

EGFL7

A new player in homeostasis of the nervous system

Frank Bicker and Mirko H.H. Schmidt*

Molecular Signal Transduction; Institute of Neurology (Edinger Institute); Johann Wolfgang Goethe University School of Medicine; Frankfurt am Main, Germany

EGFL7 drives the formation of neurons from neural stem cells. In the embryonic and adult brain this process is essential for neurogenesis and homeostasis of the nervous system. The function of adult neurogenesis is not fully understood but maybe it supports life-long learning and brain repair after injuries such as stroke. The transition of neural stem cells into mature neurons is tightly regulated. One of the essential signaling pathways governing this process is the Notch pathway, which controls metazoan development. In a recent publication, we identified a novel non-canonical Notch ligand, EGFL7, and described its impact on neural stem cells.¹ We explored the molecular mechanisms, which this molecule affects to regulate the self-renewal capacity of neural stem cells and to promote their differentiation into neurons. In this review, we discuss the implications of our findings for adult neurogenesis and illustrate the potential of EGFL7 to serve as an agent to increase neurogenesis and the self-renewal potential of the brain.

Neurogenesis

New neurons are generated throughout life from a population of dividing cells known as neural stem or progenitor cells (NSCs or NPCs) (Fig. 1).² Initially, NSCs leave the quiescent state in order to proliferate.³ The resulting daughter cells both exit or both reenter the cell cycle (symmetric cell division). Alternatively, one cell exits and the other re-enters the cell cycle (asymmetric cell division),⁴ which yields a copy of the original mother cell as well as a precursor

cell that possesses reduced stem cell-like characteristics.⁵ In dependence on the particular neural lineage these precursors are committed to, they are termed glial or neuronal precursors⁶ or alternatively, glioblasts and neuroblasts.⁷ Glioblasts form astrocytes or oligodendrocytes⁸ but neuroblasts become neurons.⁹ Subsequent to an initial phase of rapid proliferation, NPCs enter a migratory state and eventually differentiate¹⁰ but only a small proportion matures and extends axons and dendrites to establish afferent or efferent connections and synapses.¹¹

The Role of Notch Signaling in Developmental Neurogenesis

During embryonic development, the mammalian cortex is built in a layered pattern that emerges from the ventricular system. NPCs migrate out of the germinal zone along glial processes towards the surface of the brain to the nascent cortical plate, where they become neurons and integrate into the developing neocortical circuitry.¹¹ This process is governed by signaling modules such as Wnt, Hedgehog, TGF β and Notch. Especially, Notch signaling is one of the main regulators of NSCs in the developing brain.¹²

Canonical Notch signaling in mammals is based on the activation of one of the four Notch receptor isoforms (Notch1-4) by Notch ligands of the Delta (Dll1 and Dll4) or Jagged type (Jagged1 and Jagged2).¹³ These ligands initiate the proteolytic cleavage of Notch receptors (Fig. 2) by the metalloproteases ADAM10 or ADAM17.¹⁴ The resulting

Key words: EGFL7, notch, neurogenesis, neural stem cells, subventricular zone, neurospheres, self-renewal, differentiation, neurodegenerative disease

Abbreviations: Dll, delta-like; EGFL7, epidermal growth factor-like domain 7; GFAP, glial fibrillary acidic protein; Hes, hairy and enhancer of split; NeuN, neuronal nuclear antigen; NICD, notch intracellular domain; NPC, neuronal progenitor cell; NSC, neural stem cell; SGZ, subgranular zone; SVZ, subventricular zone

Submitted: 10/22/09

Revised: 12/29/09

Accepted: 12/30/09

Previously published online:

