



# Astroglia - stem cell interaction

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## Introduction

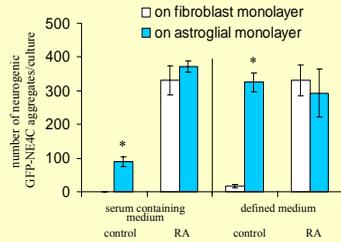
Fate specification of multipotential stem cells is governed by signals derived from their local microenvironment (1,2). The major components of the cellular milieu surrounding neural stem cells are cells of the astroglial lineage (3,4). Radial glial cells guide and regulate the migration of neuronal precursors during cortical development and were shown to possess neuronal stem cell properties, as well (5,6,7). Astroglial cells within the subventricular zone and the hippocampus of adult animals ensheat the migrating neuroblasts and are involved in the maintenance of the non-differentiated neural stem cell population (3). Stem cells, implanted into the injured or diseased brain to provide a donor source for possible nervous system repair, are encountered by reactivated astroglial cells (8,9). Hence, glia/stem cell interaction has important developmental aspects and it is of great clinical importance.

To examine the influence of astroglial cells on the fate of two stem cell types - the embryonic neuroectoderma-derived stem cells and the blastocyst-derived embryonic stem (ES) cells - we performed co-culture experiments. Immortalized neural stem cells of the GFP-NE-4C cell line and mouse GFP-ES cells (developed in our laboratories [10-15]) were co-cultured with primary astroglial cells prepared from the brains of neonatal rats or mice (16). Here we show, that under the given *in vitro* circumstances, astrocytes induce multitudinous neuronal differentiation of the neural stem cells but do not initiate extensive neuron formation by ES cells.

Refs: 1. Dorsky RI et al. *Bioessays* 2000, 22(8):708-16; 2. Song H et al. *Nature* 2002, 417:39-44; 3. Doetsch F et al. *J Neurosci* 1997 Jul 1;17(13):5046-61; 4. Alvarez-Buylla A, Garcia-Verdugo JM. *Journal of Neuroscience*. 2002; 22(3):629-634; 5. Hansen M.E. *Annu. Rev. Neurosci.* 1999; 22:511-39; 6. Noctor SC et al. *Nature* 2001; 409:6821-714-20; 7. Campbell K, and Gotz M. *Trends Neurosci*. 2002; 25(5):238-45; 8. Lundberg C, Bjorklund A. *Neuroreport* 1996; 22:700/847-52; 9. Zhang SC et al. *Nat Biotechnol*. 2001 Dec;19(12):1129-33; 10. Schlett K et al. *Int J Dev Neurosci*. 1997; 15: 95-804; 11. Schlett K, Madarász E. *J Neurosci Res*. 1997; 47: 405-415; 12. Varju P et al. *J Neurochem*. 2001; 77, 1444-1456; 13. Herberth B et al. *J Neurosci Res*. 2002; 67(5):574-82; 14. Jelini M et al. *J Neurobiol*. 2002; 51(1):54-65; 15. Tamok K et al. *Eur. J. Cell Biology* 2002; 81:403-412; 16. Kornyei Zs et al. *J. Neurosci Res*. 2000; 61: 421-429

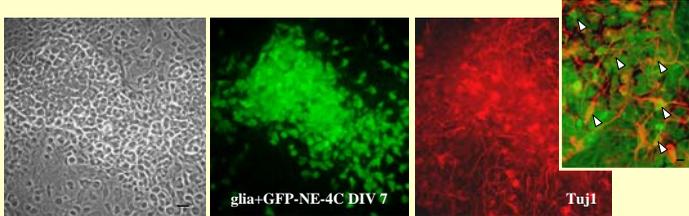
## I. Astrocytes induce neuronal differentiation of immortalized neuroectoderma-derived stem cells

In glia + GFP-NE-4C neural stem cell co-cultures abundant neuronal differentiation could be observed. However, no neuron formation occurred, when NE-4C cells were plated on fibroblast monolayers.



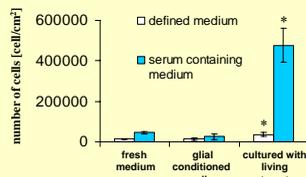
Nearly 100 % of the astroglia-induced NE-4C clusters developed into foci of abundant neuron production if cells were grown in serum free medium (NB-B27). In the presence of 5% serum, however, neurons were found only in 36±12% of the neural stem cell clusters at the end of the first week in co-culture.

Addition of all-trans retinoic acid (RA) induced neurogenesis in practically all non-differentiating clusters.

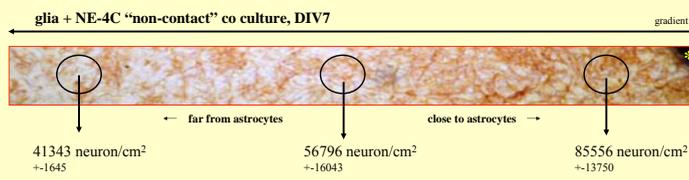
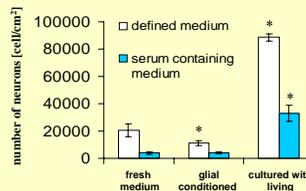


## II. Astrocytes induce neuronal differentiation of NE-4C neural stem cells through factor(s) released into the culture medium

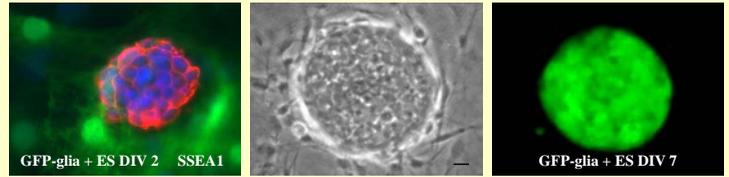
Glial conditioned medium (GCM) did not increase the overall cell number of neural progenitor cells within 48 hours after plating, neither using serum containing nor serum free (NeuroBasal-B27) medium. However, a significant increase in cell number could be observed, if neural progenitors were grown in the presence of living astrocytes.



