Mol Neurobiol (2012) 46:555–562
DOI 10.1007/s12035-012-8303-8

The Role of eNSCs in Neurodegenerative Disease

Raja Kittappa · Stefan R. Bornstein · Andreas Androutsellis-Theotokis

Received: 6 July 2012 / Accepted: 9 July 2012 / Published online: 21 July 2012

Abstract Recent progress in biology has shown that many if not all adult tissues contain a population of stem cells. It is believed that these cells are involved in the regeneration of the tissue or organ in which they reside as a response to the natural turnover of differentiated cells or to injury. In the adult mammalian brain, stem cells in the subventricular zone and the dentate gyrus may also play a role in the replacement of neurons. A positive beneficial response to injury does not necessarily require cell replacement. New findings suggest that some populations of endogenous neural stem cells in the central nervous system may have adopted a function different from cell replacement and are involved in the protection of neurons in diverse paradigms of disease and injury. In this article, we will focus on the immature cell populations of the central nervous system and the signal transduction pathways that regulate them which suggest new possibilities for their manipulation in injury and disease.

Keywords eNSCs · Neurodegenerative disease · Stem cell biology

R. Kittappa
Wellcome Trust Centre for Stem Cell Research,
University of Cambridge,
Cambridge, UK

S. R. Bornstein · A. Androutsellis-Theotokis (✉)
Department of Medicine, University of Dresden,
Dresden, Germany
e-mail: Andreas.Theotokis@uniklinikum-dresden.de

S. R. Bornstein · A. Androutsellis-Theotokis
Center for Regenerative Therapies Dresden,
Dresden, Germany

A. Androutsellis-Theotokis
European Brain Research Institute,
Rome, Italy

Introduction

There is great excitement surrounding stem cell biology and potential cell therapies to treat complex, degenerative diseases. Numerous degenerative diseases (including Alzheimer's disease, Parkinson's disease, Huntington's disease, motor neuron diseases, etc.) affect the aging nervous system and come at a tremendous personal cost to individuals and their families and a significant financial burden to the public. The most obvious question with regard to cell therapies is the source for cell replacement. Should the focus be on embryonic, fetal, or adult stem cells? Which source can best avoid the problems of immuno-compatibility, inefficient cell expansion in culture, induction of cancer, ethical issues, etc.? Recent breakthroughs have tried to tackle some problems through new means of deriving cloned human embryonic stem cell lines and the reprogramming of adult skin fibroblasts into an embryonic stem cell-like state.

A more fundamental impediment for cell therapy in neurodegenerative disease is the complex cytoarchitecture of the central nervous system (CNS). In Parkinson's disease (PD), for example, dopamine neurons that extend from the substantia nigra to the striatum, several centimeters away, die over long periods of time. Cell transplantation (generated by any method) results in their placement in one area (usually the striatum) where they act as dopamine pumps and do not recapitulate the functional nigrostriatal circuitry [1]. This problem may not be as serious in other tissues or organs with a much simpler organization where cell connectivity is less complex (e.g., liver and pancreas) or where stem cells can easily access their niche (e.g., bone marrow).

Another consideration of cell therapy comes from recent evidence from PD and amyotrophic lateral sclerosis showing that neurodegeneration is not necessarily cell-autonomous; furthermore, in several cases, grafted cells in PD patients acquire the disease (assessed by the presence of "Lewy bodies") [1–4]. While the replacement of compromised cells can alleviate disease symptoms, it does not necessarily halt disease

progression. Importantly, in a recent clinical case, grafted stem cells in the CNS of a patient did not differentiate but, instead, developed into benign tumors, highlighting possible dangers of transplanting immature cells [5].

Here, we focus on the potential for endogenous neural stem cells (eNSCs) to overcome neurodegenerative processes. Central to this review are two seemingly contradictory observations: (1) The adult mammalian brain maintains a population of eNSCs, and these may be much more plentiful and widespread than once thought. (2) Adult neurogenesis is a rare event even under injury conditions and confined to specific areas of the brain (the subventricular zone and the subgranular zone of the hippocampus) [6–18].

The apparent contradiction between abundant endogenous stem cell presence and scarce neuronal replacement raises the question: what is the function of endogenous neural stem cells, if any, outside of the hippocampus and olfactory bulb? Recent evidence points to a role for eNSCs in the protection of neurons [19, 20]. NSCs in vitro and in vivo produce neuroprotective factors including glial-derived neurotrophic factor (GDNF) [19] and sonic hedgehog [20]. Novel markers of endogenous neural stem cells show the close physical association between eNSCs and neurons, placing these immature cells in an ideal situation to provide trophic support to normal and injured neurons. We will review a work aimed at elucidating the presence and activation of eNSCs and their potential role in neuroprotection, and we will discuss a signaling pathway that is of particular relevance to the regulation of self-renewal and expansion.

Presence of Stem Cells in the Adult Central Nervous System

Determining the presence of eNSCs depends principally on two experimental approaches. The first is the identification of biomarkers for the direct detection of eNSCs. A limitation of this approach is the incomplete specificity of biomarkers for a single cell type. Even combinations of markers often are not able to define any single cell type, including the eNSC. The earliest realization of the existence of cells that can generate neurons in the adult brain came from experiments performed in the 1960s by Joseph Altman and coworkers and later by Michael Kaplan [6, 8]. These groups used radioactive thymidine to label all dividing cells in the living brain and subsequent histological examination to identify neurons that were derived from labeled cells. These early experiments showed that neurons can be generated in the adult CNS from cells that are still able to divide, pointing the way towards establishing the presence of immature cells in the adult CNS.

