). A recent study has shown that knockout of notch signaling in pro-neural PDGF/P53 results in tumor survival, indicating the fact that notch might play tumor suppressor role (8). Inactivating notch mutations are well observed in patients with gliomas and absence of notch signaling has been linked to early progression and overall survival of the tumor. From these findings, it is explicated that notch signaling role in gliomas is bi-facet and requires further explorations. Gliomas have a high rate of annual

incidence worldwide. Malignant gliomas, as per WHO guidelines, are one of the several types of gliomas, among which, astrosytomas and mixed gliomas have an annual incidence of 5 per 100,000 individuals (9, 10). Gliomas have also been characterized on the basis of severity of disease and invasiveness as grade I to grade IV. Another classical division classifies gliomas into primary and secondary glioblastomas (11, 12). Glioblastoma multiforme has a high rate of malignancy, infiltration and necrosis. Currently, Glioblastoma multiforme (GBM) treatment involves surgical excision of tumor followed by chemotherapy and radiation for a period of one year. However, this treatment has drastic side effects and poor survival out-come (13). Mutations in gliomas have been involved in regulation of variety of cellular processes such as cell growth, development, differentiation, migration, invasion and angiogenesis. Master regulators of these vital cellular processes such as the Notch, EFGR, TP53, PTEN and PDFGR are deregulated in gliomas, consequently, giving a way to malignancy (14). Evidence of the fact that tumor glial cells hijack Notch signaling components came from siRNA-based experimentations. Deprivation of Notch1 in glioma cell lines with the aid of siRNA resulted in decreased cell growth and increased apoptosis (15). Inhibition of Notch signaling also resulted in increased astrocytes number through up-regulation of glial fibrillary acidic protein (GFAP) and decrease in the endo-mesenchymal transition. A decreased expression of vimentin in glioma cell lines was observed (16). From these findings, it is clear that maintenance of a stream of un-differentiated glial cells is necessary for the tumor progression and Notch1 acts as an oncogene in gliomas. Xenograft studies have further confirmed the oncogenic properties of Notch1 gene. SiRNA-mediated inhibition of Notch1 and ligand Dll1 resulted in early death of mice, while knockdown of Jagged1 had no effect on overall survival and proliferation (17). Notch1 and its paralog Notch2 had opposite effect on growth and development of subcutaneously engrafted U251 and A172 glioma cell lines. Knockdown of Notch1 or overexpression of Notch 2 had a

slight different effect on pattern of growth of glial cells *in vitro* (18). Tenascin C (TNC) has been reported to enhance cell migration under the influence of the Notch gene in gliomas. Notch-induced transcription factor RBPj-kappa binds to TNC, which results in proliferation and migration of GBM cells (19). This transformation also raises the number of GBM based astrocytes and induces overall decreased survival rates of patients (19). All these findings point towards the use of miRNAs as therapeutic as well as a diagnostic tool for Gliomas.

The Notch signaling is triggered by ligand-receptor interaction resulting in deregulation of extra-cellular portion of the notch receptor and detachment of intracellular portion of the notch receptor by protolytic cleavage of the receptor. This protolytic cleavage is not triggered in the absence of ligands. The activity of the notch receptor at intracellular domain is monitored by (histone deacetylase) HDAC and a set of co-repressors such as NCoR and SMRT (20). Altogether, these co-repressors prevent gene activation by notch receptors. The notch receptor‘s intracellular domain (NICD) moves inside the nucleus and binds to CSL transcription factor (CBF1 in humans, suppressor of Hairless in Drosophila, LAG in the *C.elegans*) through its RAM 23 domain. In the absence of Notch activity, CSL act as co-repressor of target genes but the activity of Notch transforms it from co-repressor to co-activator. The histone acetylase transferase (HAT) and p300 are active enzymes that mediate chromatin relaxation and recruit RNA polymerase II enzyme which, in turn, promotes active transcription of target genes (21). Hairy enhancer of split and basis helix-loop-helix are the two transcription regulators of notch targeted genes. Studies have confirmed that expression of HES and bHLH are the two mediators of neuronal stem cells stemness and *de-novo* regeneration (22).