www.landesbioscience.com/journals/cc/article/11091

*Correspondence to: Mirko H.H. Schmidt;
Email: Mirko.Schmidt@kgu.de

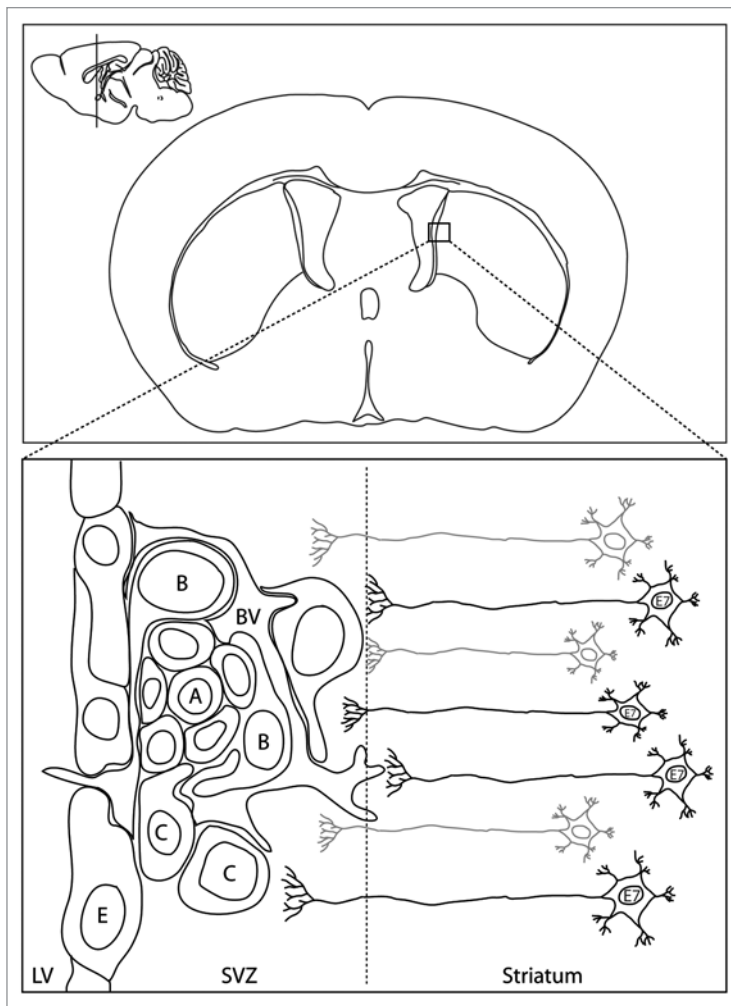


Figure 1. Localization of the SVZ in the adult murine brain. The upper picture displays a coronal section through the adult murine brain, where the longitudinal position is indicated by a vertical line through the sagittal section displayed in the upper left corner. The lower picture shows an enlargement of the SVZ and a schematic representation of the lateral wall of the ventricle. The region left to the dotted line illustrates the anatomy and cellular composition of the neurogenic niche. The lateral ventricle (LV) is lined by ependyma cells (E). Astrocytic cells (B) within the SVZ give rise to rapidly dividing transit amplifying cells (C), which themselves differentiate into the committed neuroblasts (A). Black neurons within the striatum represent EGFL7-positive cells (E7); those in light grey are negative for EGFL7. BV, blood vessel.

membrane-tethered receptor remnant is further processed by a γ -secretase-presenilin complex, which releases the cytosolic portion of Notch receptors.¹⁵ This intracellular domain of Notch (NICD) translocates into the cell nucleus, binds to the DNA-associated molecule RBP-J κ ¹⁶ and induces the replacement of transcriptional co-repressors such as Hairless or Groucho¹⁷ by co-activators such as Mastermind or Mastermind-like molecules.¹⁸ The transcriptional active ternary protein complex induces the expression of target genes of the hairy and enhancer of split (Hes) family¹⁹ which repress proneural genes.²⁰

Notch signaling inhibits neuronal differentiation in the developing central nervous system and suppresses the maturation of oligodendrocyte precursors during gliogenesis.²¹ Previous work implicated Notch signaling in the maintenance of NSCs, because stem cells isolated from the brain of Notch1 knock-out mice formed less neurospheres in vitro.²² Conversely, constitutive activation of Notch signaling by retroviral infection of NICD rescued the self-renewal capacity of NSCs in vivo and was accompanied by diminished neuronal differentiation of NSCs in vitro.²³ Recent findings suggest Notch-dependent maintenance

of NSCs is reciprocally regulated by the dynamic and oscillating expression of Hes1, which mediates the temporal oscillation of the Notch ligand Dll1 and the proneural gene *neurogenin2*.²⁴ Mimicking Notch activation through the expression of Hes1 in the developing forebrain of mouse embryos inhibited neuronal differentiation.²⁵ In addition, multipotent neural progenitor cells with stem cell potential were identified based on the determination of Notch activity as measured by the quantification of the expression levels of Hes5. This transcription factor is rather specific for NSCs and is downregulated in immature neurons or glia in vitro and in vivo.²⁶ Last, it has been described that Dll4 and Jagged1 induce non-canonical Notch signaling in fetal NSCs as measured by the elevated expression of Hes3 and Sonic hedgehog. This pathway led to an increased survival of NSCs but did not affect differentiation in vitro.²⁷

In sum, data summarized above illustrates the central position of Notch signaling in the development of the brain but raises the question on the cellular pathways that are altered to affect self-renewal and differentiation of NSCs. Maybe the cell cycle itself is altered and switched from the division mode to a differentiation program. The involvement of Notch in this context has been demonstrated by tamoxifen-induced overexpression of NICD in glial fibrillary acidic protein positive (GFAP⁺) cells in vivo, which induced the proliferation of postnatal glial precursor cells within the hippocampus.²⁸ In contrast, inhibition or genetic ablation of Notch—by tamoxifen-induced conditional knock-out in GFAP⁺ cells—promoted the exit of glial precursor cells from the cell cycle. This caused the transition of NSCs and NPCs into transit-amplifying neurogenic precursors and neurons.²⁸

Alternatively, Notch may regulate NSCs by defining a microenvironment in stem cell niches that favors self-renewal over differentiation. In fact, it has been reported that the impact of Notch signaling on gliogenesis,²⁹ proliferation^{30,31} and self-renewal³² differs in dependence of the microenvironment. The secreted protein Reelin, which regulates neuronal migration, may participate in this type of regulation. The loss of Notch signaling

resulted in a severe radial migration defect that caused the laminar displacement of neurons in so-called Reeler mice, which are deficient for Reelin.³³ Overexpression of NICD rescued the phenotype, which indicates that Reelin-induced neuronal migration in the developing cerebral cortex depended on Notch.