The rate of neuronal differentiation of NE-4C cells was not raised by the addition of GCM, whereas a significant increase in the total neuron number was observed in NE-4C cultures grown together with living astrocytes. In the latter case the rate of neuron formation was inversely proportional with the distance measured from the living astroglial cells.

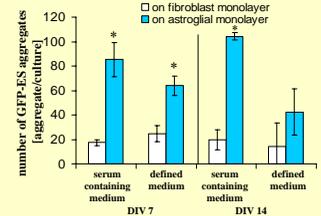


## III. GFP-ES cells form compact aggregates on the surface of astroglial monolayer



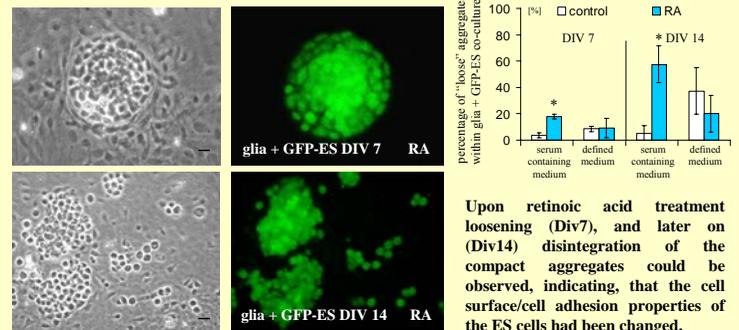
SSEA1+ GFP-ES cells plated individually onto fibroblast or astroglia monolayers formed compact aggregates within a few days. The size of the GFP-ES aggregates kept growing during the two week period of co-culturing. Most of the aggregates retained their compact structure throughout 14 days. Outmigration from the cell assemblies grown on astroglia monolayer was rarely observed.

The number of GFP-ES cell assemblies was significantly higher in astroglia + ES, than in fibroblast + ES co-cultures. The rate of aggregate formation was slightly higher in serum containing medium, than in serum free defined medium. These observations indicate, that astrocytes - particularly in cooperation with some serum factors - efficiently support the survival and growth of ES cells.



## IV. Retinoic acid initiates changes in the cell adhesion properties of ES cells

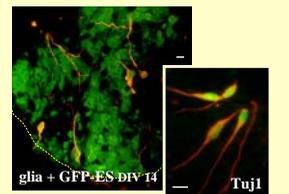
Formation of the ES cell aggregates was not affected by the persistent application of all-trans retinoic acid. However, the average size of the aggregates formed in RA treated co-cultures was smaller, than that in the control cultures.



Upon retinoic acid treatment loosening (Div7), and later on (Div14) disintegration of the compact aggregates could be observed, indicating, that the cell surface/cell adhesion properties of the ES cells had been changed.

## V. Multitudinous neuronal differentiation of ES cells in glia + ES co-cultures was not observed

In glia + ES co-cultures maintained for 14 days in serum free medium some GFP+/Tuj1+ cells were found. However, multitudinous ES-cell derived neuron-formation could not be observed.



GFP-ES cells could be induced towards neuronal fate by retinoic acid treatment, if standard ES cell differentiation protocols were used. In glia + GFP-ES co-cultures, however, retinoic acid failed to induce extensive neuron formation.

## Discussion

Our results show, that

- on the top of astroglia monolayer ES cells form persistently growing, compact aggregates, while NE-4C neural stem cells form quickly developing, but less densely packed cell clusters.
- ES cells do not mingle with the underlying astroglial cells, while NE-4C cells migrate out from the cell clusters and intermingle with the surrounding astrocytes.
- in glia/stem cell co-cultures neuron formation by ES cells rarely occurs, while most of the clusters formed by neural stem cells show extensive neuronal differentiation.
- the astroglial neurogenesis-inducing effect is mediated either by direct cell to cell communication or by soluble, short range acting factors released into the medium.
- under our co-culture conditions retinoic acid does not induce neuronal differentiation of the ES cells, but initiates changes in their cell adhesion properties, resulting in loosening and disintegration of the compact aggregates.
- retinoic acid cooperates with serum factors either in enhancing glia induced neurogenesis of neural-stem cells or mediating changes in ES cell adhesion properties.

The data indicate, that

- astrocytes promote the survival and proliferation of both ES cells and neural stem cells;
- two stem cell types - the blastocyst-derived totipotent embryonic stem cells and the neuroectoderma-derived pluripotent neural stem cells differ in their cell-to-cell adhesion properties; in their capability to make contact with astroglial cells (see poster P37); in their ability to respond to astroglia-derived factors by neuronal lineage-commitment; in their responsiveness to all-trans retinoic acid.