Biochemical and Biophysical Research Communications 386 (2009) 628–633

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of canonical Wnt signaling promotes gliogenesis in P0-NSCs

David Kunke [a,*], Vítězslav Bryja [b,c], Line Mygland [a], Ernest Arenas [b], Stefan Krauss [a,d]

[a] Centre for Molecular Biology and Neuroscience and Institute of Medical Microbiology, Rikshospitalet, 0027 Oslo, Norway
[b] Laboratory of Molecular Neurobiology, Dept. Medical Biochemistry & Biophysics, Karolinska Institutet, Scheeles väg 1, 171 77 Stockholm, Sweden
[c] Institute of Experimental Biology, Faculty of Science, Masaryk University & Institute of Biophysics AS CR, Kotlarska 2, 611 37 Brno, Czech Republic
[d] SFI-CAST Biomedical Innovation Center, Rikshospitalet, 0027 Oslo, Norway

ARTICLE INFO

Article history:
Received 8 June 2009
Available online 21 June 2009

Keywords:
Differentiation
Gliogenesis
Neuronal progenitor cells
Canonical Wnt signaling
Soluble Frizzled related protein

ABSTRACT

Wnt signaling plays an essential role in the development of mammalian central nervous system. We investigated the impact of activation/inhibition of the Wnt signaling pathway on neuronal/glial differentiation in neurospheres derived from neonatal mouse forebrains. For short term alterations, neurospheres were stimulated with recombinant Wnt-3a, Wnt-5a and the Wnt inhibitor Dickkopf-1 (Dkk1). Furthermore, neurospheres were transduced with retroviral vectors encoding Wnt-3a, Wnt-7a and their inhibitors Dkk1 and soluble Frizzled related protein-5 (sFRP5). Long-term activation of Wnt pathway by Wnt-7a or by treatment with GSK3 inhibitors promoted a moderate increase of the neuronal differentiation and blocked gliogenesis. In contrast, Wnt pathway inhibition in neurospheres, induced by retroviral overexpression of either Dkk1 or sFRP5, robustly increased the gliogenesis at the expense of neurogenesis. In summary, our data demonstrate that activation or inhibition of Wnt/β-catenin signaling in neurospheres regulates neuronal and glial differentiation, respectively. Thus, our results suggest that Wnt signaling may also contribute to regulate these processes in the neonatal brain.

Introduction

During development of the cerebral cortex, a rapid onset of massive gliogenesis starts mainly at postnatal day 0 (P0) and shortly after birth. A number of extrinsic stimuli, as well as intrinsic cellular pathways have been identified [1–10], but a more punctual description of the molecular events associated with the onset of gliogenesis remains mostly unknown [11,2,12,13]. Remarkably, progenitor cells derived from both neonatal and adult forebrain sustained expression of many secreted factors known to inhibit Wnt signaling [14,15]. However, a function of Wnt signaling in gliogenesis, has not been so far described. The Wnt signaling pathway has been previously shown to play a major role in the development of nervous system and is predominately mediated and tightly controlled by an interplay between Wnt ligands, Wnt receptors and Wnt inhibitors in a selective or an instructive manner [16–19]. Interestingly Wnt inhibitors such as Dkk1 has been shown to inhibit Wnt/β-catenin signaling (also referred as canonical Wnt signaling) by binding to and antagonizing LRP5/6 [20,21]. Instead, soluble Frizzled related proteins (sFRPs) block Wnts by prevent their binding to Frizzled receptors [22–24]. Wnt signaling regulate patterning, cell fate determination, proliferation and differentiation during the early embryonic and later stages of development [25–28]. Interestingly, neuronal progenitors respond differently to the same Wnt signals at different stages of embryonic development. [29,30,1,31,25,32]

Here, we analyzed the effects of activation/inhibition of Wnt signaling pathway on the neuronal/glial differentiation in the neurospheres derived from newborn animals. We demonstrate that long-term activation of the Wnt pathway by retroviral-mediated transgene expression [14] strongly affects differentiation. The activation of canonical Wnt signaling in neurospheres promoted neurogenesis and inhibited gliogenesis, while inhibition of canonical Wnt signaling in neurospheres increased gliogenesis. Thus, our results suggest that the inhibition of the Wnt pathway is a prerequisite for the onset of gliogenesis in the forebrain.