Last, the maturation of neurons may depend on Notch, since differentiating neurons have been described to express various Notch ligands.³⁴⁻³⁶ Transfection of primary neurons with NICD inhibited neurite outgrowth in vitro.³⁷ Vice versa, neurons targeted for conditional knockout of Notch1 in vivo exhibited shorter and irregular processes as well as protrusions that directly originated from the cell soma.²⁸ Together, this suggests that high levels of Notch signaling may keep NSCs in an undifferentiated state and once reduced, allows for neuronal differentiation.

Implications of Notch in Adult Neurogenesis

In the mature mammalian brain two regions have been described to harbor adult NSCs, namely the subventricular zones (SVZ),² which line the lateral walls of the lateral ventricles (Fig. 1), and the SGZ of the dentate gyrus in the hippocampus.^{38,39} Different types of progenitor cells have been identified within the SVZ and the SGZ, such as the quiescent B cells or the homologue Type-1 cells in the SGZ. These cells are similar to radial glial cells in embryonic development and are reminiscent of mature astrocytes in morphology and physiology. In contrast, there are the small, round and rapidly proliferating C cells (Type-2 cells in the SGZ). C cells give rise to A cells (Type-3 cells in the SGZ), which are the neuroblasts committed to a neuronal cell fate. Neuroblasts originating from the SVZ migrate along the rostral migratory stream into the olfactory bulb, where they become interneurons of the granule layer and the periglomerular region.⁴⁰⁻⁴³ In the hippocampus, SGZ-derived neural precursors become granule cells and form new axons—so-called mossy fibers—to connect to neurons in the hilus and to CA3 pyramidal cells.⁴⁴

Equally, Notch signaling is significant for adult and embryonic neurogenesis.⁴⁵

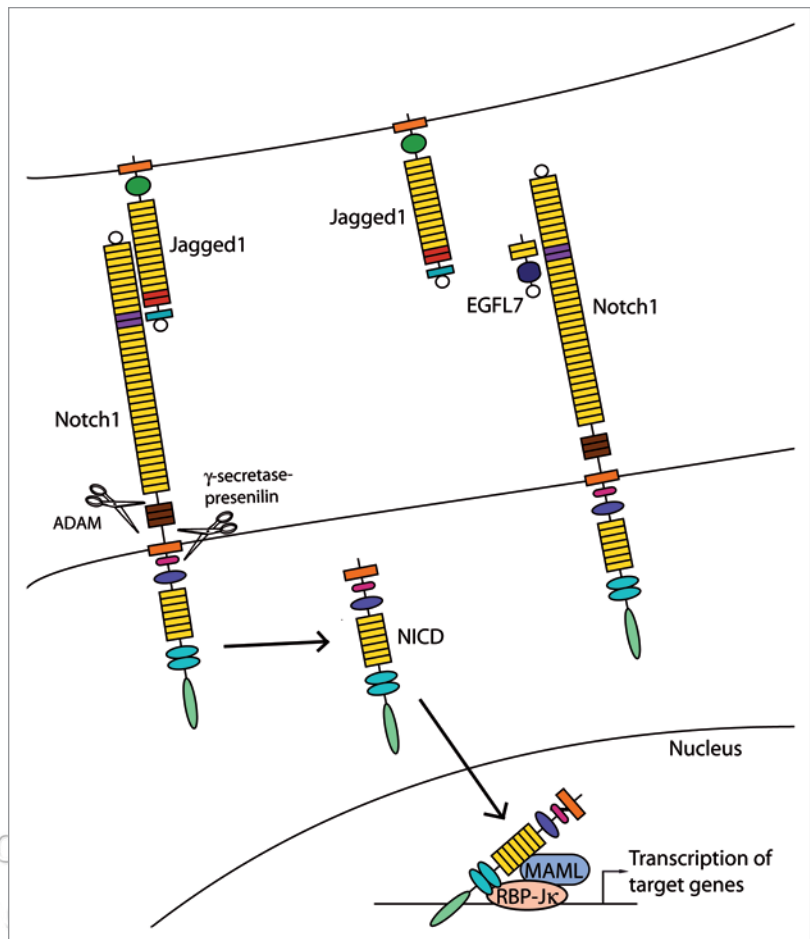


Figure 2. Activation of the Notch pathway by Jagged1 and the role of EGFL7. The Notch ligand Jagged1 activates its receptor Notch1 (left hand site) on a neighboring cell by induction of a series of proteolytic cleavage events that are initiated by metalloproteases of the ADAM type and completed by the γ -secretase-presenilin complex. This cascade releases NICD, which translocates into the cell nucleus and acts as an activator of transcription by forming a transcriptional active ternary protein complex with RBP-Jk and Mastermind-like molecules (MAML). EGFL7 blocks binding of Jagged1 to Notch1 (right hand site) and thereby abrogates the pathway.

