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AAVHSCs Transduction Does Not Significantly Elicit p53-Mediated Apoptosis or Alter Cell Cycle in Human iPSCs and Primary Cells When Compared to Non-Clade F AAV Vectors

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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. There have been efforts to gain further understanding about their tropism, cellular trafficking, and mode of actions to help identify the most safe and efficacious capsids to be used in humans. A new family of AAVs was isolated from human hematopoietic stem cells (HSCs), termed AAVHSCs. These Clade F AAVs have broad tissue tropism across different cell types and species. To further characterize the functional properties of AAVHSCs, commercially available AAVs 1-9 and AAVHSC1/4/6/7/8/9/13/15/16/17 capsids containing a self-complementary CBA-GFP payload were used to transduce four different human iPSC lines. We observed several differences between AAVHSCs and the commercially available non-Clade F AAVs (AAV 1-8). Specifically, AAV12/3/6/8 induced apoptosis, with AAV2/3/6 being the most potent that elicited irreversible cell death in iPSCs when compared to AAVHSCs at multiplicity of infection (MOI) of $1x10^5$. This finding was driven by a p53-dependent pathway as shown by p53 upregulation and its downstream targets including p21, caspase-3, and PARP after transduction. Cell death with the non-Clade F vectors was also observed at low MOI of $2x10^3$, independent of vector genomes, and it was rescued by transient p53 knockdown prior to AAV transduction. By contrast, no or little upregulation of p53 was detected when iPSCs were transduced with AAVHSCs at MOI of $5x10^5$. Upregulation of proteins involved in the DNA damage repair (DDR) pathway, such as the activation of CHK2 at threonine 68 and H2AX at serine 319 following AAV1/2/3/6/8 transduction, was also observed. Knockdown of CHK2 was insufficient to prevent apoptosis in iPSCs upon transduction, while double knockdown of p53 and CHK2 rescued cell death and the cell proliferation was enhanced at 24 hours compared to p53 knockdown alone following the non-Clade F vector transductions. This suggests that CHK2 and p53 are involved in different pathways acting synergistically to regulate cell cycle progression and apoptosis. To determine the effects AAV has on cell proliferation, BrdU was added to culture medium after AAV2 or AAVHSC15 transduction. The cells were subsequently stained for apoptosis and cell cycle markers to visualize the cell cycle distribution in a given cell population by flow cytometry. At MOI of $1x10^5$, AAV2 arrested the transduced iPSCs at G_2 / M phase with a concomitant

decrease in the proportion of cells in G_0 / G_1 and S phase while AAVHSC15 did not impact the distribution of cells within G_0 / G_1, S, and G_2 / M phases. Similarly, AAV2 also disrupted the normal cell cycle distribution in primary human fibroblasts and skeletal myoblasts/myotubes. Taken together, the results from this study suggest that AAVHSCs have different properties than AAVs from other Clades such as AAV1-8. These differences found in human iPSCs and primary cells appear to be mediated through the activation of p53 and DDR pathways that are crucial for cell cycle regulation and programmed cell death.