Years later, biomarkers including the intermediate filament, nestin (for neuroepithelial stem cell intermediate filament), were established to identify NSCs in the developing mammalian brain as well as in culture [21–24] and, subsequently, in the postnatal and adult brain [13, 14, 25–28]. Nestin allowed the direct visualization of precursor cells and NSCs without the need to rely on labeling of dividing cells and provided additional specificity. More markers were eventually identified, including Sox2, sonic hedgehog pathway components, platelet-derived growth factor (PDGF), EGFR, GFAP, Hes3, Hes5, Musashi, and CD133 [20, 28–38]. Despite the development of these useful markers, none of these markers may be individually sufficient to exclusively identify eNSCs.

The second approach to identify a stem cell population is to extract these cells from primary tissue and culture them under conditions that support survival and self-renewal. Clonal analysis of eNSCs definitively demonstrates stem cell multipotency in culture—can a colony derived from a single cell generate the three main cell types of the nervous system (neurons, astrocytes, and oligodendrocytes), for which adequate markers exist? The difficulty with this approach is that it is limited by current cell culture technology. Still, the inability to maintain a stem cell in culture does not disprove the existence of this cell in the tissue from which the culture was established. In contrast, the development of clonal growth assays for stem cells is a powerful proof of stem cell multipotency and self-renewal.

Currently, the presence of stem cells in the adult mammalian CNS is believed to be restricted to a few small regions including the subventricular zone (SVZ), the area around the lateral ventricles of the brain. In addition, a stem cell/neuronal precursor population of immature cells is also observed in the dentate gyrus of the hippocampus, but also in other areas of the postnatal CNS [6, 12, 13, 15, 32, 39–42].

The traditional focus on the stem cell field on these two neurogenic areas is largely due to the assumption, following the work of Altman, that stem cells are only spatially associated with neurogenesis, a process involving the proliferation of immature cells and their subsequent differentiation into neurons. As a consequence, perhaps, several of the biomarkers for eNSC also characterize other CNS cell types in their proliferative state [30, 43, 44]. However, it can be argued that although a stem cell may have the potential to divide and generate neurons, it still may not do either of these. Additional roles may have been adopted by eNSCs other than neuronal replacement such as modulation of neuronal function and neuroprotection. Experimental approaches that are biased towards neurogenesis may not be able to detect additional eNSC populations which are quiescent and/or located in microenvironments that do not promote neurogenesis. Evidence from several research groups suggests that widespread eNSCs or more committed progenitor cells exist throughout the adult brain and spinal cord, and treatments that increase their numbers in the living tissue also confer neuroprotection and rescue

neurons from death in models of degenerative disease and acute insults [35, 40, 45–50]. This eNSC-mediated neuroprotection occurs in the notable absence of neuronal replacement. Several efforts are under way to determine the location and numbers of these cells and to determine if targeted manipulations of these cells could represent novel therapeutic strategies.

Activating eNSCs

Several insults, including injury, ischemia, and models of degenerative disease, are capable of activating the eNSC population in the adult brain. Activation leads to an increase in the numbers of eNSCs and in some cases, such as in ischemic stroke models, to migration towards the infarct site [51–56]. In addition to the insults, various pharmacological manipulations are also able to induce the expansion of the eNSC population including basic fibroblast growth factor (bFGF), epidermal growth factor, PDGF, insulin, Wnt and Notch signaling components, and perturbation of ephrin signaling [32, 46, 57–64].

Limited neurogenesis is observed in many of these paradigms [53, 54, 65–73], and the vast majority of newly generated migrating neuroblasts in ischemic stroke models die by the time they have reached the peri-infarct area [52]; in spite of these observations, certain models that aim to enhance the natural neuronal replacement in the adult hippocampus following ischemic brain injury have generated promise in ameliorating symptoms [61]. The discrepancy between strong eNSC activation and very limited neurogenesis can be explained in a number of ways. One possible explanation is that eNSC activation is a general response to injury and does not serve a specific purpose. This seems unlikely given that adult neurogenesis seems to be of functional importance in, at least, the olfactory bulb and hippocampus. A second possibility is that eNSC activation is

