Transfer of therapeutic miRNAs within extracellular vesicles secreted from HD iPSC-derived neurons

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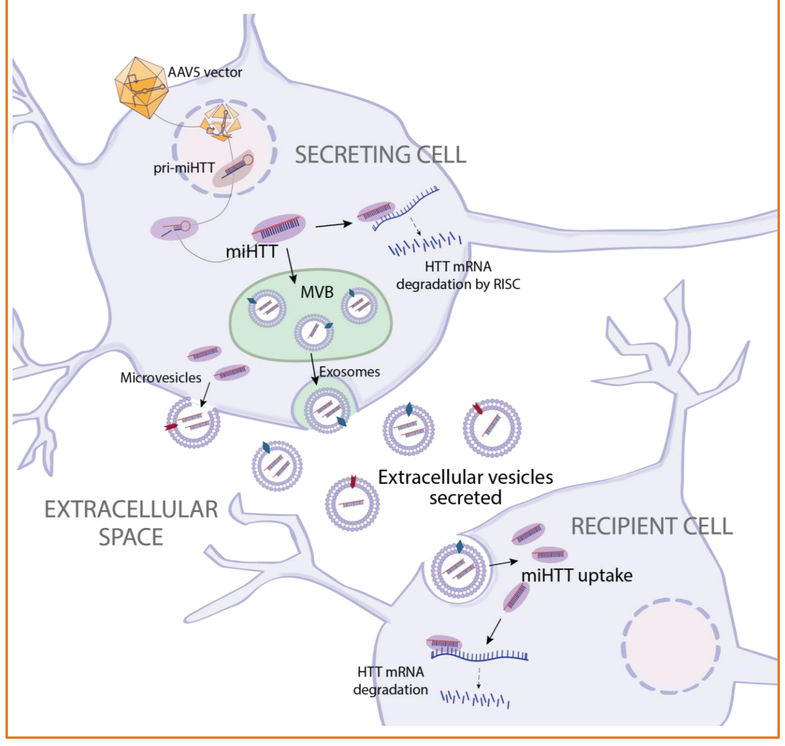
BACKGROUND

Huntington's disease (HD) is a neurodegenerative disorder caused by an autosomal dominant mutation in the huntingtin gene (*HTT*), which leads to mutant protein aggregation, toxicity and neuronal cell death. The huntingtin-lowering therapy developed by uniQure is based on an engineered micro(mi)RNA targeting HTT mRNA (miHTT)[1,2]. The expression cassette encoding the miHTT is delivered to brain cells using adeno-associated viral vector serotype 5 (AAV5-miHTT). AAV5-miHTT have demonstrated an efficient HTT lowering *in vitro* and *in vivo* in the brain of different HD animal models[3,4]. For adequate efficacy, wide distribution of therapeutic miRNAs to HD affected brain regions is of crucial importance.

Recently, extracellular vesicles (EVs), and in particular exosomes and microvesicles, have been identified as carriers of RNA species[5]. Therefore, understanding how therapeutic miRNAs are transferred between neuronal cells might be relevant for delivery, translational studies and biomarker discovery in gene therapies for brain disorders.

HYPOTHESIS: Transfer of AAV-delivered miHTT within extracellular vesicles



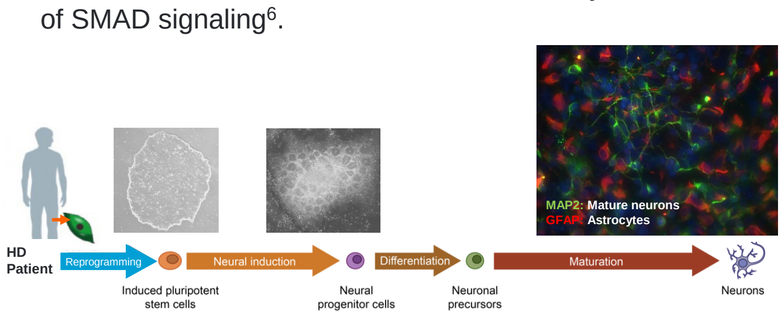
OBJECTIVES

- To investigate the **presence** of therapeutic miHTT molecules within EVs secreted from AAV5-miHTT-treated neuronal cultures from an HD patient.
- To analyze the **correlation** between secreted EV-associated miHTT levels and both viral input and cellular miHTT expression.
- To study the **transfer** of EV-associated miHTT molecules to recipient neuronal cells.

