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Functional Characterization of AAV-TT

Nelly Jolinon[1], Antonette Bennett[2], Julie Tordo[1], Patrick Aldrin-Kirk[3], Adam Dyer[1], Tomas Björklund[3], R. Michael Linden[1], Mavis Agbandje-McKenna[2] and Els Henckaerts[1]

[1]Department of Infectious Diseases, School of Immunology and Microbial Sciences, King's College London, London, UK; [2]Department of Pediatrics, Powell Gene Therapy Center, University of Florida, Gainesville, FL, USA; [3]Molecular Neuromodulation, Wallenberg Neuroscience Center, Lund University, Lund, Sweden

In an attempt to address the generally limited biological activity of AAV vectors, our lab has used an evolutionary approach to design new AAV capsids. We introduced 14 amino acids (AA) that were found to be distinct from wtAAV2 yet conserved in natural AAV2 isolates currently circulating in the human population. Vectors based on this novel capsid, AAV-TrueType (TT), have shown significantly increased transduction in the rodent CNS in comparison to AAV2, and have even outperformed AAV9 in the potential to correct a mouse model of MPSIIIC. However, the mechanisms underlying the improved transduction are not fully understood. Distinct mechanistic differences residing within these 14 AA relating to retrograde transport efficiency and viral spread have emerged in vivo between AAV-TT and AAV2, introducing the possibility to identify functional “switches” on the AAV capsid that contribute to these characteristics in animal models. In tissue culture, we investigate if the AA modifications trigger a change in receptor binding and/or cellular uptake. As expected from the arginine to serine substitutions at positions 585 and 588, AAV-TT demonstrated an inability to bind heparin sulphate proteoglycan (HSPG) and only poorly infected various immortalised cell lines, including cells from neuronal origin. Reversion to arginine at positions 585 and 588 restored in vitro transduction ability of AAV-TT, providing us with a tool to utilize in vitro. Surprisingly, AAV-TT failed to transduce primary rat cortical neurons, which was rescued by restoring HSPG binding ability of AAV-TT. Glycan array analyses and cell binding assays revealed that AAV-TT capsids do not bind any of the known AAV attachment factors. However, AAV-TT transduction requires the universal AAV receptor, with possibly a stronger dependence of AAV-TT on AAVR compared to AAV2. In summary, the defined differences between wtAAV2 and AAV-TT provide a tool to address key characteristics of AAV vector biology.