Role of Cell to Cell Connections in the course of in vitro Neuron Formation:

Gap Junction Communication by NE-4C neuroectodermal stem cells

Jelitai M.[1]*, Zs. Környei[1], K. Tárnok[2], M. Anděrová[3], E. Madarász[1]

[1]Institute of Experimental Medicine, Hung.Acad.Sci., Budapest; [2]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary; [3]Department of Neuroscience, Institute of Experimental Medicine, ASCR, Prague, Czech Republic

Jelitai@koki.hu

Introduction

Fate determination of neural stem cells is governed by signals derived from their microenvironment via multiple forms of contact and humoral cell-to-cell interactions. Gap junctions are involved in the control of both, cell proliferation and differentiation. In the primary germinative layer of the neural tube, clusters of neuroectodermal cells are interconnected by gap junctions (lo Turco and Kriegstein, 1991).

Nowadays, it is thought that communications through gap junctions may contribute to the maintenance of the proliferative state of stem cells (Cheng et al., 2004; Deisseroth et al., 2004; Duval et al., 2002). Gap junction coupling is a common feature of progenitor cells througout the whole period of neural tissue development.

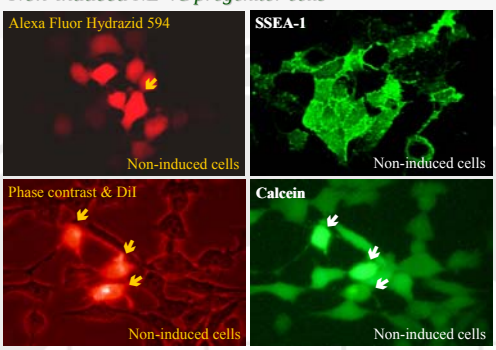
NE-4C – a p53-deficient, immortalized neuroectodermal stem cell line (Schlett and Madarasz, 1997) - has been used to study the in vitro neuron formation induced either by all-trans retinoic acid (RA) or by the presence of primary astrocytes. In the course of in vitro differentiation, NE-4C cells give rise to astrocytes in addition to neurons. The time course of gap junction formation has been related to multiple parameters of neuronal cell fate determination by NE-4C model cells.

In the recent studies, gap junction coupling and the importance of gap junction communication were investigated in the course of in vitro induced neuronal development.

Schlett K., Madarasz E. Retinoic acid induced neural differentiation in a neuroectodermal cell line immortalised by p53 deficiency. J Neurosci Res.47 (1997)
Cheng A., H.Tang, J.Cao, M.Zhu, X.Zhang, M.Rao, M.Mattson. Gap junctional communication is required to maintain mouse cortical neural progenitor cells in a proliferative state. Developmental Biology 272, 203-216 (2004)
Deisseroth K.,S.Singla,H.Toda,M.Monje,T.D.Palmer,R.C Malenka Excitation-Neurogenesis Coupling in adult neural stem/progenitor cells. Neuron 42, (2004)
Duval N., D.Gomes, V.Calabrese, A.Calabrese, P.Meda, R.Bruzzone Cell coupling and Cx43 expression in embryonic mouse neural progenitor cells. J.Cell Science 115, 3241-3251 (2002)

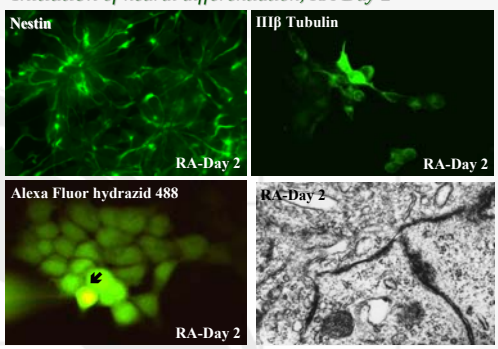
Retinoic Acid (RA) induced neuronal differentiation

Non-induced NE-4C progenitor cells



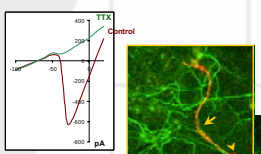
Non-induced NE-4C progenitors express several neuroectodermal stem cell markers: SSEA-1, nestin and are continuously proliferating. The cells are gap junction coupled as it can be detected by dye spreading. The cells were loaded with dyes either electrophysiologically (Alexa Flour Hydrazid 594) or by previous dye uptake (DiI & calcein) in a separate culture. The presence of gap junctions were also demonstrated by electron microscopy.

Initiation of neural differentiation, RA-Day 2

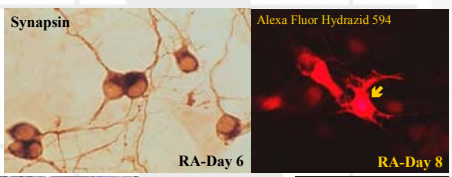


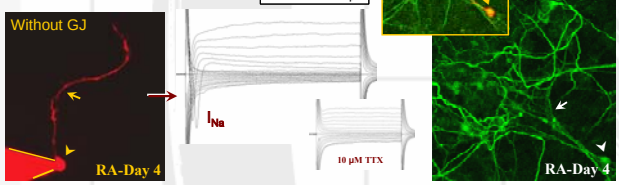
At the beginning of induction, cells arrange themselves into compact aggregates. The majority of cells are still proliferating. The first IIIβ tubulin cells appear within the aggregates. The cells within the clusters, remained coupled and form gap junctions.