METHODS

Differentiation of HD patient iPS-derived neurons

- HD iPS cells (71 CAG repeats) were induced and further differentiated into frontal brain-like neurons by dual inhibition of SMAD signaling[6].

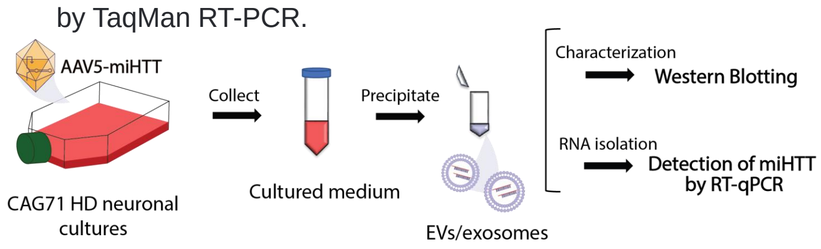


Transduction

- The HD iPS-derived neurons were transduced with AAV5-miHTT at different multiplicities of infection (MOI). Formulation buffer was used as control.
- At 20 days after transduction cells were harvested and DNA and RNA isolated. Viral genome copies (gc), miHTT and HTT mRNA expression were quantified by TaqMan qPCR. Ultra-sensitive single molecule counting assay anti-HTT 2B7 and MW1 to quantify human mutant HTT protein (IRBM).

EV precipitation from cultured medium

- At 5 and 12 days after transduction, 10 ml of cultured medium from transduced cells were collected and EVs precipitated by ExoQuick-TC™.
- EVs were characterized by Western blotting. RNA was isolated from EV pellets and miHTT levels were quantified by TaqMan RT-PCR.



RESULTS

Dose-dependent transduction and HTT lowering in HD patient iPS-derived neurons

- We observed dose-dependent levels of AAV5 transduction (**Fig. 4A**) and cellular miHTT expression (**Fig. 4B**).
- AAV5-miHTT treatment resulted in a dose-dependent lowering of HTT mRNA (**Fig. 4C**) and human mutant HTT protein (**Fig. 4D**)