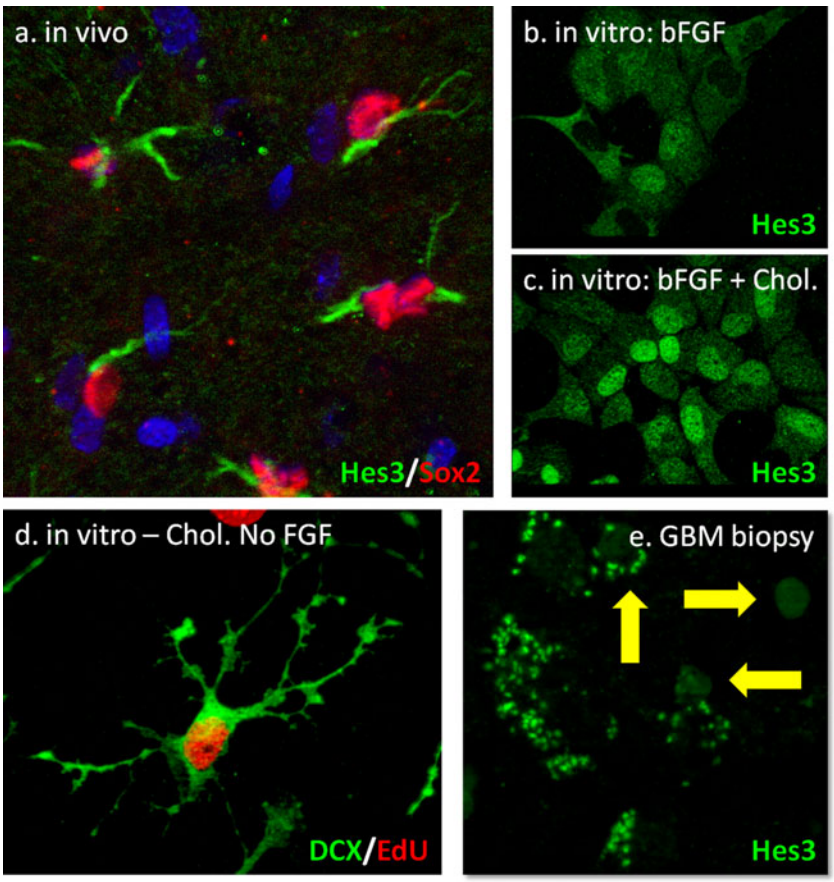
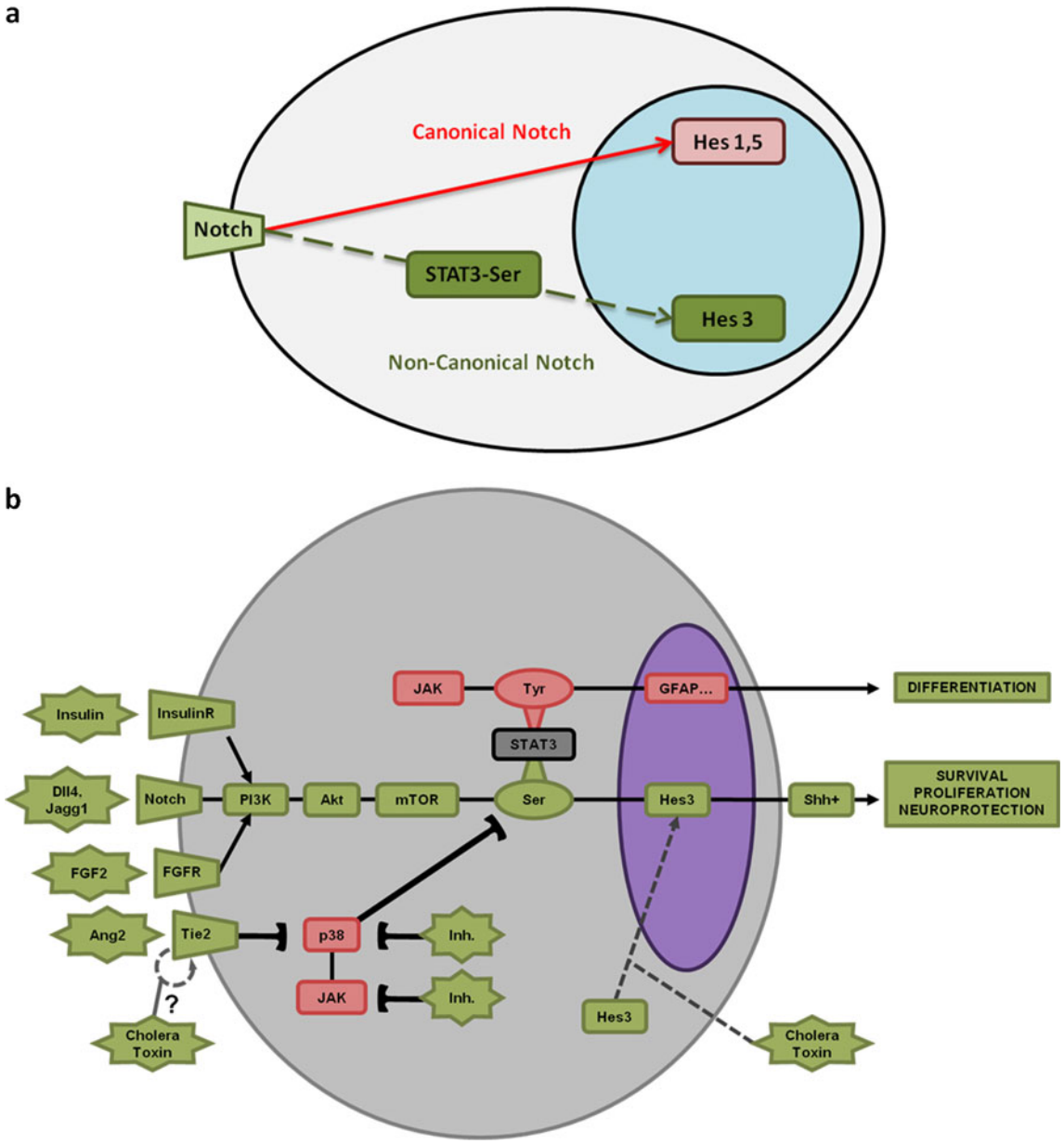


