Assessment of 3T3-L1 transduction using different AAV capsid variants

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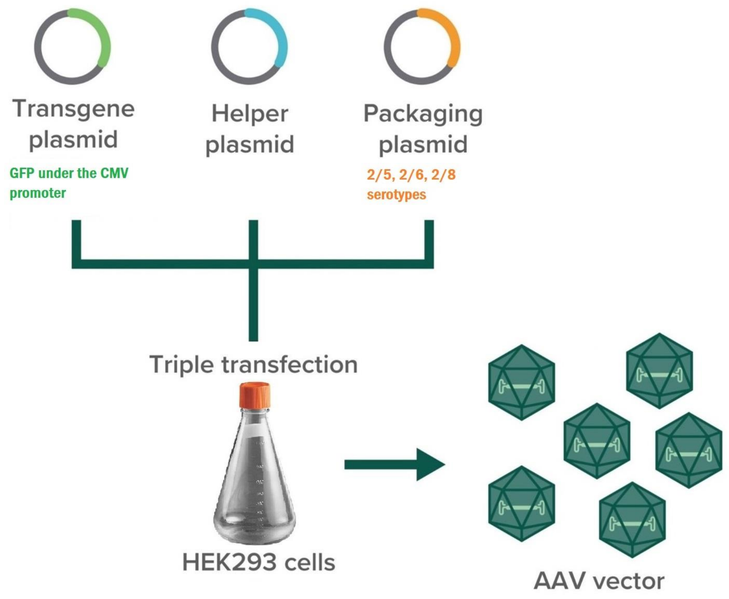
ABSTRACT

Conversion of energy-storing white adipose tissue into energy-burning beige adipose, called browning, has emerged as a promising approach in the field of obesity treatment. Adeno-associated viruses (AAV) are widely used for gene delivery in eukaryotic cells. This study focused on the transduction efficacy of AAV 2/5, 2/6, 2/8 expressing GFP in 3T3-L1 murine preadipocyte cell line. Three transduction modes were assessed: AAVs transduction in 3T3-L1 preadipocyte cells with or without further differentiation into mature adipocyte-like cells and injection of AAVs in differentiated adipocyte-like cells.

AIM OF THE WORK

The aim of this work was to evaluate a transduction efficiency of rAAV 2/5, 2/6, 2/8 vectors in 3T3-L1 preadipocyte and mature adipocyte-like cell. Evaluated serotypes will be used for viral delivery of genes coding for transcription factors into adipose tissue in order to ignite the browning program.

METHODS



AAV 2/5, 2/6, 2/8 expressing GFP vectors were produced by co-transfection of pHelper, pAAV ITR-expression with gene of GFP vector, and pAAV Rep-Cap gene certain serotype (Fig.1).

Fig. 1 AAV 2/5, 2/6, 2/8 vectors production scheme

GFP expression were analysed by live imaging microscopy using IncuCyte S3. The differentiation of 3T3-L1 into mature adipocyte-like cells was induced by adipogenic IBMX-DEX-INS cocktail according to a standard protocol.

