Identification of cell-autonomous microglia phenotypes in an induced pluripotent stem cell model of Huntington's disease

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Background

- Huntington's disease (HD) is a severe neurodegenerative disorder caused by a dominantly inherited CAG trinucleotide repeat expansion in the huntingtin gene (*HTT*). [1]
- As in other neurodegenerative diseases, neuroinflammation is a prominent sign of HD pathology. [2]
- Several positron emission tomography (PET) studies have demonstrated that microglial activation correlates with disease severity in HD patients and can be detected up to 15 years before predicted age of onset. [3,4]
- We are the first to investigate HD pathophysiology in microglia differentiated from human induced pluripotent stem cells (iPSCs).

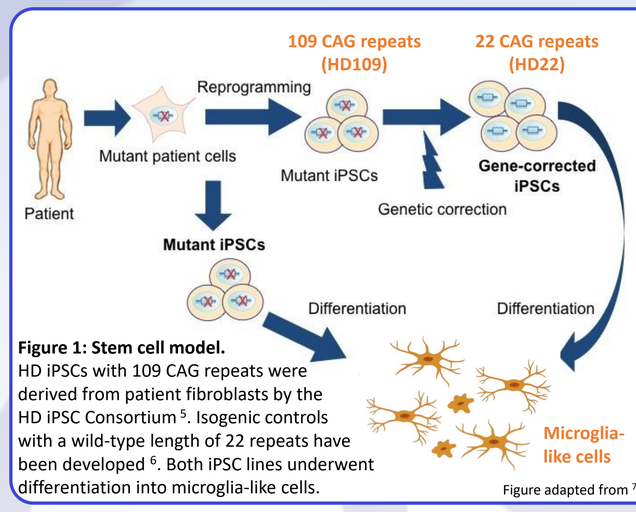

Figure 1: Stem cell model. HD iPSCs with 109 CAG repeats were derived from patient fibroblasts by the HD iPSC Consortium [5]. Isogenic controls with a wild-type length of 22 repeats have been developed [6]. Both iPSC lines underwent differentiation into microglia-like cells.

Figure adapted from [7]

Development of tools and assays to address the following research questions:

- Does the mHTT protein affect microglia morphology and function in a cell-autonomous manner?
- Do microglia have a direct effect on HD pathophysiology?

Methods

- Differentiation of HD22 and HD109 iPSCs into microglia-like cells [8]

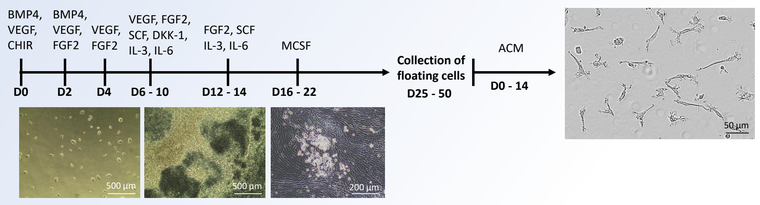

Figure 2: Microglia differentiation protocol. The first step of the differentiation promotes the formation of non-adherent primitive macrophages in the culture supernatant. Then these cells were harvested and differentiated to a microglial phenotype by the addition of astrocyte conditioned medium (ACM) for 14 days. D = day

- Immunostaining of microglia precursors (Figure 3 A) and microglia-like cells (Figure 3 C)
- Flow cytometry of microglia precursors (Figure 3 B)
- Analysis of microglia morphology using the IncuCyte S3 live-cell imaging system and IncuCyte S3 software (Figure 4 A, B)
- Phagocytosis assay using pHrodo red E. coli BioParticles in the IncuCyte (Figure 4 C-F)

