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Commentary

The hunt for novel AAV capsids with improved cardiac tropism

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The last two decades have shown an impressive progress in the development of viral vectors, leading to the approval for commercialization by the FDA of seven gene therapies based on adeno-associated viral vectors (AAVs). This was made possible due to improvements already attained in vector structure to mitigate immunogenicity, prevent off-target expression, increase transgene expression, and reduce neutralizing antibody recognition.[1,2] However, some research areas are still lagging behind, as is the case for gene therapy targeting cardiac diseases. To date, there are no approved recombinant viral vector-based therapies to treat cardiac diseases. Even cardiac disorders caused by monogenic mutations do not yet have approved viral vector-based therapies.

One of the fundamental reasons for the lack of approved therapies is the absence of AAV capsids with enhanced cardiac tropism in humans.[3] In the previous issue of *Molecular Therapy – Methods & Clinical Development*, Kok et al.[4] tried to overcome this problem by using an innovative strategy to engineer novel AAV capsids by directed evolution. They used a shuffled AAV library and screened by the highest enrichment of cardiac tropism using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). After six rounds of selection, five novel cardiotropic recombinant AAVs (rAAVs) showed more promise. The selected rAAV variants were then characterized to determine the AAV parental serotype, which revealed the selected rAAV variants to be related to AAV1 (KK01), AAV3 (KK04), and AAV6 (KK02, KK03, and KK05).

Further validation of the new enriched cardiotropic rAAV variants was performed by conducting cell entry and gene expression analyses in a competitive transduction assay, including AAV1, AAV6, and AAV9 capsids, besides the five selected novel rAAV variants. All vectors were packaged with a GFP-expressing cassette containing an exclusive barcode and transduced into hiPSC-CMs.

In the functional tests, KK04 outperformed all other capsids, including the wild-type (WT) capsids. This result was also confirmed when using a non-barcoded transgene. Curiously, the change to a specific cardiac promoter led to an improvement in the efficiency of transduction in the rAAV.KK02, similarly to the observed transduction of rAAV.KK04.

Next, the authors tested the transduction efficiency of WT and novel variant rAAVs in human cardiac organoids and human, non-human primate, and pig cardiac slices. Two days post transduction, cell entry and gene expression were assessed. Interestingly, transduction efficiency was variable across different species, but most importantly, the novel rAAV KK04 variant had the highest cell entry and increased expression in the human cardiac slices.

Although knowledge earned from rodent studies does not always end up being transferred to humans, the present mouse data warrant further investigation of these novel cardiotropic rAAV variants. Variability in cell entry and expression was observed in mouse hearts four weeks after systemic injection, compared to what was seen in cardiac organoids and tissue slices, with a superior effect for rAAV9. The reason for this unexpected result may be due to differences in the time of analysis post-transduction or to the simple fact that the engineered capsids were intended to transduce human cardiomyocytes.

The findings from Kok et al.[4] definitely help move the field forward toward having an effective cardiotropic rAAV vector. However, there are still some challenges that need to be overcome, such as having better models to test the efficacy of gene therapy in human cardiac samples with prolonged viability[5,6] and models that can successfully predict or closely recapitulate the cardiac transduction efficiency that would be seen after systemic or focal delivery. The present work represents an important step toward cardiac gene therapy, and despite some minor limitations, the hunt is still on.

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DECLARATION OF INTERESTS

The authors declare no financial interest.

REFERENCES

1. Li, C., and Samulski, R.J. (2020). Engineering adeno-associated virus vectors for gene therapy. Nat. Rev. Genet. *21*, 255–272. https://doi.org/10.1038/s41576-019-0205-4.

2. Cannatà, A., Ali, H., Sinagra, G., and Giacca, M. (2020). Gene Therapy for the Heart Lessons Learned and Future Perspectives. Circ. Res. *126*, 1394–1414. https://doi.org/10.1161/CIRCRESAHA.120.315855.

3. Ishikawa, K., Weber, T., and Hajjar, R.J. (2018). Human Cardiac Gene Therapy. Circ. Res. *123*, 601–613. https://doi.org/10.1161/CIRCRESAHA.118.311587.

4. Kok, C.Y., Tsurusaki, S., Cabanes-Creus, M., Igoor, S., Rao, R., Skelton, R., Liao, S.H.Y., Ginn, S.L., Knight, M., Scott, S., et al. (2023). Development of new adeno-associated virus capsid variants for targeted gene delivery to human cardiomyocytes. Mol. Ther. Methods Clin. Dev. *30*, 459–473. https://doi.org/10.1016/j.omtm.2023.08.010.

5. Pitoulis, F.G., Watson, S.A., Perbellini, F., and Terracciano, C.M. (2020). Myocardial slices come to age: an intermediate complexity in vitro cardiac model for translational research. Cardiovasc. Res. *116*, 1275–1287. https://doi.org/10.1093/cvr/cvz341.

6. Fischer, C., Milting, H., Fein, E., Reiser, E., Lu, K., Seidel, T., Schinner, C., Schwarzmayr, T., Schramm, R., Tomasi, R., et al. (2019). Long-term functional and structural preservation of precision-cut human myocardium under continuous electromechanical stimulation in vitro. Nat. Commun. *10*, 117. https://doi.org/10.1038/s41467-018-08003-1.

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