RESULTS

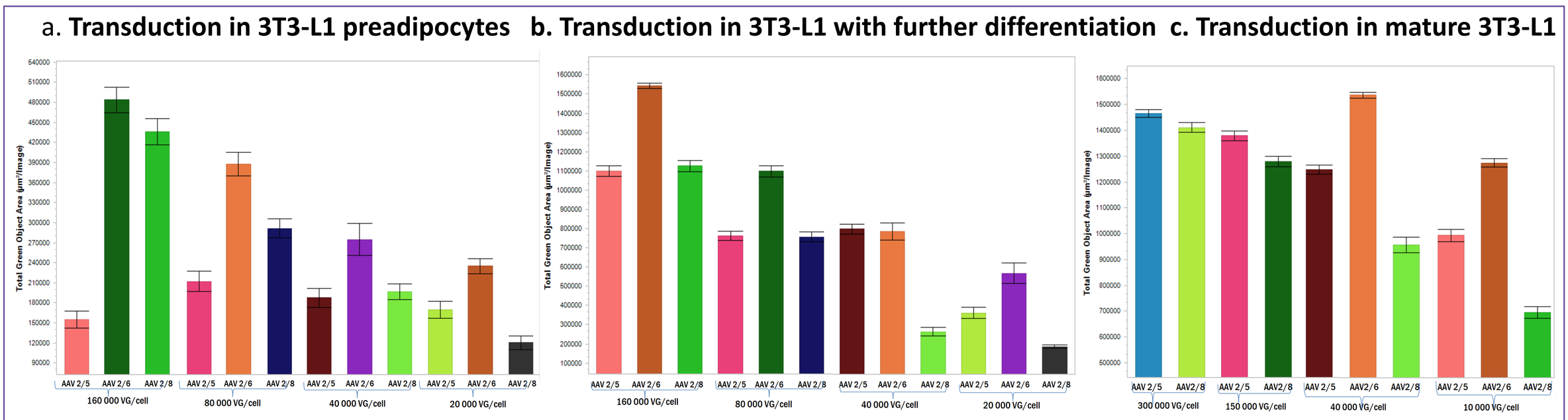


Fig. 2. Quantitation of green fluorescent objects after transduction with different AAV serotypes and concentration (VG/cell - viral genomes per cell)

CONCLUSIONS

It was AAV2/6 that demonstrated the highest transduction capacity in 3T3-L1 preadipocytes in the range of viral concentration from 2×10^4 to $1{,}6\times10^5$ VG/cell (Fig. 2a). The induction of 3T3-L1 differentiation three days after AAV transduction showed that GFP expression levels were maintained and increased, with AAV2/6 still showing the highest transduction capacity (Fig. 2b). AAV2/6 also demonstrated a higher capacity to transduce mature adipocytes (Fig. 2c). The expression of GFP under the ubiquitous CMV promoter remained stable for up to 20 days (Fig. 4). Thus, AAV2/6, compared to AAV2/5 and AAV2/8, demonstrated higher transduction efficacy in 3T3-L1 preadipocytes and mature adipocytes, proving its usefulness along with AAV8 and AAV9 for gene delivery to adipocytes.

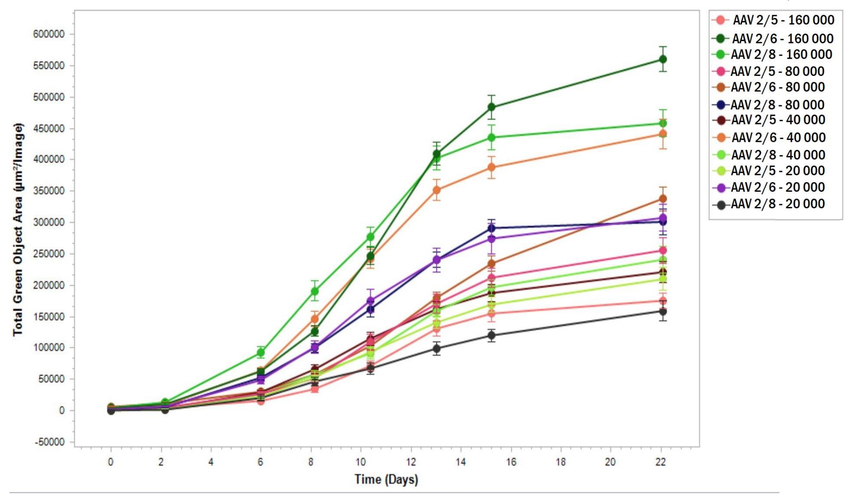


Fig. 3. Quantitation of green fluorescent objects after transduction

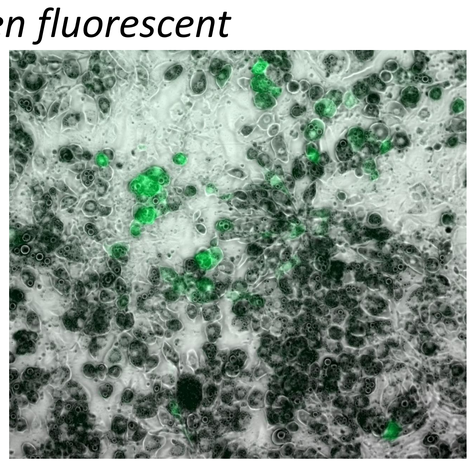


Fig. 4. Mature 3T3-L1 adipocytes express GFP after AAV8 transduction

Acknowledgments

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