Several molecules and mechanisms have been intricated to regulate Notch signaling at post-transcriptional and transcriptional levels. For instance, Hes gene family of transcription factors can curb gene expression in neuronal cells

at transcription level by maintaining asymmetric cell division using a feedback loop system. Other mechanisms such as glycosylation, proteolysis, endocytosis and degradation are also employed to modulate the Notch signaling at post-transcription level.

Proneural genes such as Ascl1 and Neurog2 have been reported to drive neurogenesis via activation of the bHLH transcription factors (23). Furthermore, these genes can also modulate the process of neurogenesis and differentiation by triggering expression of notch ligands such as Delta like 1 (Dll1). However, some studies have matched involvement of proneural genes with synthesis of neuron differentiating genes such as NeuroD. Lateral Notch signaling can be regulated by transcriptional feedback loop (24). The NICD promotes expression of Hes1 and Hes5 transcription factors that in turn leads to repression of proneuronal genes such as Ascl1 and Neurog2 (25). This inhibits the process of neuronal differentiation and lateral notch signaling. Despite the involvement of NICD-RBPj-Hes complex in repression of differentiation, this promoter complex is indispensable for early differentiation of embryonic neuronal stem cells (26). Hes-mediated oscillations creates both forward and backward loops that curtails active differentiation and synthesis of the Dll1 and Ngn2 mRNA expression (27). Using time lapse-imaging analysis, Imayoshi *et al.* determined that HES gene and proneural genes oscillations are in an inverse relation with Dll1 and Ngn2 oscillation expression. Altogether, these proneural genes tend to promote asymmetric cell division in neuronal cells that help in production as well reservation of neuronal stem cells through lateral Notch signaling (28)(29).

Interplay of Notch with other Pathways for neurogenesis in Gliomas

Notch signaling alone cannot trigger the process of neurogenesis. Therefore certain crosstalk with other cellular pathways is required for maintaining neuronal cells' growth and differentiation.

Bone morphogenetic protein (BMP) pathway curtails differentiation of glial cells and inhibits neuron growth during early phase of neurogenesis. BMP

interacts with cell surface receptor that in turn leads to activation of SMAD via phosphorylation (30). BMP signaling renders a specific latency to neuronal stem cells and similar to Notch signaling is an obligatory maintainer of neuronal stem cells (31)(32) . Both signaling pathways share a common target: transcription factor Hes and inhibitor of DNA binding (ID) genes (32). The common target gene might pose certain idleness due to the competition between HES5 and HES3, which has been documented by Hatakeyma *et al.* with his experiment on neurogenesis. Loss of RBPj mutations has revealed that both BMP as well as notch have certain discrepancy with respect to neuronal differentiation. BMP promotes abnormal cell differentiation while notch was found to be more specific (28).

Wnt signaling has been explored for its role in the neuronal differentiation and programming (33). Glycogen synthetase kinase 3 (GSK3) is the chief element responsible for ubiquitination of beta catenin with the help of axin and factors required for phosphorylation (34). However, in the absence of GSK3, Wnt is activated by its interaction with frizzeled receptor in the presence of Lrp6. This activity prevents phosphorylation of beta-catenin and aids in translocation of unphosphorylated beta-catenin to the cytoplasm, where it transcribes the targeted genes (34). Wnt and Notch signaling interplay with each other is in agonistic manner - one's activation can promote the activation of similar transcription factors. Wnt directly promotes the activation of neuronal genes such as Ngn1 and neuroD. These two genes are the mediators of differentiation while notch targeted Hes5 competes with these two to block the differentiation of neuronal cells (35). Other signaling pathways such as Hippo signaling and FOXO also interact with the Notch signaling at various levels of transcription and promote neuronal crest growth and differentiation (36). However, further efforts are still required for understanding the involvement of these pathways in neuronal stem cells upkeep and differentiate.