Materials and methods

Culture of primary mouse neural stem/progenitor cells. Neurospheres forming NSCs were prepared from neonatal forebrains of C57BL/6 mice (P0 stage) and were expanded as described previously [14]. Low passage NSCs were used throughout all the experiments.

Abbreviations: bFGF, basic fibroblast growth factor; Dkk, dickkopf; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; IRES, internal ribosome entry site; LEF, lymphoid enhancer-binding factor; NSCs, neurospheres; P, postnatal day; sFRP, secreted frizzled-related protein; TCF, T-cell factor.

* Corresponding author. Fax: +47 23074061.
E-mail address: David.Kunke@rr-research.no (D. Kunke).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.084

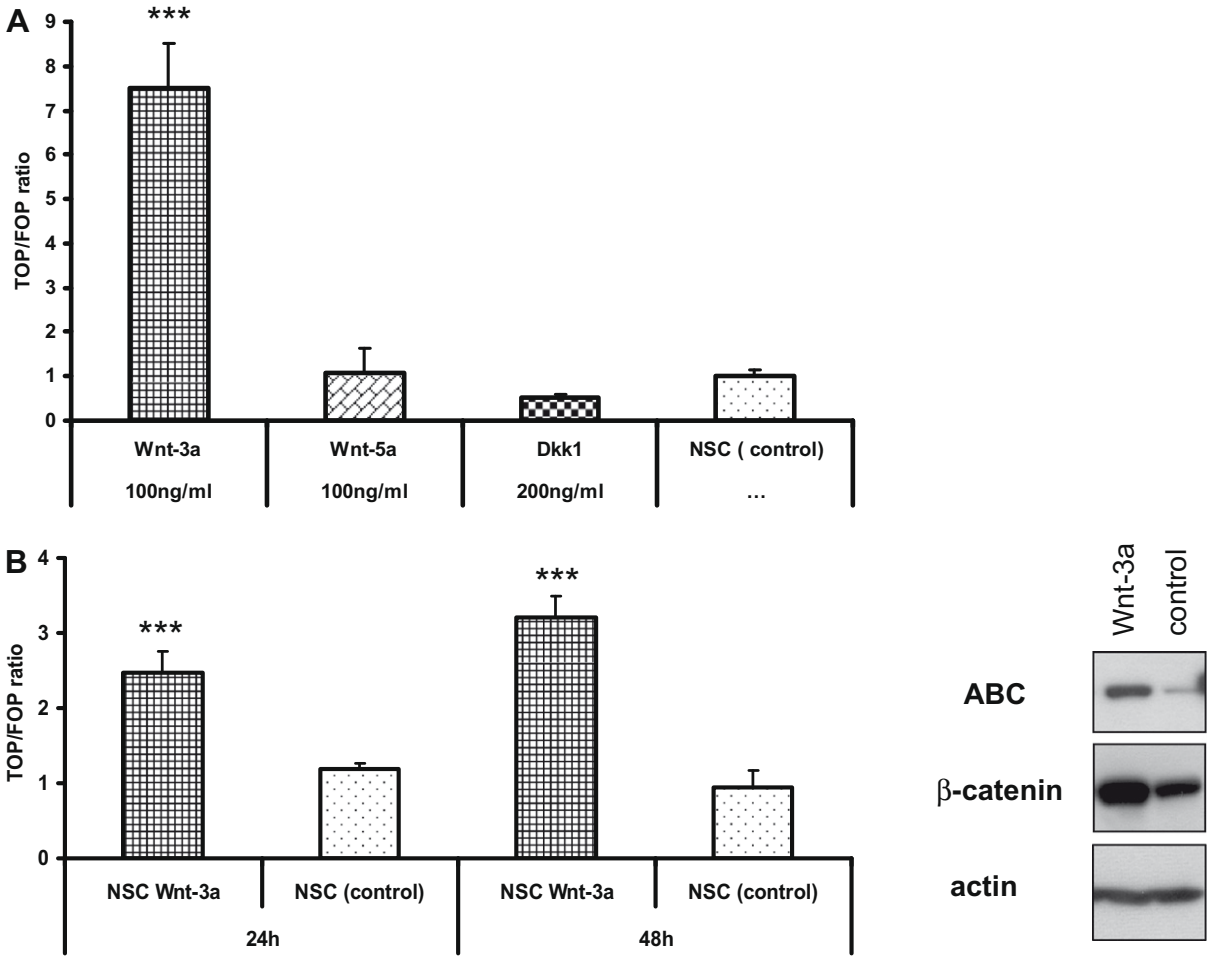

Fig. 1. Canonical Wnt signalling can be activated in P0 forebrain-NSCs by Wnt-3a. (A) Response to recombinant Wnt proteins. NSCs were plated as single cells and maintained onto retronectin coated plate in serum-free neurobasal A medium one day before transfection. Cells were co-transfected with TOP-flash or FOP-flash reporter (Upstate) and pRL-TK (Renilla-TK-luciferase vector, Promega). After 48 h cells were treated either with recombinant Wnt-3a (100 ng/ml), Wnt-5a (100 ng/ml) or Dkk1 (200 ng/ml) for 4 h at37 °C. Data are expressed as TOP/FOP ratio, normalized to Renilla activity. Statistically significant difference from control cells and other experimental conditions ($p < 0.001$) as analyzed by one-way ANOVA and with Tukey's multiple comparison post-tests. (B) Response in retroviral transduced NSCs. Wnt-3a transduced NSCs and non-transduced NSCs as control were trypsinised, plated as single cells onto retronectin coated plates in serum-free neurobasal A medium one day before being processed for co-transfection. Cells were harvested after 24 h and 48 h after transfection. Firefly luciferase measurements were normalized to Renilla activity, TOP/FOP ratio of pTOP-flash/pFOP-flash luciferase activity ($^{***}p < 0.001$). The activation of β-catenin was determined by WB as increase in total and active-β-catenin (ABC); actin was used as a loading control.

