Hienola et al., http://www.jcb.org/cgi/content/full/jcb.200602043/DC1

N-syndecan knockout mice display normal neural proliferation and differentiation

We estimated the number of proliferating cells in N-syndecan knockout and wild-type cortex at E12 by labeling all cells undergoing mitosis during a period of 6 h. This period corresponds to the cell cycle length of neural stem cells at that age (Takahashi et al., 1995). In addition, we compared the number of cortical cells between adult N-syndecan knockout and wild-type mice and the number of BrdU-labeled cells at the age of E18 (labeling age E15). There was no difference in the average cell density or in the number of $BrdU^+$ cells in the cerebral cortex between N-syndecan knockout and wild-type mice at any observed age (Table S1). Previously, we have shown that HB-GAM inhibits FGF-2 activity on neural stem cells possibly by competing with its binding to HS (Hienola et al., 2004), but the current findings (Table S1) indicate that N-syndecan is not the required HS carrier in neural stem cells.

We collected BrdU-injected knockout and wild-type E15 embryos 14 h after the BrdU injection and counted the number of BrdU/β-III–tubulin double-positive cells in the area below subplate. In the wild types, 33.8 ± 4.0% of the all BrdU-positive cells expressed β-III–tubulin as well. In knockouts, the amount of these cells was 31.1 ± 5.2%. This result suggests that the N-syndecan knockout neurons obtain the neuronal phenotype around the same time and at the similar frequency as the wild-type neurons. At P1, we observed the cells in the CP and estimated 92.0 ± 2.8% of all BrdU-positive cells to be β-III–tubulin expressing as well. The situation was not different in N-syndecan knockouts, in which the amount was 93.0 ± 4 .1%.

Table S1. Proliferation and total number of cells in the N-syndecan knockout cortex is normal

Age/sample type	N-syndecan wild type	N-syndecan knockout
E12 $BrdU^+$ ($1 \times 10^6/mm^3$)[a]	0.920 ± 0.242 ($n = 7$)	0.898 ± 0.187 ($n = 6$)
E18 $BrdU^+$ ($1 \times 10^6/mm^3$)[b]	0.351 ± 0.049 ($n = 4$)	0.392 ± 0.050 ($n = 4$)
Adult total ($1 \times 10^6/mm^3$)[c]	1.266 ± 0.211 ($n = 4$)	1.224 ± 0.184 ($n = 5$)

± SEM of independent experiments.
[a]Three injections of BrdU during 6 h.
[b]One injection of BrdU given to the mother at age E15.
[c]Age 2–3 mo.

Table S2. Differentiation of embryonic neural precursor cells after 7 d in vitro

Differentiation marker	N-syndecan wild type	N-syndecan knockout
β-III–tubulin	47.5 ± 5.0 ($n = 4$)	49.8 ± 7.0 ($n = 4$)
GFAP	60.2 ± 9.3 ($n = 4$)	66.2 ± 6.0 ($n = 5$)
Nestin	20.3 ± 6.0 ($n = 3$)	22.0 ± 1.9 ($n = 4$)
Neurofilament	31.3 ± 1.3 ($n = 4$)	35.5 ± 4.4 ($n = 4$)

Data are shown in percentages ± SEM of independent experiments.

Table S3. Distribution of layer markers in the N-syndecan knockout P10 cortex

Layer marker	N-syndecan wild type	N-syndecan knockout
Brn2, layers II–IV	42.8 ± 7.4	46.7 ± 5.4
Cadherin-8, layer V	45.1 ± 6.1	42.7 ± 7.2
N-cadherin, layer VI	56.1 ± 4.6	61.0 ± 5.6

Relative amount of all immunopositive cells in all layers. Data are presented in percentages ± SEM. $n = 4$ pups.

Neural precursor cells isolated from E15 embryos were differentiated in cell culture for 1 wk, and the number of β-III–tubulin-, GFAP-, nestin-, and neurofilament- (light and medium chain) expressing cells was counted. We could not see changes in N-syndecan knockout neuronal differentiation in vitro based on these immunomarkers (Table S2). We also used an array of different immunomarkers on histological brain sections distinguishing between different layer phenotypes in cortical cells, including Brn2 (II–IV, rabbit IgG; Santa Cruz Biotechnology, Inc.), cadherin-8 (layer V, goat IgG; Santa Cruz Biotechnology, Inc.), and N-cadherin (layer VI, rat IgG; Zymed Laboratories). The analysis was made at P10 from the motor and parietal cortex, where the immunostaining for all three markers was most prominent. According to these immunostaining data, the N-syndecan knockouts do not show any clearly displaced cells in the cerebral laminae (Table S3).

References

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