Notch signaling and microRNAs in Gliomas

MicroRNAs aid in differential growth as well as stemness of neuronal cells by collaborating with the Notch signaling. MiRNAs maintain a regular balance between self-renewal and differentiation of neuronal cells and several miRNAs such as miR-9 modulates the expression of neuron differentiation through the Notch-axis. MiR-9 targets Hes1 and modulates its expression via a negative feedback loop system (37). MiR-9 and its sister stand miR-9* have been reported to regulate whole class of notch family receptors in a negative feed-back loop system and modulate neuronal development (37). Other miRNAs such as miR-124 along with miR-9 have been associated with the expression of notch ligands. MiR-124 interacts with Jagged 2 to control the maintenance of neuronal stem cells(38). On the similar ground, miR- let-7 interacts at the transcription site and targets Hes5 and promotes glial cell differentiation (39). MiR-34 is another microRNA that has been intricated to regulate balance between neuronal cell differentiation and self-renewal via acting on Numbl (40). A little is known about the effect of long non-coding RNAs on self-renewal of neuronal cells. However, a recent study by Rani *et al.* have shown the involvement of lncRNAs in maintaining neuronal self-renewal in different animal models (41). Glioblastoma is the most prevalent type of tumor in adults. Glioma-initiating cells (GICs) are the main culprit behind triggering glioblastoma. Glioma initiating cells have characteristics of stem cells and have been closely linked to aggravate tumor progression. However, the role of GICs in tumor progression in glioblastoma, containing GICs and non-GICs, is still debatable. Wang *et al.* demonstrated the involvement of miR-33a in maintaining GICs' growth and development. Microarray based analysis confirmed that miR-33a directly targets two genes phosphodiesterase 8A (PDE8A) and UV radiation resistance-associated gene (UVRAG) in humans and xenografted cell lines of GICs. Both these genes modulated the expression of Notch signaling along with cAMP/PKA pathway. Expression analysis revealed that elevated level of miR-

33a promotes cell growth in glioblastoma having GICs via activation of Notch and PKA pathway in xenografted cell lines. Targeting miR-33a could be a suitable treatment for glioblastoma (42). miR-34c-3p and miR-34c-5p have been evidenced to hinder cellular growth and proliferation of glial cells in Glioblastoma. qPCR analysis has documented that miR-34c-3p and miR-34c-5p directly target Notch signaling-associated genes, resulting in decreased cell proliferation, increased apoptosis and hindered metastasis in glial cell lines U251 and U87, respectively. Overall pointing towards the importance of these molecules as the tool for eradication of gliomas (43).

MiR-18a* has been involved in clonal proliferation and tumor heterogeneity *in vivo*. In human glial cells, miR-18a* overexpression caused by ERK activation inhibits Delta like ligand 3 overexpression, consequently triggering Notch 1 activation. In a feedback loop system, Notch1 simulates the activation of ERK. This positive feed-back loop is modulated by miR-18a*. Activation of miR-18a* promotes expression of SHH-GLI-NANOG network, which in turn maintains a steady progeny of glioma initiating cells and enables their self-renewal. This finding indicated the crucial role of miR-18a* in the sustained growth and reproducibility of the GICs (44)(45).

Presence of low level persistent expression of Notch gene is indispensable for the prolonged cellular growth of glioblastoma cells. Notch signaling and its down-stream signaling molecules have been well documented for their involvement in long term survival of tumorous glial cells. Huber *et al*. using microarray analysis established a link between miR-21 over expression and glioblastoma cell aggressiveness. Their findings revealed that Notch/Deltex pathway, modulated by the miR-21, was inevitable for the glioma cells invasiveness and growth. DTX1 activated by the Notch canonical signaling triggers RTK/PI3K/PKB and the MAPK/ERK mitotic pathways, which in turn promote expression of anti-apoptotic proteins such as the Mcl-1. Another

finding revealed that miR-21 overexpression directly elevated the expression of ERK, thus promoted cellular growth and stamness of glioblastoma (46).

MiR-107 has been involved in induction of apoptosis and growth arrest in glioma cell lines such as U251 and A172. Chen *et al,*. using lentiviral system approach and GFP assay showed that miR-107, under the influence of P53, down regulated the expression of Notch-2 and CDK6 that resulted in suppressed tumor cell growth in glioblastoma. This collectively indicated the anti-proliferative activity of miR-107 in brain tumors (47).

