Of mice, chicken and human induced pluripotent stem cells: studying midbrain dopaminergic neuron development and survival in the context of Parkinson’s Disease

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Human midbrain dopaminergic (mDA) systems

1. Mesostriatal
   - motor control
   - Parkinson’s Disease (PD)

2. Mesocortical
   - cognition
   - Schizophrenia

3. Mesolimbic
   - motivation/reward
   - Addiction
mdDA neuron development in the mouse

Ang, Development 2006
Wurst and Prakash, DA Handbook 2010

Specification

Induction

Terminal differentiation / survival

OTX2, LMX1A/B, MSX1/2, NGN2, MASH1, EN1/2, ALDH1A1
LMX1A/B, NGN2, NURR1, EN1/2, ALDH1A1, TUBB3
LMX1A/B, NURR1, EN1/2, PITX3
TH, AADC, ALDH1A1, TUBB3

mdDA progenitor
mdDA precursor
mdDA neuron

KCND3+
VTA DA neuron

CALB1+

SNC DA neuron

Wnt1
Th+ neurons

E12.5
Midbrain
Forebrain
Hindbrain
MHB
p1
p2
p3
PSC-based approaches to DA-associated neuropsychiatric diseases
PD is an age-related progressive neurodegenerative disease

after Le et al., The Neuroscientist 2009

Age

Dopaminergic Function

mdDA progenitor → mdDA precursor → mdDA neuron

Severity of Disease

Symptoms
PD is an age-related progressive neurodegenerative disease

- **PITX3** polymorphisms: sporadic and early-onset PD (Fuchs et al., 2009; Bergman et al., 2010; Le et al., 2011; Haubenberger et al., 2011; Guo et al., 2011)
- **NR4A2** polymorphisms/mutations: sporadic and familial PD (Le et al., 2003; Zheng et al., 2003; Xu et al., 2002; Grimes et al., 2006)
- **EN1** polymorphisms: sporadic PD (Fuchs et al., 2009; Haubenberger et al., 2011)
- **FGF20** polymorphisms: familial and sporadic PD (van der Walt et al., 2004; Mizuta et al., 2008; Wang et al., 2008; IPDGC & WTCCC2, 2011; Pan et al., 2012)
Genetic mouse models
WNT1 controls two different steps in the generation of mdDA neurons \textit{in vivo}

- **Induction**
  - E9.5-10.5
  - 5-HT neurons
  - Nkx2-2
  - Gbx2
  - Otx2

- **Specification**
  - E11.5-Adult
  - WNT1
  - SHH

- **Terminal Differentiation**
  - mdDA progenitor domain
  - mdDA neurons
  - Pitx3

**WNT/β-catenin signaling**

- Joksimovic et al., Nat Neuroscience 2009
- Tang et al., Development 2009
- Tang et al., J Neurosci 2010

References:

- Prakash et al., Development 2006
- Omodei, et al., Development 2008
- Di Giovannantonio, et al., Dev Biol 2013
Chicken *in ovo* electroporation

*Huber et al., J Vis Exp 2013*
mdDA neurons appeared at the tetrapod transition during evolution

Terrestrial | Amniotes
Aquatic | Amamniiotes

modif. Marín et al., TINS (1998)
Differences in the spatiotemporal expression profile of mdDA neuron markers between chicken and mice

Klafke et al., Development 2016
Differences in the genetic regulation of mdDA neuron markers between chicken and mice

A pathway involving Wnt9a, Lmx1a, Shh, and Pitx3 is shown for E3.5 chicken, while B shows a similar pathway for E11.5 mouse.
Species-specific (evolutionary) similarities and differences in mdDA neuron development

1. Similarities:
   - Chicken and mice transcription factor (Nr4a2, Pitx3) and enzyme (Aldh1a1, Th) gene sequences are very conserved and the corresponding proteins most likely have the same biochemical functions.

2. Differences:
   - Transcriptional (and posttranscriptional/posttranslational?) regulation and epistatic relationships of these genes/signaling pathways have diverged between chicken and mice.
   - Consequently, the spatiotemporal expression patterns of these genes in the brain differ considerably between chicken and mice.

Developmental (preclinical) studies in animal models may have only very limited translational value for the human situation.
Genetic mouse models
Differences in gene regulatory signaling levels for mdDA neuron generation between mice and humans?

Similar gene expression:

La Manno et al., Cell 2016  
Pertek et al., Mol Biotechnol 2014  
Fukusumi et al., J Neurosci 2015
~51% of the transcripts enriched in the WNT-responsive mdDA domain at E12.5 encode ion channel, receptor and transporter proteins.
Differences in the spatiotemporal expression profile of activity-related mdDA neuron genes between mice and humans?

Distinct gene expression:

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La Manno et al., Cell 2016
Species-specific (evolutionary) similarities and differences in mdDA neuron development

1. Similarities:
   - WNT1/b-catenin signaling appears to be crucial for mdDA neuron generation in mice and humans.
   - Spatiotemporal expression patterns of critical components and target genes of this signaling pathway are at least similar if not identical in mice and humans.

2. Differences:
   - Human mdDA neuron development appears to require a much higher WNT/b-catenin signaling dosage compared to mouse.
   - Spatiotemporal expression patterns of electrophysiological activity-related and potential WNT/b-catenin target genes appear to differ between mice and humans (?)

Assessment of gene expression patterns, regulatory (signaling) pathways and physiological aspects in the human condition (stem cells and tissues) appears mandatory for any translational approach in human mdDA neuron development.
Human iPSCs
Calcium activities in mdDA neuron development: DACaION

Fukusumi et al., J Neurosci 2015

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1. Despite (very) similar sequences and structures as well as biochemical functions of many mdDA neuron-associated genes and/or proteins among more or less closely related animal species, crucial (post-?) transcriptional and/or (post-?) translational regulatory and/or physiological processes are/may be different!
   - Wrong conclusions about genetic, epistatic and physiological impacts on phenotypic outcome.

2. Analyses of human organs, tissues and/or cells thus appear mandatory to draw the right conclusions required for any therapeutic approach to human disease.
   - Organ/tissue/cell availability?
   - Ethical and legal issues?

3. Can organs, tissues and/or cells replace an entire organism on the systemic level?
   - Emergent properties of a whole organism/body compared to single organs, tissues, cells.
   - Exhaustive efficacy and safety (preclinical) testing of potential therapeutic agents: possible in just cells, tissues, organs?
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               Ellen Euchner
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               Olga Rempel

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Deutsche Forschungsgemeinschaft

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