Bioconjugated adeno-associated virus vectors: bridging organic chemistry and vectorology for enhanced gene therapy.

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Résumé :

For our gene therapy projects, we employed innovative chemical bioconjugation techniques to enhance the efficiency of recombinant Adeno-Associated Virus (rAAV) vectors, termed BioAAV. This was achieved by selectively modifying lysine or tyrosine residues on rAAV capsids with ligands [1].

rAAV-based gene therapy is the result of decades of biological and clinical research aimed at treating diseases such as Duchenne muscular dystrophy, Alzheimer, and retinitis pigmentosa. However, overcoming key challenges - such as broad tissue distribution, high injected doses, and immunological complications - remains crucial to fully unlocking the potential of these vectors. Integrating chemical strategies with vectorology offers a promising approach to addressing these limitations. In our studies, we leveraged covalent coupling reactions, including the nucleophilic addition of lysine amino groups, the aromatic electrophilic substitution, and the electrochemical modification of tyrosine phenol groups using isothiocyanate, diazonium salt, or N-methylluminol ligands, respectively [2-3].

Following rigorous validation of the chemical modifications through a panel of analytical assays, we evaluated the *in vivo* efficacy of these BioAAV vectors, each carrying an enhanced Green Fluorescent Protein (eGFP) reporter gene expression cassette, in the retina and brain of rodent and non-human primate models. Our findings revealed that BioAAV2 significantly improved vector transduction efficiency and gene expression following both subretinal and intrastriatal injections. In rat and NHP retina, eGFP expression levels were an order of magnitude higher with BioAAV2 compared to unmodified rAAV2. In the mouse brain, BioAAV2 resulted in a broader eGFP expression area than unmodified rAAVrh10.

Overall, our study demonstrates that lysine and tyrosine bioconjugation on rAAV vectors represents a valuable strategy for enhancing protein expression in targeted tissues, complementing genetic engineering approaches. This innovative method holds significant potential for advancing gene therapy interventions for conditions such as glaucoma, optic neuropathies, and neurodegenerative disorders.

1. Mével, M. *et al*. *Chem. Sci.* **2020**, *11*, 1122−1131. DOI: 10.1039.C9SC04189C.
2. Depienne, S. et al. *Nat. Com.* **2023**, *14*, 5122-5131. DOI: 10.1038/s41467-023-40534-0.
3. Leray, A. et al. Biomed Pharmacother. 2024, 171, 116148-116158. DOI: 10.1016/j.biopha.2024.116148.

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