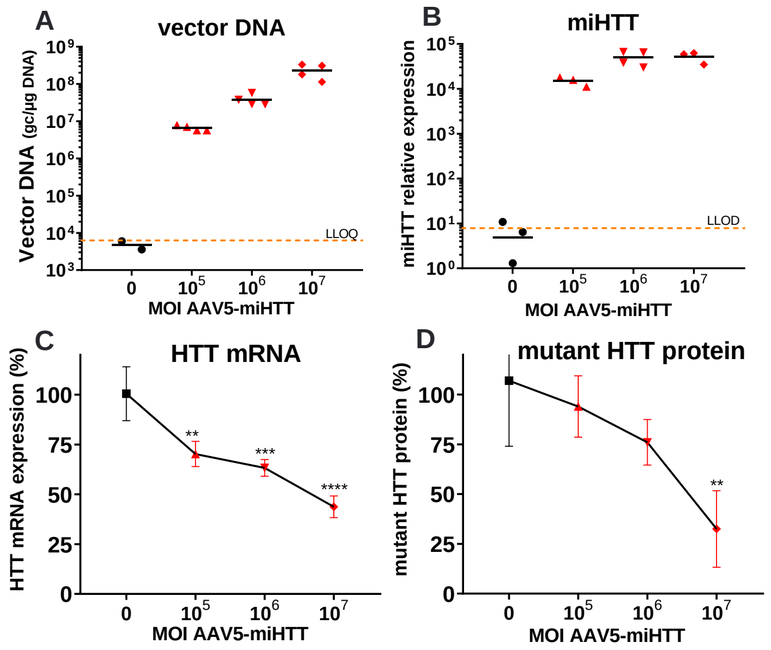


Fig. 4. Transduction and HTT lowering in HD iPS-derived neurons. ***A)*** *Vector DNA copies (gc/µg DNA).* ***B)*** *Mature miHTT expression relative to control, using U6 as reference gene.* ***C)*** *HTT mRNA expression and* ***D)*** *mutant HTT protein relative to control (* p<0.05, *** p<0.001, **** p<0.0001).*

miHTT molecules are secreted within EVs from AAV5-miHTT-treated neuronal cells

- We detected dose-dependent levels of mature miHTT molecules within EVs secreted from treated neuronal cells (**Fig. 5**).

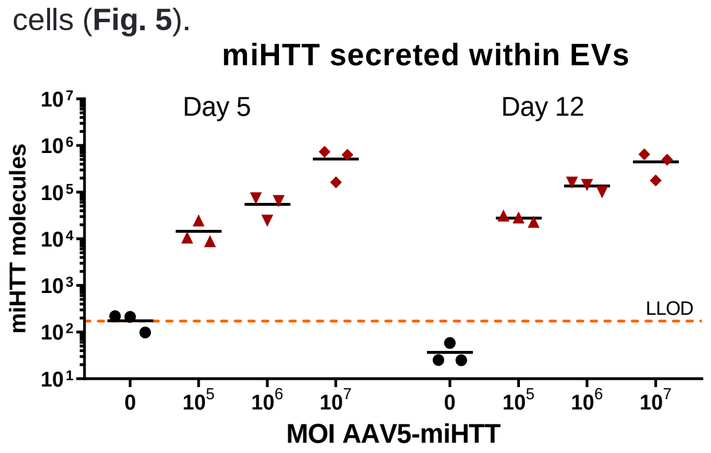


Fig. 5. Dose-dependent miHTT molecules secreted within EVs from AAV5-miHTT-treated neuronal cells. Mature miHTT molecules quantified by standard curve-based RT-qPCR, using miR-16 as reference gene.

Characterization of EVs by Western blotting

- Vesicles precipitated from cultured medium were positive for EV/exosomes, microvesicles and RISC-protein markers; and negative for cellular markers.

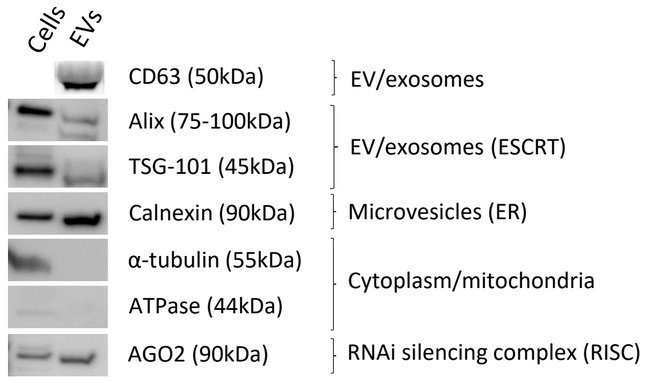


Fig. 6. Western blot characterization of EV/exosomes precipitated from cultured medium of HD patient neuronal cells. Cell lysates were used as positive controls.

EV-associated miHTT levels strongly correlated with viral dose and cellular miHTT expression

- Data from independent experiments showed robust correlations between extracellularly secreted miHTT molecules (EV-miHTT) and viral dose (**Fig. 7A**), and cellular miHTT expression (**Fig. 7B**).