Fig. 1 Dynamic regulation of Hes3 in different cell states. **a** In the adult rat striatum, Hes3 is expressed in a subset (approx. 10 %) of GFAP+/Sox2+ cells and is excluded from the nucleus of most cells. **b** When placed in culture, in conditions that support their self-renewal and proliferation by including bFGF, mouse fetal NSCs express Hes3 which, in most cells, is localized both in the nucleus and cytoplasm. The chosen image shows cells that also exclude Hes3 from the nucleus with a morphology suggesting that they are spontaneously differentiating towards the glial lineage. **c** When cells from **b** are treated with cholera toxin (*Chol.*), nuclear Hes3 localization greatly increases. **d** As a consequence of cholera toxin treatment, cells (including 15 % of the cells that express the early neuronal marker doublecortin, *DCX*) maintain the proliferative state even 1 week after bFGF withdrawal. **e** In human glioblastoma biopsies, Hes3 immunoreactivity is observed in intracellular particles (which also co-express prominin) and in the nucleus (*arrows*). Images are reproduced from a previously published work [35, 81, 111]



merely an evolutionary remnant from more primitive vertebrates (fish and tailed amphibians) that are able to induce massive and consequential neurogenesis. Zebrafish seems to produce new neurons throughout their lifetimes throughout the brain and can replace neurons destroyed by a physical lesion [74, 75]. Urodele amphibians are able to functionally regenerate a severed spinal cord or the entire complement of midbrain dopamine neurons after a 6-OHDA lesion [76]. It is notable that cell replacement occurs in these instances despite the complicated cytoarchitecture of the adult brain, suggesting that under the appropriate circumstances, these cell types could be replaced and reform functional circuits in the adult mammalian brain. Neither of these scenarios excludes the possibility that eNSCs could be manipulated, genetically or otherwise, to regenerate lost neurons. Finally, perhaps, eNSCs serve a purpose other than neuronal replacement—neuroprotection, as discussed above, by secreted factors produced by the activated eNSCs, suppression of inflammation in the damaged tissue [77–79], or clearance of cellular and extracellular debris to facilitate tissue remodeling.

The best experimental evidence for a non-neurogenic role for eNSCs supports a neuroprotective effect. Transplanted NSCs have been used to assess the effects of increasing the numbers of NSCs in the adult brain. Grafted NSCs express GDNF [19], a neuroprotective cytokine, raising the possibility of a neuroprotective role for grafted and, possibly, eNSCs.

Initiatives to probe the potential neuroprotective roles of eNSCs have been hindered by the established belief that eNSCs are few and very limited in their localization (discussed previously). The establishment of new biomarkers

◀ **Fig. 2** Canonical and non-canonical signaling pathways regulate neural stem cell numbers in vitro and in vivo. **a** Simplified diagram showing canonical and non-canonical pathways that regulate NSCs. In the canonical Notch pathway, ligand–receptor binding leads to the proteolytic cleavage of the intracellular domain of the Notch receptor and release of the intracellular domain (ICD) into the cytoplasm [87]. Notch ICD interacts with other proteins and the resulting complex regulates gene transcription of a number of target genes, including Hes1 and Hes5. In the non-canonical Notch signaling branch, a series of second messengers are activated with fast kinetics (minutes); hallmarks of the pathway are the phosphorylation of STAT3 on the serine residue, in the absence of any detectable phosphorylation on the tyrosine residue, and a subsequent increase in the transcription of the Hes/Hey gene family member Hes3. In the original experiments that elucidated this non-canonical Notch signaling pathway, blockade of the proteolytic release of the Notch ICD by use of a gamma secretase inhibitor also blocked the induction of STAT3-serine phosphorylation; however, this result does not prove that the non-canonical branch is dependent on the release of Notch ICD. It is possible that conformational changes on the Notch receptor other than ICD release may be responsible for the initiation of the non-canonical Notch pathway. We also note that of all the members of the Hes/Hey gene family, Hes3 was the only one whose mRNA levels changed detectably upon stimulation of cultured fetal rodent NSCs with soluble versions of the Notch ligands Delta4 and Jagged1 [20]. **b** More detailed diagram describing a non-canonical Notch signaling pathway operating in NSCs. [We note in advance that *arrows* do not represent direct protein–protein interactions]. Notch receptor activation leads to the phosphorylation of Akt and PI3 kinase within 5 min. These events are followed by the phosphorylation of mTOR within approximately 20 min and subsequent phosphorylation of STAT3 on the serine residue (peak at 40 min). No detectable phosphorylation on the tyrosine residue was observed. Within 1 h, an increase in Hes3 mRNA was observed, and elevation was stable 7 h after that. One day following Notch activation, elevated sonic hedgehog protein expression was observed, with even greater levels on the next day. These treatments had a powerful positive effect on cell survival and expansion of cell number. Additional treatments chosen based on the prediction that they can promote STAT3-serine phosphorylation in the absence of STAT3-tyrosine phosphorylation included the Tie2 ligand angiopoietin 2 and insulin. A JAK kinase inhibitor and a p38MAP kinase inhibitor also promote activation of this pathway. All these treatments also promoted NSC survival [20, 35, 46, 111]. Lack of STAT3-tyrosine phosphorylation was a major factor in predicting treatments because this modification induces gliogenic differentiation [107–110]. Cholera toxin was also shown to regulate the non-canonical pathway in two ways: First, treatment of NSC cultures with the active toxin increased immunoreactivity for Tie2, an input into the STAT3/Hes3 pathway, and second, the same treatment induced the nuclear localization of Hes3 both in the presence and the absence of supporting mitogen in the culture medium. Cholera toxin had particularly powerful effects on cell number expansion [81]