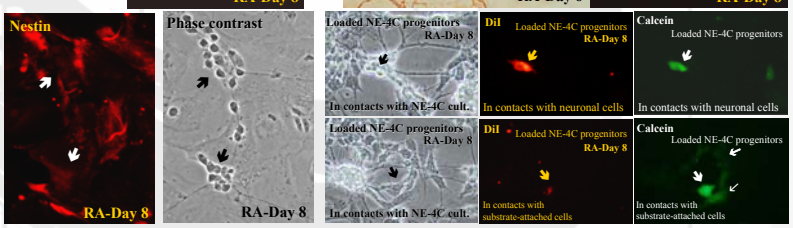
Maturating NE-4C neurons



Coupled	Non-coupled cells	
36	2	Number of rec. cells
0 %	0 %	Na current [%]
45	9	Number of rec. cells
11,1 %	33,3 %	Na current [%]
22	36	Number of rec. cells
9,1 %	72,2 %	Na current [%]
5	35	Number of rec. cells
0 %	100 %	Na current [%]







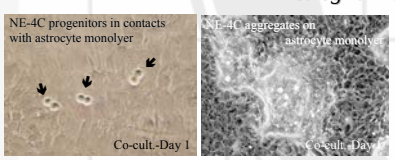
After the onset of RA-induced differentiation, heterogeneous populations of cells developed in the cultures. Neuronal precursors displaying IIIβ tubulin immunoreactivity (green) and TTX sensitive Na currents (I_{Na}), cease gap junction coupling.

Gap junction coupling is retained between morphologically non-differentiated cells, which do not show neuron specific current patterns (arrows: Alexa Fluor Hydrazid 594 loaded cells)

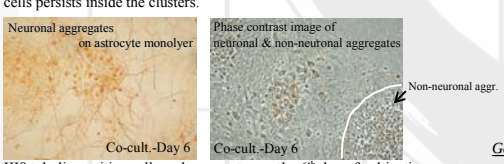
On the 8th day of RA induced differentiation, a subpopulation of cells displayed differentiated, neuronal features. Besides several neuronal markers as IIIβ tubulin, NF, MAP2, NeuN, these cells express presynaptic proteins like synapsin, and display neuron specific currents. Coupling have been never detected between such cells..

Substrate-attached cells and some morphologically non-differentiated cells, express nestin filament and are coupled but exlusively with non-differentiated cells.

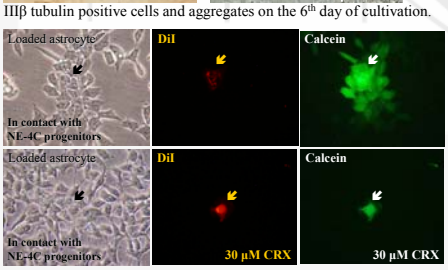
Astroglia induced neuronal differentiation



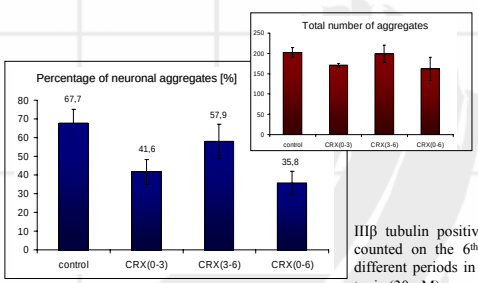
Initially, individual NE-4C cells form aggregates, as a result of local proliferation. Neurons are formed within the aggregates, but a significant amount of non-differentiated cells persists inside the clusters.

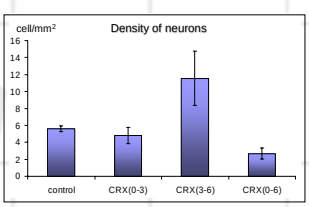


IIIβ tubulin positive cells and aggregates on the 6th day of cultivation.



30 μM CRX blocks the dye spreading between a single astrocyte (loaded with DiI and calcein) and non-induced NE-4C cells.





IIIβ tubulin positive cells, neuronal and non-neuronal aggregates were counted on the 6th day of co-culturing. The cultures were grown for different periods in the presence of CRX gap junction inhibitor at a non-toxic (30 μM) concentration.

Conclusion

Gap junction communication by NE-4C neuroectodermal stem cells

Retinoic acid induced neuronal differentiation

1. NE-4C neural stem cells communicate with each other via gap junctions.
2. With the advancement of neuronal development, differentiating cells cease gap junction communication with neighboring cells.
3. Gap junction coupling persists between substrate-attached, non-differentiated cells.

Astroglia induced neuronal differentiation

4. Astrocytes take an active part in the regulation of neurogenesis and gap junction communication plays an important role in the process.
5. Gap junctions are readily formed between stem cells and astrocytes upon co-plating.
6. Neuron formation occurs inside the homotypic aggregates of NE-4C cells
7. Blockage of gap junction communication interferes with the neuron formation, depending on the developmental stage of NE-4C cells.

- Blockage of gap junction communication during the initial period (0-3 days) of astrocyte induced neuron formation, when the majority of NE-4C cells are still proliferating, reduces both the number and size of neuronal aggregates.
- Blockage of gap junction communication in the later phase (3-6 days) of astrocyte induced differentiation, when neuronal maturation of committed NE-4C precursors takes place, results in an increase in the number of neurons.

The observations indicate that communication through gap junctions supports the maintenance of the proliferative, progenitor state of NE-4C neuroectodermal cells.