Notch signaling is necessary for angiogenesis, fate determination and survival of the cancer. Cross talk between microRNAs and Notch delineates the very framework of cancer stemness. By using bioinformatics and biological approaches, Chen *et al.,* found miR-524-5p directly modulates the expression of two downstream targets of Notch Pathway: Jagged1 and Hes1. Knockdown of either Jagged1 or Hes1 was inversely related to miR-524-5p expression. This finding suggested that miR-524-5p was a sole modulator of Notch signaling, which acted by negatively regulating Jagged1 and Hes1expression. This modulation resulted in increased apoptosis and decreased cell growth in glial cells (48).

Hes1 is reported to be a target of miR-199-5p in medulloblastoma. Down regulation of miR-199-5p is a hallmark in majority of medulloblastoma. This down regulation is mediated by epigenetic methylation changes in the promoter region of miR-199-5p. Hes1 has been reported to alter the binding site of miR-199-5p promoter region, resulting in advance metastasis and prolonged cell growth. Reverse-phase protein assay in various cell lines of medulloblastoma has revealed the interplay between miR-199-5p and Akt signaling pathway that in turn negatively regulates expression of apoptotic machinery of the cell (49). Furthermore, in xenograft models it has been established that miR-34a has the ability to induct its influence on a number of signaling cascades that harbour key process of cell division and migration of stem cells (50).

Involvement of microRNAs and notch was further evidenced by Kefas *et al.* Their finding brought to lime light the crucial relationship between miR-326 and notch 1 in gliomas. Notch1 was modulating the expression of miR-326 in a negative feedback loop system to hinder the efficacy of its targeted genes. Thus, proving the fact that miR-326 could be used as a potential therapeutic target for gliomas (51).

In murine model of glioma and human glioma cells, microRNA expression is pivotal for the growth and development of cancer stem cells. However, it has come to light less lately that miR-145 down regulation increases the expression of BNIP3. BNIP3 under the influence of Notch signaling promotes growth and development of glioma cells in both murine and human glioma tissues. Inhibition of BNIP3 by forced over expression of miR-145 results in elevated apoptosis, which suggests that miR-145 down regulation by BNIP3/Notch is the main reason for survival and recurrence of gliomas. These findings focus on development of miR-145 as a novel approach for the eradication of gliomas (52).

Role of miR-34a as mediator of trans-differentiation has recently been reported. MiR-34a acts as an inducer by targeting notch ligand Dll1 that in turn promotes cellular differentiation in to vascular endothelial cells. Notch 1 activity is greatly reduced in glioma cell lines by induction of miR-34a mimics. miR-34a transfection to the U251 cell lines results in decrease tube formation of glioma stem cells by hindering the expression levels of Notch1 and Dll1 (53).

Epidermal growth factor like receptor (EGFR) signaling is the key cascade that is perturbed in gliomas. Recent study has shed light on the involvement of miR-524-3p and miR-524-5p in suppression of this pathway in gliomas. Mutations in the EGFR pathway, especially in EGFRvIII, result in abrogated cell growth in gliomas. However, miR-524-3p and miR-524-5p over expression greatly attenuate glioma differentiation but overexpression of Notch/TGF beta/Hippo pathways result in tumor stemness. EGFRvIII mutation suppresses the

expression of miR-524-3p and miR-524-5p at histone level causing overexpression of EFGR. Overexpression of Notch/TGF beta/Hippo pathways aids in the activation of c-Myc that binds to promoter region of EGFRvIII, promoting cellular growth. All this suggested the importance of miR-524-3p and miR-524-5p diagnostic marker for glioma development (54).

MicroRNAs can trigger as well inhibit the growth of glial cells. A recent report has shed light on the bifacet role of microRNAs in gliomas. MiR-92a-3p up regulation is directly linked to the growth and development of gliomas. However, its activity is different under the influence of different signaling cascades. MiR-92a-3p promotes cancer stemness via modulation of Notch signaling cascade. Contrary to this, miR-92a-3p mediated activation of beta catenin/Wnt pathway results in increased apoptosis in glioma stem cell in vitro (55).