and improved cultured techniques allowed the recent identification of a widespread population of endogenous immature cells throughout the brain and spinal cord. The elucidation of a novel signal transduction pathway that regulates the survival and numbers of cultured NSCs and eNSCs provides novel means to activate eNSCs and maintain them in culture.

A cell population may expand as a result of increased proliferation and/or due to transcriptional changes that cause the appearance of biomarkers that identify that cell population. In the case of eNSCs, various insults, cognitive and physical tasks, and pharmacological treatments described previously are able to increase eNSC proliferation. Direct genetic manipulations of cell cycle genes also regulate eNSC numbers, providing direct support to this viewpoint [80]. Therefore, an activated eNSC is often regarded as a proliferating eNSC. Recent work suggests that, in part, posttranscriptional/post-translational changes in eNSCs contribute to the increase in their numbers following various pharmacological stimuli. Single injections of Notch or Tie2 receptor ligands in the lateral ventricles of adult rats induced a several fold expansion in the number of Hes3+ cells within 5 days. In animals that were given twice daily injections of BrdU on days 2, 3, and 4, to label dividing cells, only approximately 15 % of total Hes3+ cells were labeled with BrdU [35]. Although it is possible that BrdU labeling of dividing cells was not perfectly efficient, and although BrdU was not given throughout the entire duration of the eNSC activation experiment, the small percentage of BrdU labeled new Hes3+ cells suggests the possibility that a relatively large number of cells may be able to adopt a stem cell identity by gene expression changes. This result suggests that the number of eNSCs in the adult CNS cannot be easily determined based on the presence or absence of a particular marker or combinations of markers. It is possible, for example, that a quiescent population of eNSCs does not express an identifying marker, and that conditions that activate these cells induce biomarker expression; eNSCs may already be in the tissue, but in a non-identifiable state. Hes3 expression itself shows dynamic regulation. Hes3+ cells in the CNS parenchyma generally exclude Hes3 from the nucleus, exhibiting purely cytoplasmic expression (Fig. 1a). When placed in culture, under mitogenic support, however, Hes3 is found in both the cytoplasm and nucleus (Fig. 1b) [35]. Cholera toxin, a treatment that greatly promotes cultured NSC proliferation, even in the absence of mitogen, powerfully increases nuclear Hes3 presence (Fig. 1c). Cholera toxin also induces the proliferation of cultured neural stem cells and neuronal precursors (identified by expression of the microtubule-associated protein doublecortin; Fig. 1d), raising the intriguing possibility that nuclear Hes3 may regulate cell proliferation [81]. This possibility may be supported by the observation that in the aggressive brain cancer glioblastoma multiforme grade IV, putative cancer stem cells (identified by expression of the glycoprotein prominin) co-express Hes3, and that although most of the Hes3 signal is in the cytoplasm, a nuclear fraction is clearly identifiable (Fig. 1e). Elucidating the signaling mechanisms that regulate NSC numbers will uncover novel biomarkers which will help determine the numbers of eNSCs in the adult CNS.

Knowledge of oncogenic pathways has provided valuable clues to the regulation of neural stem cells in vitro and in vivo. A meaningful example is the regulation of eNSCs in the adult SVZ by the tumor suppressor, p53 [82]. Genes and signaling pathways that play important roles during embryonic development are often also implicated in tumorigenesis

and have also become recognized as regulators of stem cell functions in the adult brain. The Notch signaling pathway, for example, is involved in pattern formation in developing tissues [83–87], but also regulates the expansion and differentiation of neural stem cells in culture and in the living CNS [20, 88], and is also involved in tumorigenesis [89]. Both the receptor family (Notch) and the receptor ligands are membrane bound, necessitating cell-to-cell contact for stimulation. Such requirements may also define the exact architecture of the microenvironment and may contribute to the tight physical interactions between different cell types within it.

The most studied branch of the Notch signaling pathway (often referred to as "canonical") involves the proteolytic cleavage of the intracellular domain of the Notch receptor (Nicd) following contact with the ligand, subsequent interaction of Nicd with proteins including recombining binding protein suppressor of hairless (Rbpsuh), and transcriptional activation of target genes, including Hes1 and Hes5, two members of the Hes/Hey family of basic helix-loop-helix transcription factors [83, 87, 90–96]. In the developing CNS, these processes limit the formation of neurons and promote the differentiation of progenitor cells to glia [97–103].