Various components of Notch signaling are expressed in the SVZ and SGZ, e.g., the Notch1 receptor or its ligands Jagged1 and Delta1.⁴⁶ The ablation of Jagged1 or Notch1 in adult NSCs led to a decrease in the self-renewal potential of NSCs accompanied by a reduced expression of Hes1 and Hes5.⁴⁷ The mechanisms of Notch-mediated regulation of NSCs in vivo are subject of ongoing research. State of the art is that under physiological conditions, Notch signaling keeps ependymal cells, which line the lateral ventricles, quiescent.⁴⁶ Upon middle cerebral artery occlusion these cells give rise to neuroblasts and astrocytes and this way, may contribute to repair processes of damaged brain areas upon injury. Inhibition of Notch signaling

caused the mobilization of the cells of the ependyma in untreated animals, while the activation of Notch signaling by NICD blocked their mobilization in response to stroke.⁴⁶ Data indicate that Notch signals responsible for the activation of cells within the neurogenic niche in response to brain damage. A deeper understanding of these processes may offer opportunities for future regenerative therapies.

Epidermal Growth Factor-Like Domain 7 (EGFL7) Affects NSCs via the Notch Pathway¹

Most recently, we studied the novel secreted molecule EGFL7 in the context of NSC regulation. Previously, EGFL7—also

known as VE-statin, Zneu1 or Notch4-like protein—was described as an endothelial cell-derived factor that inhibited the platelet-derived growth factor-induced migration of human aortic smooth muscle cells.⁴⁸ Later, a gene trap vector screen identified EGFL7 as an essential factor of vessel development.⁴⁹ The *egfl7* gene covers about 11.6 kb of the mouse genome where it is located on chromosome 2.⁴⁸ Furthermore, the gene encodes for two biologically active microRNAs in intron 7, miR-126 and miR-126*, which promote angiogenesis via the downregulation of inhibitors of proliferation like Spred1 in endothelial cells.⁵⁰ The EGFL7 protein is 278 amino acids long and has a molecular mass of 30 kD. EGFL7 carries an amino-terminal secretion signal that targets the molecule to the extracellular space by trafficking through the endoplasmic reticulum and the Golgi apparatus. Additionally, mature EGFL7 contains an N-terminal EMI domain that is succeeded by two EGF-like domains and a coiled-coil region.

EGFL7 is conserved among species and has been implicated in the formation of the vascular system. High levels of EGFL7 were detected in endothelial progenitors and in endothelial cells of developing vessels in human, mouse and zebrafish embryos.⁴⁹ Consequently, an essential role for EGFL7 in endothelial lineage determination and vascular development has been suggested. In contrast, expression of EGFL7 in adult mice was observed in a small subset of vessels in highly vascularized organs such as the lung, heart, uterus, ovary and kidney, which may reflected the diminished amount of vascular remodeling in adult organisms.⁴⁹ In general, EGFL7 is silenced in quiescent vessels⁴⁹ but it is upregulated in the proliferating, migrating and remodeling endothelium during physiological angiogenesis⁵¹ or by pathological stimuli such as arterial injury⁵² or hypoxia.⁵³ EGFL7 loss-of-function studies in zebrafish⁵¹ and mice⁵⁴ revealed severe vascular defects, as EGFL7 knock-down fish displayed disorganized lumens or no lumen at all. In mice, the knock-out of EGFL7 led to 50% lethality in utero, which was due to severe systemic edema. Furthermore, the surviving pups displayed a delayed postnatal

vascularization of the retina. Nevertheless, these phenotypes were recently attributed to the loss of the *egfl7* encoded proangiogenic miRNAs.^{50,55,56}

In our recent manuscript¹ we suggest a role of EGFL7 beyond the vascular system. Expression studies indicated the presence of EGFL7 in non-endothelial tissues such as the murine brain or mouse neuroblastoma cells.⁴⁹ This suggested EGFL7 could have a function within the central nervous system, which prompted us to analyze the expression pattern of EGFL7 in the adult murine brain. Quantitative RT-PCR from various brain regions displayed remarkable amounts of *egfl7* in the adult brain. Especially, high levels of EGFL7 were detected in brain areas with large amounts of neurons such as the cortex. In order to determine the cellular source of EGFL7, we performed immunohistochemistry of adult murine brains. Cells that were positive for EGFL7 in the cortex were also positive for the neuronal marker NeuN but were negative for the oligodendrocyte marker O4, the astrocyte marker GFAP or for the endothelial cell marker platelet/endothelial cell adhesion molecule 1 (PECAM-1). Thus, we were able to identify mature neurons as a source of brain-derived EGFL7.

Interestingly, regions like the SVZ with a lack of neurons contained low levels of EGFL7 as compared to the surrounding striatum. Consistently, NSCs isolated from the SVZ and cultured in form of neurospheres expressed only very low levels of EGFL7. Furthermore, increased activation of Notch signaling by overexpression of NICD in NSCs decreased the endogenous EGFL7 level, while the inhibition of Notch by DAPT had the opposite effect. Last, EGFL7 expression and high levels of Notch signaling were mutually exclusive during the differentiation of NSCs in vitro.