Retroviral vectors used for transduction, fluorescent cell sorting. In the bicistronic viral vector plasmid pSF91–GCSh–EGFP [14] containing enhanced green fluorescence protein (EGFP) under control of the poliovirus IRES, gene coding for gGCS was either deleted by NotI and self-ligated (pSF91–IRES–GFP) or NotI fragments containing cDNA coding for the appropriate gene were ligated (a 1578-bp fragment by mWnt-3a, a 1771-bp fragment by mWnt-7a, a 1112-bp by mDkk1, a 1544-bp fragment by msFrp5 and a 1909-bp fragment by mShh). For production of retroviral particles the Phoenix-Eco packaging cell line was transfected using FuGene6 [33] with the pSF91–IRES–GFP or above described bicistronic EGFP retroviral plasmids. Culture supernatants containing viral particles were collected at 48 h after transfection. Recombinant fibronectin-coated plates (10 μg/cm^2 rFN (Retronectin, Takara Shuzo Co.) were preloaded with retroviral supernatants [14] twice for one hour at 37 °C with fresh supernatant. The supernatant was then removed, and the plates washed with PBS before addition of the NSCs as single cells. Subsequently the population of successfully transduced gfp positive cells was obtained by subjecting the neurospheres dissociated to single-cell suspension to fluorescent cell sorting a FACStar Flow cytometer (Becton–Dickinson, San Jose, CA, USA) according to standard method.

Luciferase reporter gene assays. Neurospheres were plated as single cells on retronectin in 48-well-plate. The following day, cells were co-transfected totally with 0.48 μg plasmid DNA by using

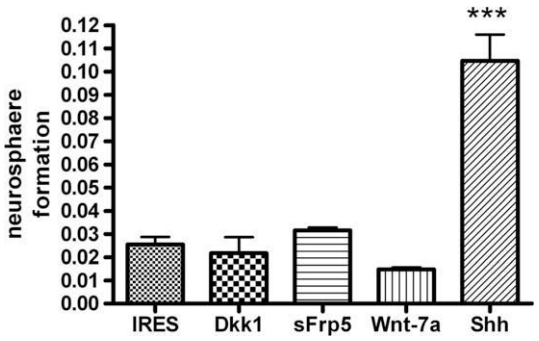

Fig. 2. Modulation of Wnt signalling does not affect proliferation of P0 forebrain-NSCs. The growth rate of all transduced cells was determined in parallels. All data are mean values ± SD. from three independent experiments. Statistically significant difference from all other control and experimental conditions ($p < 0.001$) as analyzed by one-way ANOVA and Bonferroni post-tests.