

Expression of neurogenic and region specific genes during the in vitro induced neurogenesis of NE-4C cloned neuroectodermal cells

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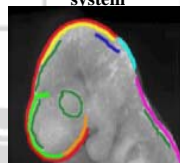


Neural stem cells keep their regional commitment in vitro

•Hitoshi S, Tropepe V, Ekker M, van der Kooy D. Neural stem cell lineages are regionally specified, but not committed, within distinct compartments of the developing brain. *Development*. 2002 Jan;129(1):233-44
 •Yasushi Nakagawa, Tadahiro Kaneko, Toshihiko Ogura, Taiga Suzuki, Masaaki Torii, Kozo Kaibuchi3, Ken-ichi Arai, Shun Nakamura and Masato Nakafuku Roles of cell-autonomous mechanisms for differential expression of regionspecific transcription factors in neuroepithelial cells. *Development* 122, 2449-2464 (1996)

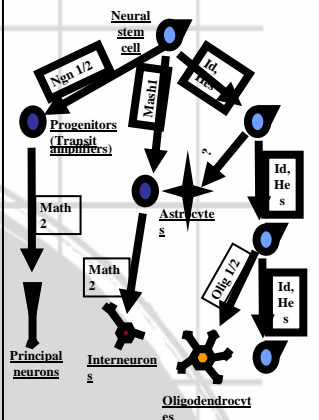
What sort of correlations exist in the expression of region specific and neurogenetic genes during neuronal differentiation?

Region specific gene expression in the developing central nervous system



- Dlx2 (distal-less homeobox 2) Ventral forebrain
- Emx2 (empty spiracles homolog 2) Dorsal forebrain
- Pax6 (paired box gene 6) Forebrain, Hindbrain
- Otx2 (orthodenticle homolog 2) Forebrain, Midbrain
- Otx3 (orthodenticle homolog 3) (Dmbx1) Forebrain, Midbrain
- En1 (engrailed1) Midbrain
- Gbx2 (gastrulation brain homeobox 2) Midbrain
- Hoxb2 (homeo box B2) Hindbrain

Role of bHLH transcription factors in neural cell fate determination



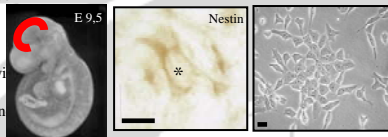
NE-4C a neuroectodermal stem cell line

- Cloned from culture of forebrain/midbrain vesicle of E9.5 p53^{-/-} mouse embryo

-Self renewing

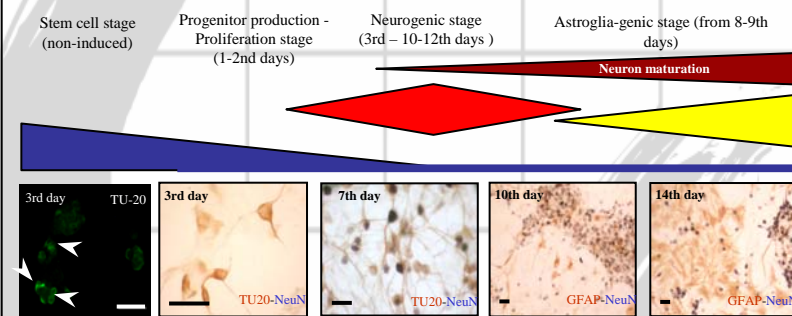
-Nestin and SSEA-1 immunoreactivity

-Potential to give rise to neurons and astroglial cells in vitro and in vivo

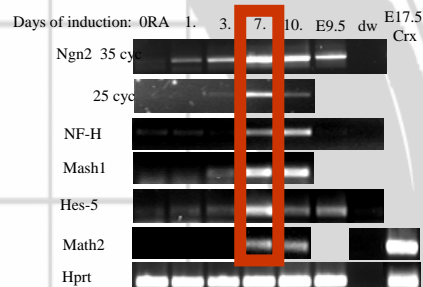


(Schlett K., Madarász E. *J Neurosci Res.* 47: 405-415 (1997). * Schlett K, et al. *Int J Dev Neurosci.* 15: 795-804 (1997). * Herberth B et al. *Neurosci Res.* 2002 Mar 1;67(5):574-82. * Jelítai M., et al. *J Neurobiol.* 2002 Apr;51(1):54-65. * Tárnok K., et al. *Eur. J. Cell Biology* 2002, July 81, 403-412 * K. Demeter, B. et al. *Experimental Neurology* 188 (2004) 254-267)