In order to elucidate the function of EGFL7 in NSC, we performed biochemical analyses and identified EGFL7 as a novel non-canonical ligand of Notch receptors. As described above, Notch governs metazoan development and the maintenance of NSC, which prompted us to investigate, if EGFL7 directly affects these biological parameters. In general, the self-renewal potential of NSCs depends on the

interaction of the Notch1 receptor with its ligand Jagged1.⁴⁷ Interestingly, we found that EGFL7 competed with Jagged1 for Notch1 binding and thereby antagonized Jagged1-induced Notch signaling. In order to analyze the effect of EGFL7 on adult NSC,⁵ we created an adenoviral infection system to express EGFL7 in neurospheres. This system offered the advantage of low cytotoxicity and we observed that the virus majorly infected the rapidly proliferating NPCs but not the stem cells. Nevertheless, EGFL7 secreted by NPCs was still able to affect NSCs. We found that the self-renewal potential of NSCs was strongly decreased upon infection of neurospheres with adenovirus encoding for EGFL7. The reduced self-renewal potential was paralleled by a decreased proliferation rate and a reduced level of Notch signaling as measured by quantitative RT-PCR of the Notch target gene *hes5*. The overall rate of apoptosis on the other hand remained unaltered.

The effect of EGFL7 on Notch in NSCs raised the question, if EGFL7 impaired the ability of NSCs to differentiate into the three neural cell lineages, namely neurons, astrocytes and oligodendrocytes. We found that adult NSCs plated on coverslips that had been coated with EGFL7 differentiated into all three cell types. Clearly, EGFL7 favored the formation of neurons as compared to control. Furthermore, additional oligodendrocytes were formed. These cells displayed a mature morphology with distinct sprouts and branches in contrast to the small and round oligodendrocytes that formed on control coverslips, which resembled us of precursor cells. The additional neurons and oligodendrocytes were formed at the expense of the number of astrocytes. Indeed, the effect of EGFL7 on the differentiation pattern of NSCs was expected, because a reduction of Notch signaling during the differentiation of NSCs had been reported to be paralleled by an increased formation of neurons and enhanced maturation of oligodendrocyte precursors.²¹ Likely, the alterations we observed reflected EGFL7's impact on Notch signaling in NSCs. In conclusion, EGFL7 altered the self-renewal and multipotency as well as the differentiation potential of NSCs by its impact on Notch-mediated signal transduction (Fig. 3).

Perspectives

Adult neurogenesis is induced by various kinds of brain injury.² In a mouse model of Parkinson's disease, a decreased survival of newborn neurons was observed in the SVZ and the SGZ, whereas cell proliferation was not affected.⁵⁷ In another study, the experimental depletion of dopamine in rodents decreased NPC proliferation.⁵⁸ Consistently, the numbers of proliferating cells in the subependymal zone, the subgranular zone and the olfactory bulb were reduced in postmortem brains of individuals with Parkinson's disease.⁵⁸ In case of Alzheimer's disease, controversial observations on the level of adult neurogenesis have been reported.⁵⁹ The over-expression of a mutated form of APP led to a decreased rate of neurogenesis in the murine SVZ and the SGZ.⁶⁰ However, an increased rate of neuronal differentiation was measured within the hippocampus of human patients.⁶¹ In models of focal and global ischemia in rodents, neurogenesis was enhanced in both neurogenic regions.^{2,62} However, about 80% of the neurons that formed as a consequence of the ischemic shock died after four weeks,⁶² despite the total number of proliferating cells was strongly increased upon ischemia.⁶³ Currently, it is still controversially discussed if adult neurogenesis effectively contributes to repair processes after brain injury.

Conversely, physiological neurogenesis in the adult brain is slightly more effective as about half of the newborn neurons survive for longer than a month.⁶⁴ Despite an excess of neural progenitors are formed, only a minor part of these cells mature into functional neurons.⁶⁵ This suggests that maturation and integration of neurons into a functional neuronal network are critical steps in adult neurogenesis. The chronic deterioration of patients' health in neurodegenerative diseases implies that the brain is not capable of compensating for the loss of neurons by endogenous regenerative mechanisms.⁶⁶ Although the mammalian brain seems capable to repair itself the capacity of adult neurogenesis is not comprehensive enough to be considered restorative. In order to promote functional recovery after brain injury, transplantation and manipulation strategies may be

complemented with agents that promote the recruitment and navigation of mobilized NPCs to their target regions. Most importantly, such an agent should support the survival and maturation into neurons in order to create a functional neuronal network within the damaged brain region. Apparently, the ultimate production of effectively integrated functional neurons fails despite adult neurogenesis is stimulated by most kinds of brain injury as described above. Under pathological conditions, more NPCs but less terminally differentiated neurons are detected.⁶² Putatively, the brain attempts to repair its damage but an elusive mechanism prevents this by limiting the amount of functional neurons that integrate into the neuronal network. If endogenous factors limit the amount of tissue repair, these factors may be modulated to increase the regenerative potential of the brain. Indeed, to increase the endogenous capacity of the brain to self-repair is one of the most exciting concepts of restorative therapies

and offers therapeutic approaches for the treatment of neurodegenerative disorders such as stroke, Parkinson's, Huntington's or Alzheimer's disease.

