Self complementary adeno associated virus

Self-complementary adeno-associated virus (scAAV) is a viral vector engineered from the naturally occurring adeno-associated virus (AAV) to be used as a tool for gene therapy.

Use of recombinant AAV (rAAV) has been successful in clinical trials addressing a variety of diseases. This lab-made progeny of rAAV is termed “self-complementary” because the coding region has been designed to form an intra-molecular double-stranded DNA template. A rate-limiting step for the standard AAV genome involves the second-strand synthesis since the typical AAV genome is a single-stranded DNA template.

However, this is not the case for scAAV genomes. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. The caveat of this construct is that instead of the full coding capacity found in rAAV (4.7-6kb)[5] scAAV can only hold about half of that amount (≈2.4kb).

In gene therapy application utilizing rAAV, the virus transduces the cell with a single stranded DNA (ssDNA) flanked by two Inverted Terminal Repeats (ITRs). These ITRs form hairpins at the end of the sequence to serve as primers to initiate synthesis of the second strand before subsequent steps of infection can begin. The second strand synthesis is considered to be one of several blocks to efficient infection.

Additional advantages of scAAV include increased and prolonged transgene expression in vitro and in vivo, as well as “higher in vivo DNA stability and more effective circularization.

Self-complementary adeno-associated virus (scAAV) vectors, which do not express any viral gene and are not linked with any known disease in humans, are attractive therapeutic gene delivery vectors in intervertebral disc degeneration (IVD). However, scAAV-based silencing of catabolic or inflammatory factor has not yet been investigated in human IVD cells. Therefore, we used scAAV6, the most suitable serotype for transduction of human nucleus pulposus (NP) cells, to knockdown the major catabolic gene (ADAMTS4) of IVD degeneration. IVD degeneration grades were determined by preoperative magnetic resonance imaging. Lumbar NP tissues of degeneration grade III were removed from 12 patients by nucleotomy. NP cells were isolated and cultured with low-glucose. Titre of recombinant scAAV6 vectors targeting ADAMTS4, transduction efficiencies, transduction units, cell viabilities and expression levels of target genes were analysed using quantitative PCR, fluorescence microscopy, fluorescence-activated cell sorting, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assays, quantitative reverse transcription PCR, western blot and enzyme-linked immunosorbent assays during 48 days of post-transduction. Transduction efficiencies between 98.2% and 37.4% and transduction units between 611 and 245 TU/cell were verified during 48 days of post-transduction ($p<0.001$). scAAV6-mediated knockdown of ADAMTS4 with maximum 87.7% and minimum 40.1% was confirmed on day 8 and 48 with enhanced the level of aggrecan 48.5% and 30.2% respectively ($p<0.001$). scAAV6-mediated knockdown of ADAMTS4 showed no impact on cell viability and expression levels of other inflammatory catabolic proteins. Thus, our results are promising and may help to design long-term and less immunogenic gene therapeutic approaches in IVD disorders, which usually need prolonged therapeutic period between weeks and months [1].

1)

Shenegelegn Mern D, Tschugg A, Hartmann S, Thomé C. Self-complementary adeno-associated virus

serotype 6 mediated knockdown of ADAMTS4 induces long-term and effective enhancement of aggrecan in degenerative human nucleus pulposus cells: A new therapeutic approach for intervertebral disc disorders. PLoS One. 2017 Feb 16;12(2):e0172181. doi: 10.1371/journal.pone.0172181. PubMed PMID: 28207788.