Both HD22 and HD109 iPSCs differentiate into microglia-like cells

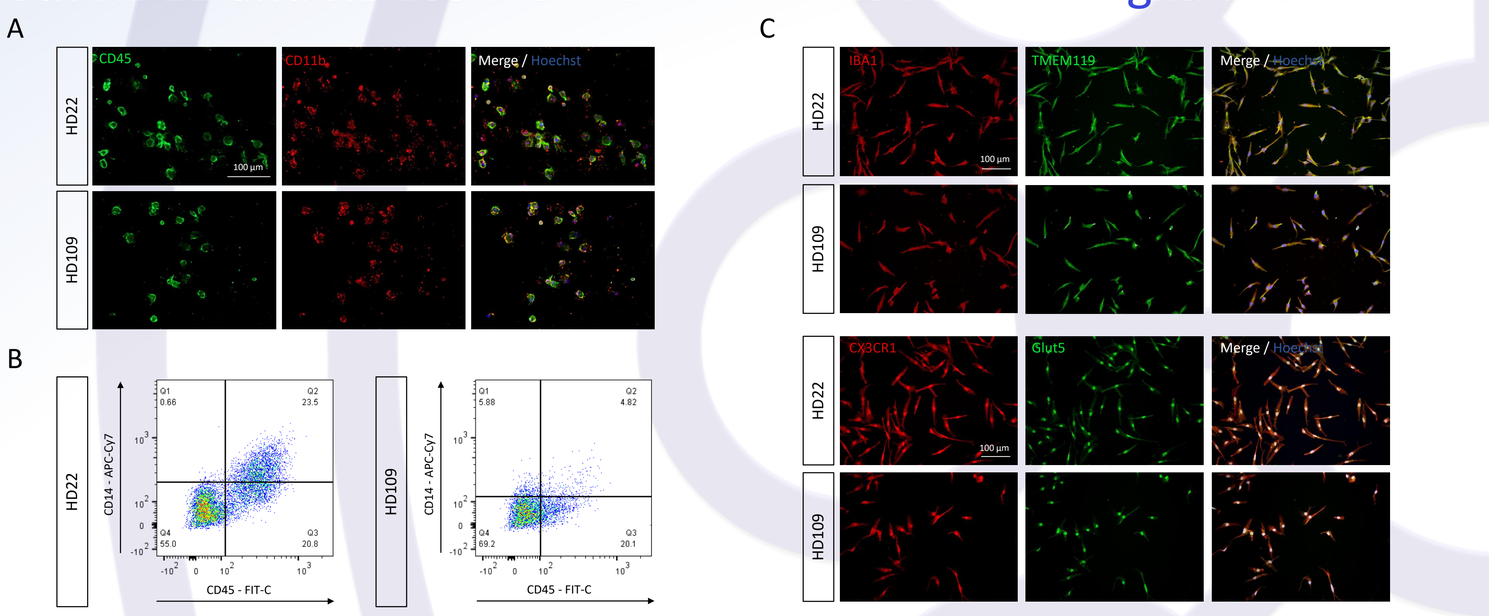

Figure 3: **Characterisation of iPSC-derived microglia precursors and microglia-like cells.** (**A**) Non-adherent microglia precursor cells were harvested from the differentiation, adhered to slides by cytospinning and immunoassayed for the myeloid lineage markers CD11b and CD45. Scale bar: 100 µm. (**B**) Precursor cells were stained for the surface markers CD14 and CD45 and underwent flow cytometry. (**C**) Microglia-like cells were immunoassayed for the microglia markers IBA1 and TMEM119, CX3CR1 and Glut5. Scale bars: 100 µm.

Preliminary functional analysis

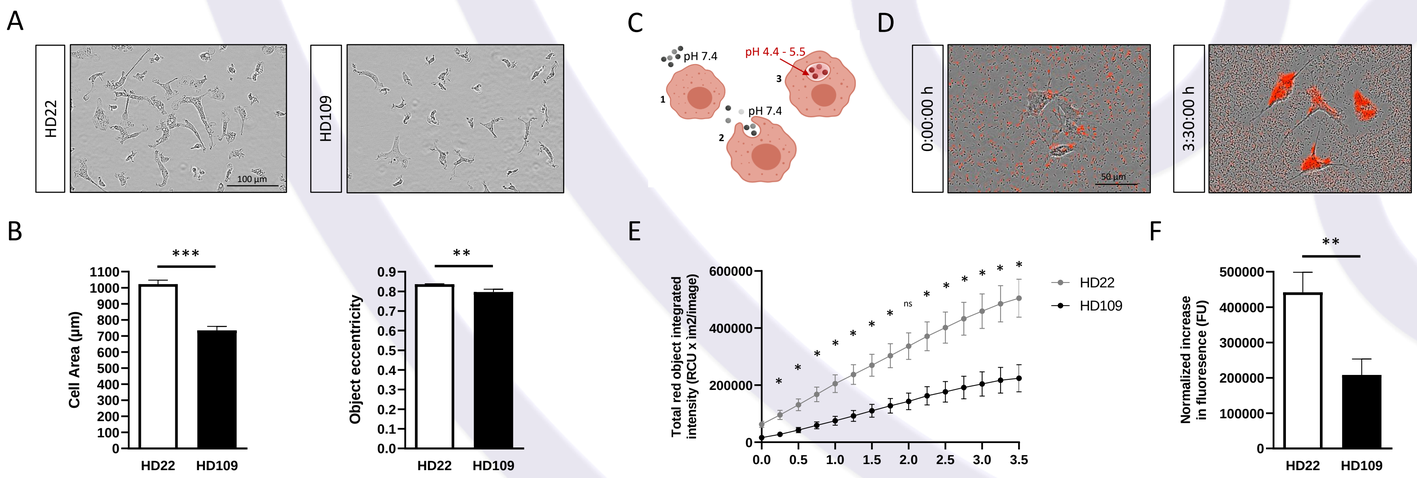

Figure 4: Morphological and functional analysis of iPSC-derived microglia-like cells. Incucyte S3 bright-field and red fluorescent live-cell imaging. (**A**) Representative images of D14 HD22 and HD109 microglia-like cells. Scale bar = 100 µm. (**B**) HD109 microglia-like cells exhibited significantly decreased cell complexity determined by decreased cell area and increased eccentricity. (**C**) Schematic overview of the phagocytosis of pHrodo red E. coli BioParticles. Graphic made using BioRender. (**D**) Representative images of the start (0.0h) and end (3.5h) point of the phagocytosis assay. Scale bar = 50 µm. (**E**) HD109 microglia-like cells phagocytose significantly fewer pHrodo conjugated E.coli beads within a timeframe of 3.5h than control cells. (**F**) Increase in fluorescence after 3.5h by the uptake of beads is significantly higher in HD22 compared to the HD109 microglia-like cells. Data are expressed as mean ± SEM. Statistical analysis was performed using two-way ANOVA with Sidak's multiple comparison test or unpaired Student´s t-test. D = day

Conclusions

- **Both, HD22 and HD109 iPSCs differentiate into microglia like-cells**
- **The initial observations suggest a cell-autonomous effect of mutant HTT on microglia morphology and activity and are consistent with previous human and mouse data [9]**
- **Thus these results support the use of human iPSC-derived microglia as a model to study neuroinflammation in HD**

References

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