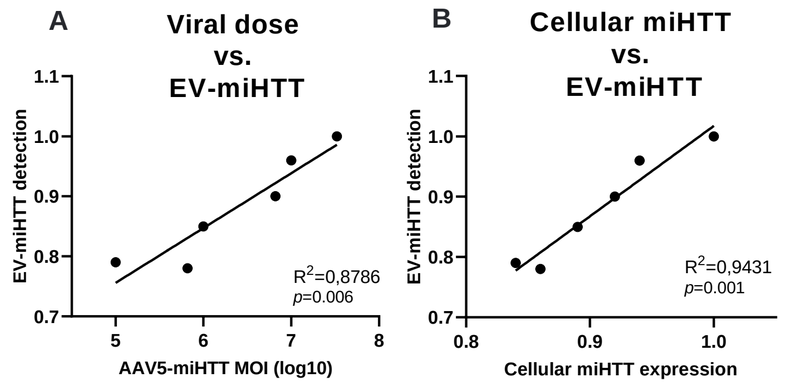
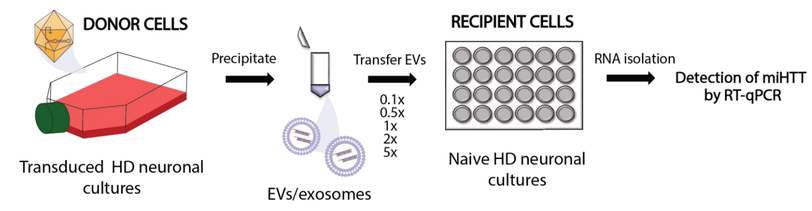


Fig. 7. Correlation analysis between viral dose, cellular miHTT and secreted EV-miHTT. Each dot represent the average of an independent experiment performed in triplicate (n=6). Cellular miHTT and EV-miHTT are showed as relative expression values.

Therapeutic miHTT molecules within EVs can be taken up by recipient neuronal cells

- EVs precipitated from cultured medium were pooled and transferred in different concentrations to recipient naïve HD neuronal cells.
- After 24h, RNA was isolated and uptake miHTT molecules were quantified by TaqMan RT-qPCR.



- Mature miHTT molecules were detected in recipient cells 24h after transfer of EVs. miHTT levels were increased with higher input of EVs (**Fig. 9**).

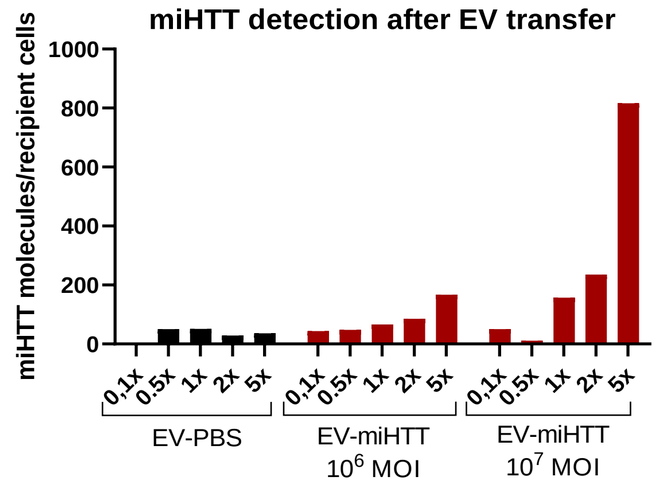


Fig. 9. Transfer of EV-associated miHTT molecules to recipient neuronal cells. Mature miHTT molecules transferred to recipient cells were quantified by RT-qPCR and compared to transfer of EVs precipitated from control cells (EV-PBS).

CONCLUSIONS

- AAV5-miHTT efficiently transduces HD patient iPS-derived neuronal cells and reduces human HTT mRNA and protein
- Therapeutic miHTT molecules are present in a dose-dependent manner within EVs secreted from AAV5-treated neuronal cells.
- Extracellular therapeutic miHTT levels secreted from treated cells strongly correlate with viral dose and cellular miHTT expression.
- Therapeutic miHTT molecules within EVs can be uptake by naïve HD neuronal cells in a dose-dependent manner.

Therapeutic miRNAs are secreted within EVs and transferred between neurons, contributing to non-viral distribution

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