Natural Compounds as Therapeutic Option for Gliomas

Treatment of GBM has been a hall mark and therapies related to treat this anomaly currently includes gamma secretase inhibitors, blocking antibodies and decoys. Although these traditional approaches are quite promising and are under clinical trials, still very little is known of the natural compounds and their use in eradication of glioblastomas. Here we summarized few of the current natural compounds and their efficacy in treating brain tumors. Biological activity of *Angelica sinensis* has been well documented in Chinese medicines. It is a natural compound that has the ability to induct apoptosis. n-Butylidenephthalide (BP) an extract of *Angelica sinensis* has been documented to initiate apoptosis in GBM. BP induces mitochondrial based apoptosis through upregulation of Nur77. This activation results in acute apoptosis and increases growth arrest in brain tumors (56) . Isolates of *Cranberry presscake*, known as Flavonoid-rich fraction (Fr6) and pronathocyanidins (PAC), have been indicated to trigger apoptosis in colorectal cancer cell lines (57). Thymoquinone (TQ), a product of *Nigella sativa* seed oil, has been reported to induce autophagy in glial cells

independent of caspase involvement (58). The activity of many cellular cascades such as the NOTCH, m-TOR and Nuclear Kappa B has been inhibited by the activity of niclosamide in pre-glioblastoma cell lines. Niclosamide are the synthetic toxic compound that actively hinder membrane permeability in glial cells (59). *Caesalpinia sappan* derived compound brazilin has the ability to trigger apoptosis via downregulation of caspase-3 and caspase-7 in glial cell lines. Dose dependent increase of Brazilin levels resulted in overexpression of PARP that led to growth arrest and apoptosis in glial cells (60). Resveratrol has been implicated to play decisive role in the inhibition of tumor growth and apoptosis. Two isoforms of resveratrol namely hopeaphenol and r2-viniferin were observed to induce apoptosis and growth arrest via activation of molecular caspases (61). *Scutellaria baicalensis* based compound namely Wogonin has been investigated for its anti-proliferative capabilities. Wogonin has been reported to elicit influence via activation of ROS pathway in glial cells. ROS activation results in suppressed growth and low cell viability in glial cell lines and human gliomas. This finding suggested the involvement of Wogonin in modulating key process of DNA damage and protein synthesis (62). Curcumin has also been reported to influence Notch signaling via inhibition of proliferation in tumorous cells. Antitumor B (Zheng Sheng Ping) a compound product of Chinese herbal medicine has been reported to show massive apoptotic properties by inhibiting the expression of Notch receptor in gliomas (63).

Conclusion

In this review, we discussed the Notch signaling pathway in relation to miRNAs and gliomas. Furthermore, we shed light on the possible natural compounds that can be extensively useful for the treatment of gliomas. Concisely, the Notch signaling network is regulated by miRNAs at various levels. Targeting these components could be an effective way to eliminate the disease. Notch signaling

is crucial for development and differentiation of neuronal cells. Aberrations in the downstream component of Notch signaling pathway, ultimately, promote irregular cell growth and increase tumor susceptibility. MiRNAs in many cases have been established to promote oncogenic properties and this could possibly a decisive approach to culminate gliomas. However, several studies have also demonstrated miRNAs as an inhibitor of tumor growth. This bifacet role of miRNAs in glioblastoma is really confusing. Due to this stumbling block, the outlook for new therapeutic strategies is currently bleak. The components of Notch signaling cascade assume a significant role not just in the control of neuronal differentiation but also in metastasis of gliomas. Yet, the accurate molecular mechanisms even in this settled capacity are not clear and need advanced examination. In this review, we showed an outline of how Notch signaling pathway has been connected to neurogenesis and diseases in the brain, concisely depicting Notch signaling network and its direction at various levels. The role of Notch and components of the pathway is less clear and often the data and interpretations are contradictory and confusing. One reason for the discrepancies between findings may be because Notch signaling is central to many processes and interacts with different signaling pathways. In this review, we also demonstrated an outline of how Notch signaling pathway can be targeted with natural compounds. Natural compounds possess unique properties that enable them to be highly target specific with almost little or no cytotoxicity. Exploring natural compound to combat tumor is an eye-catching idea that will significantly enhance our understanding of disease prevention. In addition to this, natural compounds pose low cytotoxicity and limited side effects, which make them excellent tool for therapeutic use. Furthermore, Notch regulation of epigenetic mechanisms and feedback of epigenetics and miRNAs onto the Notch pathway make the effects of signaling cell-type- and context-dependent. Much more work is needed in order to maximize our understanding and to take

advantage of the potential of novel Notch signaling pathway related diagnostic and therapeutic approaches.