In our opinion, EGFL7 is a promising agent in this context. As an inhibitor of Jagged-induced Notch signaling and a factor secreted by mature neurons it could act as a local environmental modulator within the brain. As a component of the extracellular matrix, it may create a micromilieu around neurons that triggers bypassing NPCs to differentiate. Simultaneously, EGFL7 acts proneurogenic and promotes the formation of neurons from NSCs. EGFL7 could be exploited as a therapeutic target to stimulate NSCs and NPCs to differentiate into mature neurons in vivo. This way, EGFL7 may contribute to neuronal regeneration and could have a beneficial effect on functional brain recovery. Furthermore, EGFL7 is a bona fide secreted modulator of Notch signaling, which is of advantage if compared to other Notch ligands that are usually transmembrane

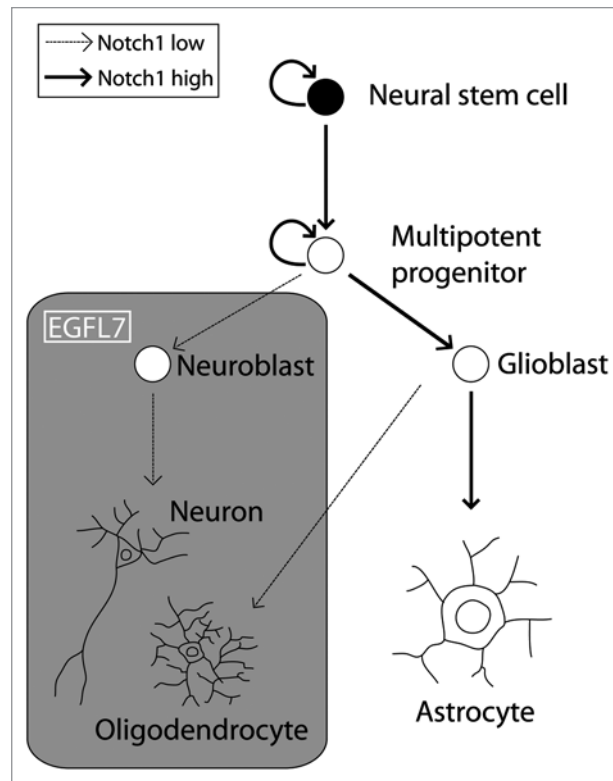


Figure 3. Role of Notch signaling in cell fate decisions of NSCs. Notch affects the self-renewal potential and the differentiation pattern of NSCs. High levels of Notch (solid lines) promote self-renewal and trigger glial differentiation of NSCs. In contrast, low levels of Notch (dotted lines) favor neuronal differentiation and the maturation of oligodendrocyte precursors. EGFL7 supports an environment with low levels of active Notch (grey box).

molecules and cannot be simply administered in their recombinant forms. Decoys of Notch receptors and ligands exist but these compounds have the disadvantage to act unpredictably as Notch agonists and antagonist, which depends on their physiological state, i.e., if they are fully soluble or associated with the extracellular matrix and the signaling cell surface.⁶⁷ EGFL7 decreases Notch in NSCs in both states, which makes this protein especially interesting for clinical applications. Further, it will be interesting to elucidate if EGFL7 acts solely on NSCs and NPCs or if the EGFL7-Notch1 axis plays a role in neuronal communication as well. Most likely, this is the case as EGFL7 appears to be constitutively expressed at significant levels in some types of neurons. However, it needs to be elucidated if neuronal EGFL7 mediates intercellular communication or if it acts on EGFL7-positive neurons in an autocrine fashion. Maybe it has a role in the transmission of electric signals between neurons or it keeps neurons in an immobile state but this is speculative. Too little is known about the function of EGFL7 in vivo and future work using transgenic and knock-out animals needs to address, in which way EGFL7 affects neural stem cells as well as neurons. We will unravel if EGFL7 supports the integration of newly formed neurons in the existing neuronal network and how it affects homeostasis of the nervous system.

Acknowledgements

This work was financially supported by the German Research Foundation DFG within the framework of Transregional Collaborative Research Centre 23 (sub-project A4) and the Excellence Cluster 147 "Cardio-Pulmonary Systems".

References

1. Schmidt MHH, Bicker F, Nikolic I, Meister J, Babuke T, Picuric S, et al. Epidermal growth factor-like domain 7 (EGFL7) modulates Notch signalling and affects neural stem cell renewal. *Nat Cell Biol* 2009; 11:873-80.
2. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008; 132:645-60.
3. Nowakowski RS, Caviness VS Jr, Takahashi T, Hayes NL. Population dynamics during cell proliferation and neurogenesis in the developing murine neocortex. *Results Probl Cell Differ* 2002; 39:1-25.
4. Huttner WB, Kosodo Y. Symmetric versus asymmetric cell division during neurogenesis in the developing vertebrate central nervous system. *Curr Opin Cell Biol* 2005; 17:648-57.

