“Development of Neural and Neural Crest Progenitor Cells from Human Pluripotent Stem Cells”

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Characteristics of Human Embryonic Stem Cells

- Distinct morphology
- Specific transcription factors and cell surface epitopes
- Usually maintain normal karyotypes
- Three embryonic germ lineage differentiation

Human Embryonic Stem Cells

4-cell Morula Blastocyst Gastrulation

1981
Evans MJ and Kaufman MH. Nature

1998
Martin GR. PNAS

Thomson JA, et al. Science

Ethical objections
Face immune rejection after transplantation

So what’s the problem?

1. Ethical objections
2. Face immune rejection after transplantation

Discovery of Induced Pluripotent Stem Cells

The Shared Nobel Prize in Physiology or Medicine 2012 “for the discovery that mature cells can be reprogrammed to become pluripotent”

Patient Specific Induced Pluripotent Stem Cells

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

differentiated donor cells

ips cells

Pluripotency induction

repaired ipS cells

Adult cells
Signals to Stem Cells

Matrix Molecules

Soluble Factors

hPSCs

Self-Renewal

Differentiation

Other Cells


Early Neural Tissue Development

BMPs

BMPR- II

BMPR- I

Nucleus

SMAD4

SMAD5

DNA-BPs

Id1,2,3

Neural Differentiation of hESCs

Noggin

Dorsomorphin

hESCs

Neural progenitors

Noggin

Noggin

TGF/EGF

hESCs

Time in differentiation:

0

1 wk

2-3 wks

4-5 wks

hESC-derived neural progenitor cells express markers for early embryonic neural stem cells – radial glial cells

Neural Differentiation of hESCs

GABAergic neurons, Glutaminergic neurons, Glial cells

Neural Differentiation of hESCs

GABAergic neurons, Glutaminergic neurons, Glial cells

Differentiation Potential of hESC-Derived Neural Progenitors
Neural Crest Development

Neural Crest Differentiation from hPSCs

Specificity of Neural Crest Differentiation Protocol

Efficient Protocol to Derive Neural Crest Progenitors

Neural Crest Progenitors are Multipotent

Activation of FGF8 and Inhibition of Notch Induce Migratory NC Phenotypes
Inhibition of Notch Induces NC-Derived Neurons

Peripheral neurons

Peripheral sensory neurons

Autonomic neurons

To sum up...

- Neural and neural crest stem cells could be efficiently derived from hESCs.
- Both neural and neural crest stem cells could be precursors for various types of neurons
- Robust and efficient protocol for the derivation of premigratory neural crest-like cells (pNCCs)
- pNCCs are multipotent and able to generate neural crest derivative cells, including peripheral sensory neurons.
- Modulation specific signaling pathways could induce migratory neural crest cells and possible other neural crest derivatives.

Notch and Neural Crest Differentiation

hPSCs → OCT4, NANO→ NCCs → Notch, NGN2, NGN3

FGFR

Migratory NC cells

Brn3a, Phox2b

Neural crest-derived neurons

Challenging of Neural Crest Differentiation

Acknowledgments

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Dr. Ras Trokovic
Dr. Kirmo Wartiovaara
Eila Korhonen

END
### Modeling neurological disorders by using iPSCs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target cell</th>
<th>Successful differentiation into target cells?</th>
<th>Neurological pathology</th>
<th>Drug test</th>
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<tbody>
<tr>
<td>Fragile X syndrome</td>
<td>ND</td>
<td>ND</td>
<td>Loss of FMR1 expression</td>
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<tr>
<td>Prader-Willi syndrome</td>
<td>Neuron</td>
<td>Yes</td>
<td>Imprint disorder</td>
<td>ND</td>
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<td>Rett syndrome</td>
<td>Neuron</td>
<td>Yes</td>
<td>Loss of response, neural spine density,</td>
<td>ND</td>
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<tr>
<td>Familial dysautonomia</td>
<td>Neural crest cells</td>
<td>Yes</td>
<td>Loss of neural crest cells</td>
<td>ND</td>
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<tr>
<td>Friedreich's ataxia</td>
<td>Motor neuron</td>
<td>Yes</td>
<td>ZNF217 gene expression</td>
<td>ND</td>
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<tr>
<td>Huntington's disease (HD)</td>
<td>Neuron</td>
<td>Yes</td>
<td>Imprint disorder</td>
<td>ND</td>
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<tr>
<td>Parkinson's disease (PD)</td>
<td>Neuron</td>
<td>Yes</td>
<td>Loss of neurones, formation, loss of QAP2 gene expression</td>
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<tr>
<td>Spinal muscular atrophy</td>
<td>Motor neuron</td>
<td>Yes</td>
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<td>ND</td>
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</table>

**Semi-autonomous disorders**

<table>
<thead>
<tr>
<th>Neurodegeneration</th>
<th>Target cell</th>
<th>Successful differentiation into target cells?</th>
<th>Neurological pathology</th>
<th>Drug test</th>
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<tbody>
<tr>
<td>Synucleinopathies</td>
<td>Motor neuron</td>
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<td>Parkinson's disease (PD)</td>
<td>Neuron</td>
<td>ND</td>
<td>Not shown</td>
<td>ND</td>
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<tr>
<td>Alzheimer's disease (AD)</td>
<td>Neuron</td>
<td>ND</td>
<td>Increase risk of Aβ2 to Aβ42</td>
<td>Yes</td>
</tr>
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</table>

**ND** not determined.