More recently, a second branch of the Notch signaling pathway was identified, where Notch activation by soluble ligands led to the activation of a second messenger cascade which includes the fast (within minutes) activation of the kinases, PI3 kinase, Akt, and mTOR [20]. Phosphorylation of mTOR is followed by phosphorylation on the serine residue of signal transducer and activator of transcription (STAT) 3, a member of the family of STAT proteins [104] (Fig. 2a).

STAT3 regulates a wide range of cellular functions and has roles in both the nucleus and cytoplasm. STAT3 has two phosphorylation sites, on a serine and a tyrosine residue. Tyrosine phosphorylation is a staple of cell survival pathways of many cell types, including transformed cells and cancer cell lines [104, 105] as well as embryonic stem cells [106]. In contrast, phosphorylation on the serine site is largely auxiliary and can be prevented without substantial effects (reviewed in Levy and Darnell [104]). However, in cultured neural stem cells, STAT3-Tyr phosphorylation leads to the differentiation of the cells into glia [107–110]. This raised the possibility that in neural stem cells, STAT3-Ser phosphorylation is of primary importance to their survival as shown by transient transfection experiments with STAT3 mutant constructs [20]. A number of studies followed that used STAT3-Ser phosphorylation as a predictor of putative treatments that increase the numbers of eNSCs, identifying angiopoietin-2 and insulin as novel treatments in vivo [35, 46, 111] (Fig. 2b).

The two branches of the Notch signaling pathway regulate distinct members of the bHLH family of transcription factors. Hes5, downstream of the canonical pathway, has been proposed as a biomarker of eNSCs [36]. Hes3, downstream of the non-canonical Notch pathway and with strong correlation downstream of STAT3-Ser phosphorylation, has also been proposed as a novel biomarker [35]. Future studies may address how the equilibrium between Hes5 and Hes3 (and, possibly, other members of the bHLH family) defines particular subpopulations of eNSCs or particular states (e.g., quiescence vs. proliferation) of these cells. Understanding how particular Hes/Hey gene family members define particular states of neural stem cells will be an important step towards elucidating the mechanisms of controlling eNSCs, made more difficult by the redundancy observed within this gene family [112].

Conclusions

While the potential of eNSCs to elicit cell replacement after brain injury or neurodegenerative disease has generated a lot of excitement, there is scant evidence that this actually occurs in the mammalian brain, to a consequential extent, or key relevance to disease or injury progress. Indeed, as described in this review, recent evidence suggests that an important function for eNSCs is to promote neuroprotection by neurotrophic signaling. From an evolutionary standpoint, this neuroprotective role may compensate for a loss in the capacity of eNSCs for cell replacement. These concepts may not be confined to neural tissue but may also regulate the repair of other organs such as the liver [113]. Continued investigation of the pathways regulating the neuroprotective function of eNSCs may lead to new therapies for the injured or diseased brain.

Acknowledgments The work was supported by the Else Kröner-Fresenius-Stiftung and the Centre for Regenerative Therapies, Dresden (AA-T) and the European Commission Project NeuroStemcell (RK).

References

1. McKay R, Kittappa R (2008) Neuron 58:659–661
2. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Nat Med 14:504–506
3. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Bjorklund A, Widner H, Revesz T, Lindvall O, Brundin P (2008) Nat Med 14:501–503
4. Yamanaka K, Boillee S, Roberts EA, Garcia ML, McAlonis-Downes M, Mikse OR, Cleveland DW, Goldstein LS (2008) Proc Natl Acad Sci USA 105:7594–7599
5. Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L, Paz N, Koren-Michowitz M, Waldman D, Leider-Trejo L, Toren A, Constantini S, Rechavi G (2009) PLoS Med 6:e1000029
6. Altman J, Das GD (1965) J Comp Neurol 124:319–335
7. Schlessinger AR, Cowan WM, Gottlieb DI (1975) J Comp Neurol 159:149–175