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Table 1: List of Tumor promoting miRNAs that enhance Notch mediated Proliferation

Tumor Promoting miRNA	Target	Reference
miR-524-3p and miR-524-5p	EFGRvIII/c-MYC	(54)
miR-34a	Dll 1	(53)

miR-18a*	ERK	(45)
miR-21	Mcl-1	(64)
miR-33a	PDE8A/UVRAG	(42)
miR-92a-3p	Notch Domain	(55)
Let-7	Hes5	(39)

Table 2: List of Tumor Suppressor miRNAs that enhance Notch mediated Apoptosis

Tumor Suppressor miRNA	Target	Reference
miR-107	CDK6/Notch 2	(65)
miR-524-5p	Jagged 1/Hes1	(48)
miR-199-5p	Hes1	(66)
miR-326	Notch 1	(51)
miR-92a-3p	B-Catenin	(55)

Cyclin Dependent Kinase 6, Hairy Enhancer of Split 1

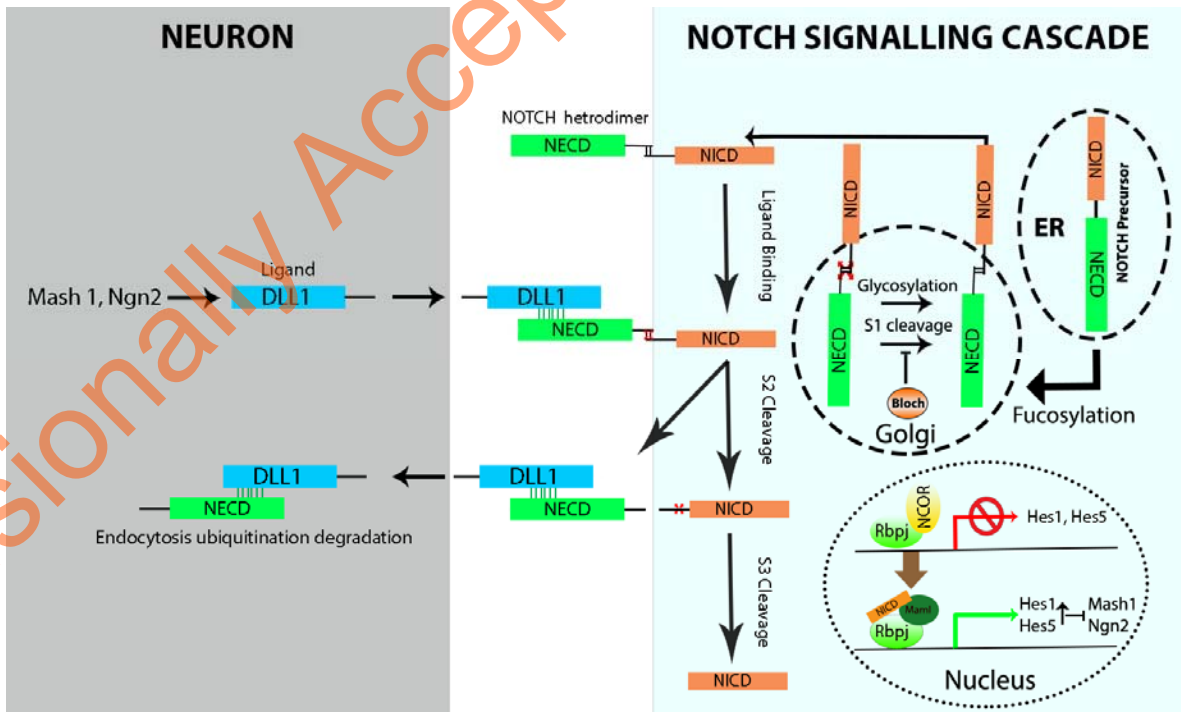

Figure 1: Regulation of Notch signaling is a strict mechanism controlled at several levels. Notch signaling is initiated by the Ascl1 and Ngn2 two transcriptional activators of bHLH family. Expression of Ascl1 and Ngn2 in turn activates expression of Dll1 ligand. Dll1 ligand activation triggers Notch

