Herinneringen Annual Meeting

IPSCs based model for understanding the environmental toxin's effect on sporadic Alzheimer

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Introduction

In Alzheimer's disease (AD), the number one cause of dementia worldwide, progressive loss of neurons in the hippocampus and cortex causes a progressive decline in cognitive and behavioral functions. In AD brain (Aβ)-42 plaques and phosphorylated TAU plaque can be found. Early onset AD (<10%) is caused by inherited mutations in for instance APP or PSEN1/2, while the more frequent late-onset AD, also called sporadic AD (sAD) is associated with risk factors, such as the presence of the APOE4 allele or mutations in inflammation coupled genes, or without known link to genetic mutations. It is believed that toxins from the environment or inflammation contribute to the development of AD.

To model the effect of toxins or inflammation in AD development, animal models can be used; or recently also induced pluripotent stem cells (iPSC). In 'Herinneringen', iPSC obtained from sAD patients will be differentiated to cortical neurons, which will be exposed to environmental toxins (Cu^{++} and Fipronil sulfonate) or inflammatory cytokines (IFNγ, IL1β and TNFα), and the effect of these toxins/cytokines on the expressed transcriptome as well as soluble miRNAs evaluated. If specific gene networks and/or miRNAs are seen deregulated (also in the brain of mice exposed to the same toxins; and primary human brain or CSF samples), follow up studies will determine the role of these deranged pathways in neurodegeneration.

Methods

Timeline for Cortical neuron differentiation

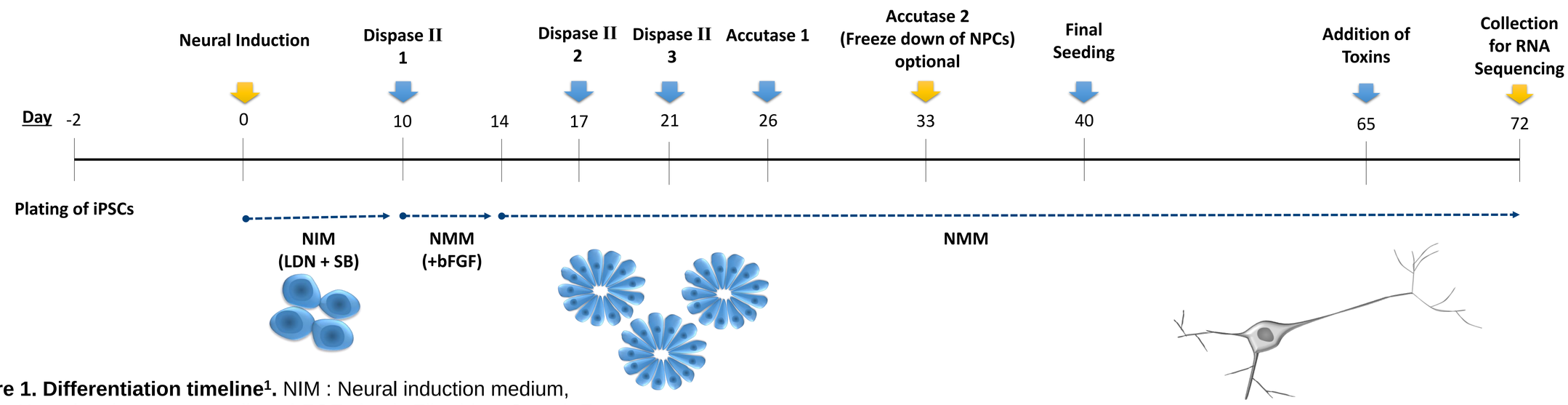

Figure 1. Differentiation timeline[1]. NIM : Neural induction medium, NMM : Neural maintenance medium, bFGF : basic Fibroblast Growth Factor