8. Kaplan MS, Bell DH (1984) J Neurosci 4:1429–1441
9. Privat A, Drian MJ (1975) Brain Res 88:518–524
10. Vaysse PJ, Goldman JE (1990) Neuron 5:227–235
11. Levison SW, Goldman JE (1993) Neuron 10:201–212
12. Cameron HA, Woolley CS, McEwen BS, Gould E (1993) Neuroscience 56:337–344
13. Luskin MB (1993) Neuron 11:173–189
14. Lois C, Alvarez-Buylla A (1993) Proc Natl Acad Sci USA 90:2074–2077
15. Kuhn HG, Dickinson-Anson H, Gage FH (1996) J Neurosci 16:2027–2033
16. Palmer TD, Takahashi J, Gage FH (1997) Mol Cell Neurosci 8:389–404
17. Palmer TD, Ray J, Gage FH (1995) Mol Cell Neurosci 6:474–486
18. Marmur R, Mabie PC, Gokhan S, Song Q, Kessler JA, Mehler MF (1998) Dev Biol 204:577–591
19. Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY (2002) Nat Biotechnol 20:1103–1110
20. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R, McKay RD (2006) Nature 442:823–826
21. Temple S (1989) Nature 340:471–473
22. Hockfield S, McKay RD (1985) J Neurosci 5:3310–3328
23. Lendahl U, Zimmerman LB, McKay RD (1990) Cell 60:585–595
24. Cattaneo E, McKay R (1990) Nature 347:762–765
25. Reynolds BA, Weiss S (1992) Science 255:1707–1710
26. Taupin P, Gage FH (2002) J Neurosci Res 69:745–749
27. Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D (1994) Neuron 13:1071–1082
28. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Cell 97:703–716
29. D'Amour KA, Gage FH (2003) Proc Natl Acad Sci USA 100 (Suppl 1):11866–11872
30. Pevny LH, Nicolis SK (2010) Int J Biochem Cell Biol 42:421–424
31. Ahn S, Joyner AL (2005) Nature 437:894–897
32. Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, Alvarez-Buylla A (2006) Neuron 51:187–199
33. Sakakibara S, Imai T, Hamaguchi K, Okabe M, Aruga J, Nakajima K, Yasutomi D, Nagata T, Kurihara Y, Uesugi S, Miyata T, Ogawa M, Mikoshiba K, Okano H (1996) Dev Biol 176:230–242
34. Temple S, Raff MC (1985) Nature 313:223–225
35. Androutsellis-Theotokis A, Rueger MA, Park DM, Mkhikian H, Korb E, Poser SW, Walbridge S, Munasinghe J, Koretsky AP, Lonser RR, McKay RD (2009) Proc Natl Acad Sci USA 106:13570–13575
36. Basak O, Taylor V (2007) Eur J Neurosci 25:1006–1022
37. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW (1997) Blood 90:5002–5012
38. Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL (2000) Proc Natl Acad Sci USA 97:14720–14725
39. Bull ND, Bartlett PF (2005) J Neurosci 25:10815–10821
40. Lee A, Kessler JD, Read TA, Kaiser C, Corbeil D, Huttner WB, Johnson JE, Wechsler-Reya RJ (2005) Nat Neurosci 8:723–729
41. Pekcec A, Loscher W, Potschka H (2006) Neuroreport 17:571–574
42. Wang YZ, Plane JM, Jiang P, Zhou CJ, Deng W (2011) Stem Cells 29:907–912
43. Lin RC, Matesic DF, Marvin M, McKay RD, Brustle O (1995) Neurobiol Dis 2:79–85
44. Clarke SR, Shetty AK, Bradley JL, Turner DA (1994) Neuroreport 5:1885–1888
45. Ourednik V, Ourednik J, Flax JD, Zawada WM, Hutt C, Yang C, Park KI, Kim SU, Sidman RL, Freed CR, Snyder EY (2001) Science 293:1820–1824
46. Androutsellis-Theotokis A, Rueger MA, Mkhikian H, Korb E, McKay RD (2008) Cold Spring Harb Symp Quant Biol 73:403–410
47. Wexler E, Palmer T (2002) Trends Neurosci 25:225–227
48. Klein C, Butt SJ, Machold RP, Johnson JE, Fishell G (2005) Development 132:4497–4508
49. Merkle FT, Mirzadeh Z, Alvarez-Buylla A (2007) Science 317:381–384
50. Alvarez-Buylla A, Kohwi M, Nguyen TM, Merkle FT (2008) Cold Spring Harb Symp Quant Biol 73:357–365
51. Magavi SS, Leavitt BR, Macklis JD (2000) Nature 405:951–955
52. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Nat Med 8:963–970
53. Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, Dragunow M, Connor B, Faull RL (2003) Proc Natl Acad Sci USA 100:9023–9027
54. Jin K, Peel AL, Mao XO, Xie L, Cottrell BA, Henshall DC, Greenberg DA (2004) Proc Natl Acad Sci USA 101:343–347
55. Liu J, Solway K, Messing RO, Sharp FR (1998) J Neurosci 18:7768–7778
56. Zhang RL, Zhang ZG, Chopp M (2008) Neuropharmacology 55:345–352
57. Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH (1997) J Neurosci 17:5820–5829
58. Martens DJ, Seaberg RM, van der Kooy D (2002) Eur J Neurosci 16:1045–1057
59. Holmberg J, Armulik A, Senti KA, Edoff K, Spalding K, Momma S, Cassidy R, Flanagan JG, Frisen J (2005) Genes Dev 19:462–471
60. Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D (1996) J Neurosci 16:2649–2658
61. Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, Tamura A, Kirino T, Nakafuku M (2002) Cell 110:429–441
62. Jiao JW, Feldheim DA, Chen DF (2008) Proc Natl Acad Sci USA 105:8778–8783
63. Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie AR, Gage FH (2005) Nature 437:1370–1375
64. Ehm O, Goritz C, Covic M, Schaffner I, Schwarz TJ, Karaca E, Kempkes B, Kremmer E, Pfrieger FW, Espinosa L, Bigas A, Giachino C, Taylor V, Frisen J, Lie DC (2010) J Neurosci 30:13794–13807
65. Gould E, Reeves AJ, Graziano MS, Gross CG (1999) Science 286:548–552
66. Chen Y, Ai Y, Slevin JR, Maley BE, Gash DM (2005) Exp Neurol 196:87–95
67. Chen J, Magavi SS, Macklis JD (2004) Proc Natl Acad Sci USA 101:16357–16362
68. Cooper O, Isacson O (2004) J Neurosci 24:8924–8931
69. Fallon J, Reid S, Kinyamu R, Opole I, Opole R, Baratta J, Korc M, Endo TL, Duong A, Nguyen G, Karkehabadhi M, Twardzik D, Patel S, Loughlin S (2000) Proc Natl Acad Sci USA 97:14686–14691
70. Jin K, LaFevre-Bernt M, Sun Y, Chen S, Gafni J, Crippen D, Logvinova A, Ross CA, Greenberg DA, Ellerby LM (2005) Proc Natl Acad Sci USA 102:18189–18194
71. Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA (2001) Proc Natl Acad Sci USA 98:4710–4715
72. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA (2002) Proc Natl Acad Sci USA 99:11946–11950
73. Jin K, Mao XO, Sun Y, Xie L, Greenberg DA (2002) J Clin Invest 110:311–319

