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Functional Characterization of AAVHSCs Compared to AAV serotypes: Activation of Cellular Pathways *In vitro* and *In Vivo* Transduction Properties

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Adeno-associated virus (AAV) has been successfully used in the clinic to deliver functional copies of genes to treat various diseases. Efforts to gain further insights about their tropism, cellular trafficking, and mode of actions are critical to identify and select safe and efficacious capsids to be used in humans. A family of AAVs isolated from human hematopoietic stem cells (HSCs) called AAVHSCs has broad tissue tropism across multiple cell types in mice and non-human primates. We examined and compared functional properties of AAVHSCs and AAV9 (AAVs of the same Clade F) and AAVs of other clades including AAVs 1-8 capsids containing a self-complementary CBA-GFP transgene in human iPSC. AAV1/2/3/6/8 induced apoptosis, with AAV2/3/6 being the most potent in eliciting cell death in iPSCs when compared to other AAVs. This finding was driven through a p53-dependent pathway including its downstream targets p21, caspase-3, and PARP. By contrast, no or little upregulation of p53 was detected in iPSC transduced with AAVHSCs. Considering that changes in p53, p21, CHEK2 and cleaved Parp were similar amongst AAV1/2/3/6, we selected AAV2 as representative for AAV1, AAV3 and AAV6. AAV2 impacted cell proliferation as demonstrated by cell cycle arrest at G2/M phase with a concomitant decrease in the proportion of cells in G0/G1, S and G2/M phases while no impact was observed in iPSC, primary human fibroblasts or skeletal myoblasts/myotubes transduced with AAVHSC15. Upregulation of p53 and cell death were not related to the amount of virus present in the cells but rather intrinsic differences in the intracellular transport to the nucleus between AAV2 and AAVHSCs in iPSC. To begin assessing the *in vivo* translation of these findings, a biodistribution study was performed in mice using CBA-Luc packaged in AAV2/5/6/8 and AAVHSC capsids as well as an editing mF8-Luc vector packaged in AAV6 and AAVHSC15. AAVHSC7/15/17 were determined to be the most efficient liver transducers among capsids tested, as determined by luciferase expression and vector genomes. These data provide further insights into the mechanisms of AAVHSC transduction and expression that are being applied in the selection of AAVHSC capsids in the development of genetic medicine therapeutics.