SECRETED THERAPEUTICS: MONITORING DURABILITY OF AAV5-MiHTT GENE THERAPY IN HUNTINGTON DISEASE

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Background
Huntington disease (HD) is a fatal neurodegenerative disorder caused by an autosomal dominant mutation in the huntingtin gene (HTT), which leads to mutant HTT (mHTT) protein aggregation, toxicity and neuronal cell death. The HTT-lowering therapy developed by uniQure is based on an engineered microRNA targeting HTT mRNA (miHTT) and delivered by adeno-associated viral vector serotype 5 (AAV5-miHTT)1. AAV5-miHTT has demonstrated a long-term efficient HTT lowering in vitro and in vivo in the brains of different HD animal models after one-time infusion.2,3 The preclinical development of AAV5-miHTT is accompanied by translational challenges, and clinical biomarkers indicative of dosing and therapeutic efficacy in the central nervous system are very much needed.6

Recent studies show that extracellular vesicles (EVs) have been identified as carriers of RNA species, including microRNAs, which are secreted into biological fluids directly from cells.7 EV-associated microRNAs are becoming promising biomarkers for diagnosis and therapeutics in brain diseases.

Objectives
In this study, we investigated the potential use of EV-associated therapeutic microRNAs as suitable measurements to monitor the expression and durability of AAV-delivered therapeutic microRNAs in the brain.

- To study the secretion of therapeutic microRNAs from AAV5-miHTT-treated neurons.
- To characterize the association of therapeutic microRNAs with EVs and soluble proteins.
- To investigate the longitudinal detection of microRNAs in cerebrospinal fluid (CSF) of non-human primates after one-time AAV5-miHTT brain infusion.

Methods
Differentiation of HD patient iPSC-derived neurons
- Induced pluripotent stem cells (iPSCs) derived from an HD patient were induced and further differentiated into frontal brain-like neurons by dual inhibition of SMAD signaling (Fig. 2).2

Transduction of neurons and EV isolation
- The HD iPSC-derived neurons were transduced with AAV5-miHTT at different multiplicities of infection (MOI).
- 10 mL of cultured medium from transduced neuronal cells was collected and EVs were isolated by precipitation (ExoQuick-TC®), or size-exclusion chromatography (SEC).8

AAV5-miHTT treatment in non-human primates
Cynomolgus monkeys (Macaca fascicularis) were injected with formulation buffer or two doses of AAV5-miHTT locally in the caudate and putamen (100 µl/region, low dose 2x10¹¹ gc/brain and high dose 2x10¹² gc/brain) (n=8).

Results
Successful AAV5-miHTT transduction results in HTT lowering and EV-associated secretion of microRNA molecules
- AAV5 dose-dependent levels of cellular microRNA expression (Fig. 3A) and HTT protein lowering (Fig. 3B) were detected in iPSC-derived neurons.
- Therapeutic microRNA molecules were secreted in a dose-dependent manner from AAV5-treated neuronal cells at 5 and 12 days after transduction (Fig. 3C).

Characterization of EVs
- Vesicles precipitated from cultured medium were positive for EV and RNA-induced silencing complex (RISC-) protein markers by western blot (Fig. 4A) and visualized by transmission electron microscopy (TEM). Cell lysates were used as positive controls.

Therapeutic microRNA molecules are associated with EVs and soluble proteins
- Therapeutic microRNA molecules were detected in association with EVs and soluble proteins, efficiently separated by size-exclusion chromatography (SEC) (Fig. 5).

Intrastriatal injection of AAV5-miHTT results in widespread distribution of miHTT in the brain of non-human primates
- Non-human primates received a one-time intrastriatal infusion of AAV5-miHTT and CSF was collected at different time points (Fig. 6A).
- Widespread distribution of therapeutic microRNA molecules were detected across main brain areas (Fig. 6B).

Detection of therapeutic microRNA molecules in CSF confirms secretion and durability of one-time AAV5-miHTT therapy
- Dose-dependent mature microRNA molecules were detected in CSF EVs up to six months after intrastriatal injection in non-human primates (Fig. 7).

Conclusions
- AAV5-miHTT efficiently transduces HD patient iPSC-derived neurons, and results in HTT protein lowering.
- Therapeutic microRNA molecules are secreted in a dose-dependent manner from AAV5-treated neuronal cells in association with EVs, as well as soluble proteins.
- Therapeutic microRNA molecules are detected in CSF up to six months after one-time intrastriatal injection of AAV5-miHTT in non-human primates.

The detection of EV-associated microRNA molecules in CSF suggests this is a promising translational marker to monitor long-term expression of AAV5-miHTT therapy in the brain.

References