74. Kizil C, Kaslin J, Kroehne V, Brand M (2012) Dev Neurobiol 72 (3):429–461
75. Chapouton P, Jagasia R, Bally-Cuif L (2007) Bioessays 29:745–757
76. Berg DA, Kirkham M, Beljajeva A, Knapp D, Habermann B, Ryge J, Tanaka EM, Simon A (2010) Development 137:4127–4134
77. Ryu JK, Cho T, Wang YT, McLarnon JG (2009) J Neuroinflammation 6:39
78. Martino G, Pluchino S, Bonfanti L, Schwartz M (2011) Physiol Rev 91:1281–1304
79. Hirsch EC, Hunot S (2009) Lancet Neurol 8:382–397
80. Salomoni P, Calegari F (2010) Trends Cell Biol 20:233–243
81. Androutsellis-Theotokis A, Walbridge S, Park DM, Lonser RR, McKay RD (2010) PLoS One 5:e10841
82. Meletis K, Wirta V, Hede SM, Nister M, Lundeberg J, Frisen J (2006) Development 133:363–369
83. Heitzler P, Simpson P (1991) Cell 64:1083–1092
84. Cagan RL, Ready DF (1989) Genes Dev 3:1099–1112
85. Simpson P (1990) Development 109:509–519
86. Cabrera CV (1990) Development 110:733–742
87. Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Science 284:770–776
88. Imayoshi I, Kageyama R (2011) Mol Neurobiol 44:7–12
89. Pierfelice TJ, Schreck KC, Eberhart CG, Gaiano N (2008) Cold Spring Harb Symp Quant Biol 73:367–375
90. Louvi A, Artavanis-Tsakonas S (2006) Nat Rev Neurosci 7:93–102
91. Ruohola H, Bremer KA, Baker D, Swedlow JR, Jan LY, Jan YN (1991) Cell 66:433–449
92. Greenwald I, Rubin GM (1992) Cell 68:271–281
93. Spana EP, Doe CQ (1996) Neuron 17:21–26
94. Campos-Ortega JA, Jan YN (1991) Annu Rev Neurosci 14:399–420
95. Kidd S, Kelley MR, Young MW (1986) Mol Cell Biol 6:3094–3108
96. Chiba S (2006) Stem Cells 24:2437–2447
97. Haddon C, Smithers L, Schneider-Maunoury S, Coche T, Henrique D, Lewis J (1998) Development 125:359–370
98. Morrison SJ, Perez SE, Qiao Z, Verdi JM, Hicks C, Weinmaster G, Anderson DJ (2000) Cell 101:499–510
99. Tanigaki K, Nogaki F, Takahashi J, Tashiro K, Kurooka H, Honjo T (2001) Neuron 29:45–55
100. Justice NJ, Jan YN (2002) Curr Opin Neurobiol 12:64–70
101. Stump G, Durrer A, Klein AL, Lutolf S, Suter U, Taylor V (2002) Mech Dev 114:153–159
102. Kamakura S, Oishi K, Yoshimatsu T, Nakafuku M, Masuyama N, Gotoh Y (2004) Nat Cell Biol 6:547–554
103. Taylor MK, Yeager K, Morrison SJ (2007) Development 134:2435–2447
104. Levy DE, Darnell JE Jr (2002) Nat Rev Mol Cell Biol 3:651–662
105. Kiuchi N, Nakajima K, Ichiba M, Fukada T, Narimatsu M, Mizuno K, Hibi M, Hirano T (1999) J Exp Med 189:63–73
106. Niwa H, Burdon T, Chambers I, Smith A (1998) Genes Dev 12:2048–2060
107. Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD, Greenberg ME (1997) Science 278:477–483
108. Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD (1996) Genes Dev 10:3129–3140
109. Song MR, Ghosh A (2004) Nat Neurosci 7:229–235
110. Rajan P, McKay RD (1998) J Neurosci 18:3620–3629
111. Androutsellis-Theotokis A, Rueger MA, Park DM, Boyd JD, Padmanabhan R, Campanati L, Stewart CV, LeFranc Y, Plenz D, Walbridge S, Lonser RR, McKay RD (2010) PLoS One 5: e9414
112. Hatakeyama J, Bessho Y, Katoh K, Ookawara S, Fujioka M, Guillemot F, Kageyama R (2004) Development 131:5539–5550
113. Woo DH, Kim SK, Lim HJ, Heo J, Park HS, Kang GY, Kim SE, You HJ, Hoeppner DJ, Kim Y, Kwon H, Choi TH, Lee JH, Hong SH, Song KW, Ahn EK, Chenoweth JG, Tesar PJ, McKay RD, Kim JH (2012) Gastroenterology 142:602–611