Stages of the retinoic acid (RA) induced neuronal differentiation of the NE-4C cell line



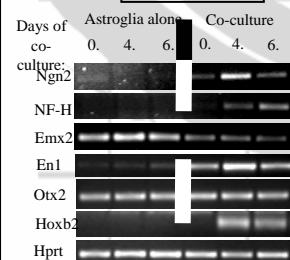
Change of the neurogenic gene expression during retinoic acid induced neuronal differentiation of the NE-4C cell line



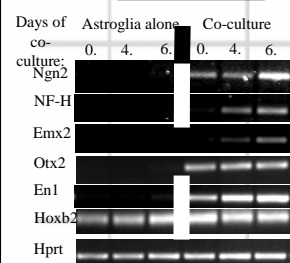
Stages of the astroglia induced neuronal differentiation of the NE-4C cell line in coculture

Stem cell stage (non-induced) Proliferation stage (1-3rd days) Neurogenic stage (from 3-4th days)
 (In details: Környei et al. ISDN congress 2004 Edinburgh)

Forebrain astroglia



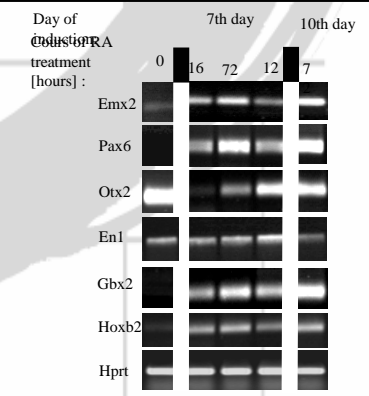
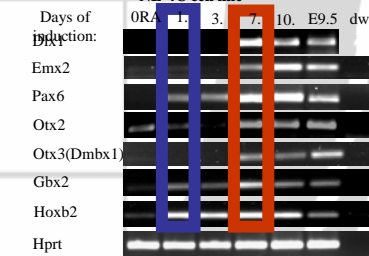
Hindbrain astroglia



Conclusions

- All trans retinoic acid can induce two pathways for neuronal differentiation of NE-4C neuroectodermal cells as it is indicated by the expression of both neurogenins and Mash1, specific for dorsal and ventral fates of anterior CNS neurons, respectively.
- The Pax6, Hoxb2 and Gbx2 region specific genes are induced at an early, progenitor stage of the in vitro neuronal differentiation suggesting some roles for these genes in the commitment of the neuronal fate.
- The elevation in the levels of Emx2, Dlx1, Otx3 transcripts coincides with a later state of neuronal development when the majority of postmitotic precursors is generated.
- The investigated region specific genes displayed highly different responsiveness to RA:
 - * Otx2 is repressed soon after addition and throughout the presence of RA
 - * The expression of Pax6, Hoxb2 and Gbx2 is upregulated soon after addition of RA and the elevated expression persists after the withdrawal of RA
 - * The "posteriorising" effect of all trans retinoic acid did not repress the expression of Emx2, Dlx1 and En1 genes.
- Astrocytes obtained from different (forebrain or hindbrain) regions maintained their regional specificity as it was indicated by the expression of Otx2, Emx2 by forebrain glia and Hoxb2b by hindbrain astrocytes. The regional differences did not influence the neuron-inductive capacity of glia cells.
- In co-cultures with astrocytes, NE-4C cells maintained their Otx2 and En1 expression, regardless of the regional specificity of astrocytes.
- In the course of astroglia-induced neurogenesis, Hoxb2 and Emx2 is activated at defined stages of neuron formation, regardless of the regional specificity of astrocytes.

Changes in region specific gene expression during retinoic acid induced neuronal differentiation of the NE-4C cell line



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