5. Gotz M, Huttner WB. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol* 2005; 6:777-88.
6. Goldman SA. Adult neurogenesis: from canaries to the clinic. *J Neurobiol* 1998; 36:267-86.
7. Gage FH, Ray J, Fisher LJ. Isolation, characterization and use of stem cells from the CNS. *Annu Rev Neurosci* 1995; 18:159-92.
8. Lee JC, Mayer-Proschel M, Rao MS. Gliogenesis in the central nervous system. *Glia* 2000; 30:105-21.
9. Lenington JB, Yang Z, Conover JC. Neural stem cells and the regulation of adult neurogenesis. *Reprod Biol Endocrinol* 2003; 1:99.
10. Ghashghaei HT, Lai C, Anton ES. Neuronal migration in the adult brain: are we there yet? *Nat Rev Neurosci* 2007; 8:141-51.
11. Olson EC, Kim S, Walsh CA. Impaired neuronal positioning and dendritogenesis in the neocortex after cell-autonomous Dab1 suppression. *J Neurosci* 2006; 26:1767-75.
12. Yoon K, Gaiano N. Notch signaling in the mammalian central nervous system: insights from mouse mutants. *Nat Neurosci* 2005; 8:709-15.
13. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* 2009; 137:216-33.
14. Ladi E, Nichols JT, Ge W, Miyamoto A, Yao C, Yang LT, et al. The divergent DSL ligand Dll3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *J Cell Biol* 2005; 170:983-92.
15. De Strooper B, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, et al. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 1999; 398:518-22.
16. Honjo T. The shortest path from the surface to the nucleus: RBP-Jkappa/Su(H) transcription factor. *Genes Cells* 1996; 1:1-9.
17. Ehebauer M, Hayward P, Martinez-Arias A. Notch signaling pathway. *Sci STKE* 2006; 2006:7.
18. Wu L, Aster JC, Blacklow SC, Lake R, Artavanis-Tsakonas S, Griffin JD. MAMLI, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat Genet* 2000; 26:484-9.
19. Kageyama R, Nakanishi S. Helix-loop-helix factors in growth and differentiation of the vertebrate nervous system. *Curr Opin Genet Dev* 1997; 7:659-65.
20. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol* 2003; 194:237-55.
21. Louvi A, Artavanis-Tsakonas S. Notch signalling in vertebrate neural development. *Nat Rev Neurosci* 2006; 7:93-102.
22. Alexson TO, Hitoshi S, Coles BL, Bernstein A, van der Kooy D. Notch signaling is required to maintain all neural stem cell populations—irrespective of spatial or temporal niche. *Dev Neurosci* 2006; 28:34-48.
23. Hitoshi S, Alexson T, Tropepe V, Donoviel D, Elia AJ, Nye JS, et al. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev* 2002; 16:846-58.
24. Shimojo H, Ohtsuka T, Kageyama R. Oscillations in notch signaling regulate maintenance of neural progenitors. *Neuron* 2008; 58:52-64.
25. Ishibashi M, Moriyoshi K, Sasai Y, Shiota K, Nakanishi S, Kageyama R. Persistent expression of helix-loop-helix factor HES-1 prevents mammalian neural differentiation in the central nervous system. *EMBO J* 1994; 13:1799-805.
26. Basak O, Taylor V. Identification of self-replicating multipotent progenitors in the embryonic nervous system by high Notch activity and Hes5 expression. *Eur J Neurosci* 2007; 25:1006-22.
27. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoepfner DJ, Ravin R, Poser SW, et al. Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature* 2006; 442:823-6.
28. 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.
29. Ge W, Martinowich K, Wu X, He F, Miyamoto A, Fan G, et al. Notch signaling promotes astroglialogenesis via direct CSL-mediated glial gene activation. *J Neurosci Res* 2002; 69:848-60.
30. Furukawa T, Mukherjee S, Bao ZZ, Morrow EM, Cepko CL. *rax*, *Hes1* and *notch1* promote the formation of Muller glia by postnatal retinal progenitor cells. *Neuron* 2000; 26:383-94.
31. Scheer N, Groth A, Hans S, Campos-Ortega JA. An instructive function for Notch in promoting gliogenesis in the zebrafish retina. *Development* 2001; 128:1099-107.
32. Morrison SJ, Perez SE, Qiao Z, Verdi JM, Hicks C, Weinmaster G, et al. Transient Notch activation initiates an irreversible switch from neurogenesis to gliogenesis by neural crest stem cells. *Cell* 2000; 101:499-510.
33. Hashimoto-Torii K, Torii M, Sarkisian MR, Bartley CM, Shen J, Radtke F, et al. Interaction between Reelin and Notch signaling regulates neuronal migration in the cerebral cortex. *Neuron* 2008; 60:273-84.
34. Henrique D, Adam J, Myat A, Chitnis A, Lewis J, Ish-Horowicz D. Expression of a Delta homologue in prospective neurons in the chick. *Nature* 1995; 375:787-90.
35. Myat A, Henrique D, Ish-Horowicz D, Lewis J. A chick homologue of Serrate and its relationship with Notch and Delta homologues during central neurogenesis. *Dev Biol* 1996; 174:233-47.
36. Dunwoodie SL, Henrique D, Harrison SM, Beddington RS. Mouse Dll3: a novel divergent Delta gene which may complement the function of other Delta homologues during early pattern formation in the mouse embryo. *Development* 1997; 124:3065-76.
37. Sestan N, Artavanis-Tsakonas S, Rakic P. Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. *Science* 1999; 286:741-6.
38. Lledo PM, Alonso M, Grubb MS. Adult neurogenesis and functional plasticity in neuronal circuits. *Nat Rev Neurosci* 2006; 7:179-93.
39. Ninkovic J, Gotz M. Signaling in adult neurogenesis: from stem cell niche to neuronal networks. *Curr Opin Neurobiol* 2007; 17:338-44.
40. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999; 97:703-16.
41. Gage FH. Mammalian neural stem cells. *Science* 2000; 287:1433-8.
42. Carlen M, Cassidy RM, Brismar H, Smith GA, Enquist LW, Frisen J. Functional integration of adult-born neurons. *Curr Biol* 2002; 12:606-8.
43. Carleton A, Petreanu LT, Lansford R, Alvarez-Buylla A, Lledo PM. Becoming a new neuron in the adult olfactory bulb. *Nat Neurosci* 2003; 6:507-18.
44. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci* 2004; 27:447-52.
45. 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.
46. Carlen M, Meletis K, Goritz C, Darsalia V, Evergren E, Tanigaki K, et al. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. *Nat Neurosci* 2009; 12:259-67.