Different stages of cortical neuron differentiation

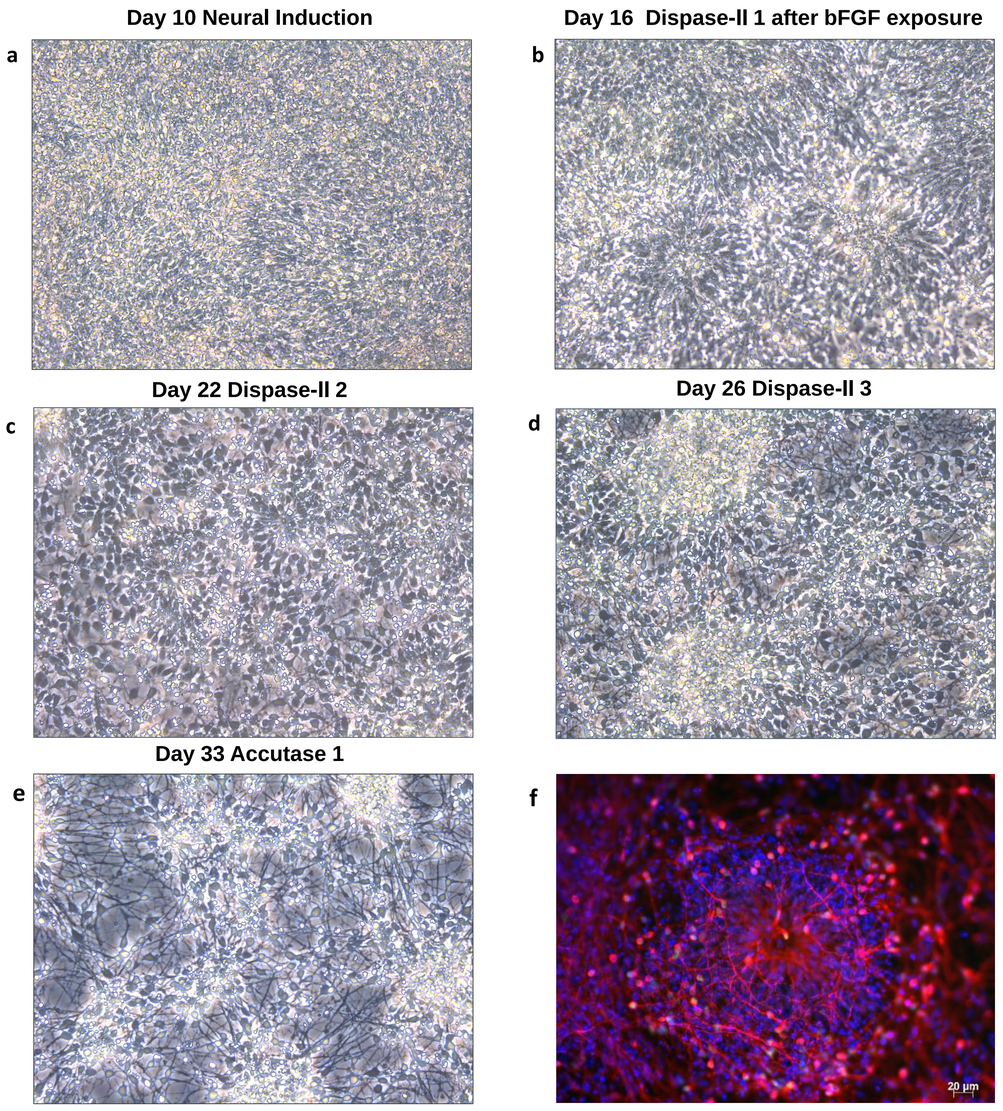

Figure 2. Cell morphology : **a**. Cells at Day 10 of differentiation before dispase II **b**. Cells at Day 16 of differentiation 4 days after exposure to bFGF. **c**. Cells at Day 22 of differentiation after 2nd dispase II step. **d**. Cells at Day 26 of differentiation after 3rd dispase II step. **e**. Cells at Day 33 of differentiation after 1st Accutase step. **f.** Immunostaining image of cortical neuron with Tuj1 (red) and Ctip2 (green).

IPSCs lines used in the project

Herinneringen code	Line name	Age of donor	Sex of donor
Group 1: Aged individuals with no AD (between 70 and 90 years of age)			
HER-01	HPSI0115i-zihe_1	75-79	Female
HER-02	HPSI0214i-rayr_1	75-79	Male
HER-03	UCSD223i-NDC1-1	86	Male
HER-04	SCRP6007i	88	Female
Group 2: Patient with sporadic AD (between 70 and 90 years of age)			
HER-06	UCSD234i-SAD2-3	83	Male
HER-07	AD8K213 (RIKEN HPS0258)	76	Male (not yet available)
HER-08	AD-iPS5	82	Female (not yet available)
HER-12	CW50119	84	Male (ordered, MTA OK)
HER-13	CW50120	82	Male (ordered, MTA OK)
HER-14	CW50133	87	Female (ordered, MTA OK)
HER-15	CW50165	78	Female (ordered, MTA OK)
Group 3: Patient with known (ApoE4 / 4)			
HER-09	iPSC APOE4/4 individual	75	Male
HER-10	TAU 3x mutant line	24	Male
HER-11	Cellectis ChiPSC6B	24	Male
Group 4: Individual with protective (Islandic) APP mutation (A673T, c.2017G>A)			
HER-05	01279.A27 knock-in	Unknown	Male (not available as iPSC)

Treatment and collection of samples

Treatment for 7 Days – Alternate day medium collection for analysis

Toxins

- Copper(II) chloride.
- Fipronil Sulfonate .
- Cytokines Mix (IL1β, TNFα, IFNγ).

Analysis from medium and cell pellet

- Total TAU measurement by ELISA.
- Aβ protein measurement.
- Micro RNA.
- mRNA from cell pellet.

Future perspective

By comparing the transcriptome as well soluble miRNAs in cortical neurons derived from healthy, sAD and patients with familial AD (with genetic risk factors) exposed to environmental risk factors, with those in aged mouse brain and CSF following exposure to the same toxins, and with those of brain and CSF of sAD patients, we will uncover candidate transcriptional pathways and/or regulatory miRNAs that may underlay sAD.

Reference 1: Adapted protocol from Shi, Y. *et al*. (2012). Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nature Protocols*, 7(10), 1836-46,