activation in adjacent cells. Notch receptor undergoes three stages of cleavages which is modulated and monitored by glycosylation in Golgi complex and endoplasmic reticulum. S1 cleavage is done by furin like proteases enzymes that are negatively regulated by Botch results in the production of two domains Notch Extracellular membrane (NECD) and Notch intracellular domain (NICD). Binding of NECD to Dll1 results in the activation of downstream signaling which includes cleavage of NICD by S2. S2 cleavage is mediated by ADAM metalloproteinases. A third cleavage is mediated by gamma secretase that transfer NICD to the nucleus. NICD binds with CSL complex Rbpj in mice that in turn assemble an activator complex containing Maml which promotes the expression of target genes Hes1 and Hes5. In neuronal stem cells NICD is being recycled to prevent fate determination.

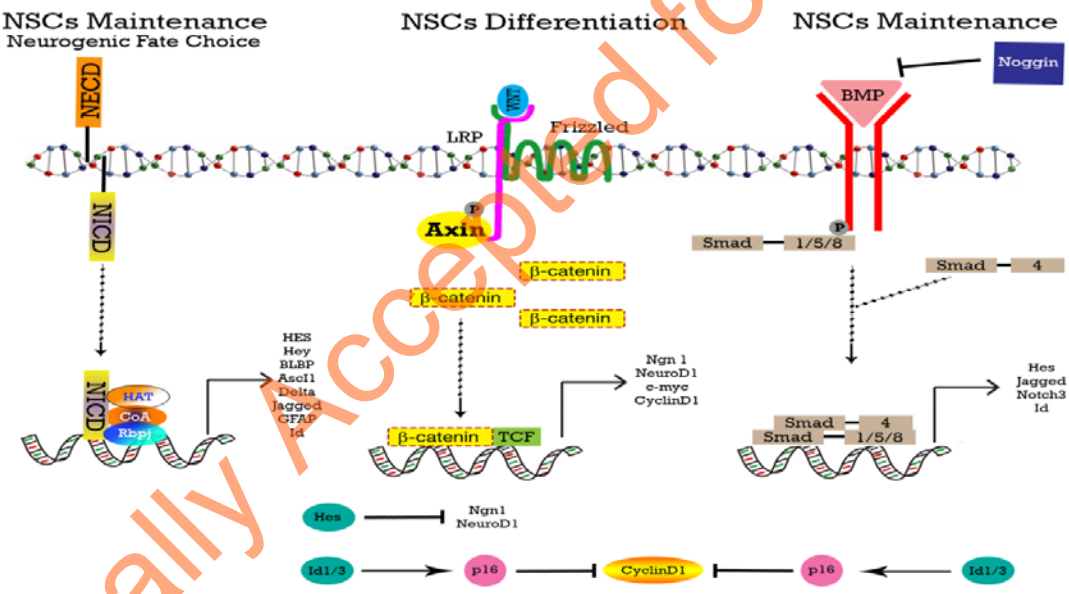

Figure 2: Notch interplays with BMP and other signaling pathways such as Wnt signaling. Notch activation results in the activation of the HES family. This signaling enables differentiation as well as maintenance of self-renewal to the NSCs. Wnt signaling aids in the proliferation and differentiation of neuronal genes. Hes inhibits the expression of the proneural genes. Notch crosstalk with BMP and Wnt signaling. Notch signals to the nucleus and induces transcription of its target genes, including those encoding Hes and Hey family members. Notch signaling is essential in NSC maintenance. Wnt signaling in neurogenesis promotes NSC differentiation by inducing transcription of proneural and neural differentiation genes. Proneural gene expression is inhibited by the Hes. BMP works in concordance with the Notch signaling altogether Notch and BMP activates CyclinD1 and P16. While Wnt work opposite to the BMP signaling.