47. Nyfeler Y, Kirch RD, Mantei N, Leone DP, Radtke F, Suter U, et al. Jagged1 signals in the postnatal subventricular zone are required for neural stem cell self-renewal. *EMBO J* 2005; 24:3504-15.
48. Soncin F, Mattot V, Lionneton F, Spruyt N, Lepretre F, Begue A, et al. VE-statin, an endothelial repressor of smooth muscle cell migration. *EMBO J* 2003; 22:5700-11.
49. Fitch MJ, Campagnolo L, Kuhnert F, Stuhlmann H. Egfl7, a novel epidermal growth factor-domain gene expressed in endothelial cells. *Dev Dyn* 2004; 230:316-24.
50. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Dev Cell* 2008; 15:261-71.
51. Parker LH, Schmidt M, Jin SW, Gray AM, Beis D, Pham T, et al. The endothelial-cell-derived secreted factor Egfl7 regulates vascular tube formation. *Nature* 2004; 428:754-8.
52. Campagnolo L, Leahy A, Chitnis S, Koschnick S, Fitch MJ, Fallon JT, et al. EGFL7 is a chemoattractant for endothelial cells and is upregulated in angiogenesis and arterial injury. *Am J Pathol* 2005; 167:275-84.
53. Gustavsson M, Mallard C, Vannucci SJ, Wilson MA, Johnston MV, Hagberg H. Vascular response to hypoxic preconditioning in the immature brain. *J Cereb Blood Flow Metab* 2007; 27:928-38.
54. Schmidt M, Paes K, De Maziere A, Smyczek T, Yang S, Gray A, et al. EGFL7 regulates the collective migration of endothelial cells by restricting their spatial distribution. *Development* 2007; 134:2913-23.
55. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. *Dev Cell* 2008; 15:272-84.
56. Kuhnert F, Mancuso MR, Hampton J, Stankunas K, Asano T, Chen CZ, et al. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. *Development* 2008; 135:3989-93.
57. Winner B, Rockenstein E, Lie DC, Aigner R, Mante M, Bogdahn U, et al. Mutant alpha-synuclein exacerbates age-related decrease of neurogenesis. *Neurobiol Aging* 2008; 29:913-25.
58. Hoglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, et al. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat Neurosci* 2004; 7:726-35.
59. Verret L, Jankowsky JL, Xu GM, Borchelt DR, Rampon C. Alzheimer's-type amyloidosis in transgenic mice impairs survival of newborn neurons derived from adult hippocampal neurogenesis. *J Neurosci* 2007; 27:6771-80.
60. Haughey NJ, Nath A, Chan SL, Borchard AC, Rao MS, Mattson MP. Disruption of neurogenesis by amyloid beta-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. *J Neurochem* 2002; 83:1509-24.
61. Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, et al. Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci USA* 2004; 101:343-7.
62. Lindvall O, Kokaia Z. Stem cells for the treatment of neurological disorders. *Nature* 2006; 441:1094-6.
63. Zhang RL, Zhang ZG, Zhang L, Chopp M. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience* 2001; 105:33-41.
64. Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG. Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur J Neurosci* 2002; 16:1681-9.
65. Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA. Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol* 2003; 460:563-72.
66. Steiner B, Wolf S, Kempermann G. Adult neurogenesis and neurodegenerative disease. *Regen Med* 2006; 1:15-28.
67. Hicks C, Ladi E, Lindsell C, Hsieh JJ, Hayward SD, Collazo A, et al. A secreted Delta1-Fc fusion protein functions both as an activator and inhibitor of Notch1 signaling. *J Neurosci Res* 2002; 68:655-67.

©2010 Landes Bioscience.
Do not distribute.