Is gene therapy coming of age?

Building the next generation of gene transfer vectors

The past decade has seen a resurgence in the field of cell and gene therapy, with three treatments being approved by the European Medicines Agency and the US Food and Drug Administration and a flood of candidate therapies nearing the end of Phase 3 clinical trials, a number of which are demonstrating promising results. Strimvelis, a gene therapy marketed by GlaxoSmithKline Plc, is a treatment for ADA-SCID, an X-linked immunodeficiency genetic disorder caused by a defective adenosine deaminase. The treatment works by adding back a correct version of the deaminase enzyme in the patient's own lymphocytes using a retroviral vector and transplanting corrected cells back into the patient.

In general, viruses from the retroviridae family, including lentiviruses, are used to genetically modify cells *ex vivo*, for subsequent delivery of corrected cells back to the patient. The other two approved treatments are based on direct *in vivo* gene therapy approaches, where the defective gene has been inserted into an adeno-associated viral (AAV) vector and placed under the control of commonly used viral promoters to drive high level, constitutive expression. They include Luxturna, recently approved for retinal dystrophy, and Glybera for lipoprotein lipase deficiency, which is no longer on the market.

Each one of these approved therapies is based on the use of a first-generation gene transfer vector, and as a result they are associated with severe limitations. In this article, the author discusses recent advances in the sector that allow superior engineering of AAV-based gene transfer vectors at multiple levels. Ultimately facilitating the rational design of vectors that are optimised to transduce the target tissue, delivering the therapeutic cargo to as many target cells as possible and then subsequently expressing the therapeutic protein to the precise levels required, thus mediating the optimal dose to achieve an effective treatment.

Delivering the therapeutic gene cargo

When developing AAV vectors for gene delivery, it is important that the capsid employed is capable of evading the immune response, primarily through not being recognised by neutralising antibodies to AAV that are prevalent in the general population. Then by minimising antigen presentation of viral peptides to T cells through MHC responses. Secondly, the chosen viral serotype/capsid has to reach the appropriate cell type, with minimum transduction of off-target cell types, whose transduction may serve to reduce the pool of vector available for transduction of the target cells.

Thirteen serotypes of AAV have thus far been identified, and most of these have been engineered into recombinant vectors for gene therapy purposes (please see Table 1 for a list of most commonly used vectors with their associated tissue tropism). These serotypes differ in their tropism, or tissues that they preferentially transduce, making AAV a very useful system for preferentially targeting specific cell types. However, it should be noted that different AAV serotypes infect multiple different tissues and cell types, meaning that for optimal selective targeting for gene therapy it is best to use a number of different strategies.

It is also possible to pseudotype AAV viral vectors, i.e., the mixing of a capsid and genome from different AAVs. Typically, the genome from AAV2 is commonly used for most gene therapy uses and is pseudotyped with the desired serotype in order to achieve the desired tissue selectivity. It is also possible to create hybrid capsids derived from multiple different serotypes, which also alter viral tropism. For instance, AAV-DJ contains a hybrid capsid derived from eight serotypes and shows a higher transduction efficiency in vitro than any wild type serotype and *in vivo* it is broadly infective across multiple tissues.

More recently, a number of groups have employed methods of *in vitro* evolution and *in vivo* selection of AAV capsids that target different tissues or cell types, and even de-target cells where transduction is detrimental to the therapy[1]. Capsid gene libraries are created by *in vitro* scrambling and shuffling of the capsid genes of all the AAV serotypes. Screening of hybrid capsid libraries is performed directly *in vivo* by panning in adult mice after intravenous injection of the shuffled capsid library that packaged their own coding sequences. The AAV variants that display enrichment in the desired tissue are subsequently retrieved by capsid gene PCR and then characterised for their tissue tropisms. This directed evolution and *in vivo* selection method is a powerful tool for generating new AAV capsids displaying higher levels of selective transduction.

The current level of technology in capsid engineering results in enhanced tropism for particular tissue types, but does not completely eliminate off-target transduction. In order to add an extra level of cell selectivity, it is possible to express the therapeutic transgene in the particular cell type of interest by transcriptional targeting. This is the process whereby the gene is transcribed from a promoter that is selectively active in the target cell type. When combined with targeted transduction using engineered AAV capsids/serotypes, this significantly improves the immune-stimulatory profile of the gene medicine, with fewer viral particles transducing antigen presenting cells and no gene expression occurring due to the selective activity of the gene promoter.

Rational Engineering of Synthetic Promoters

The genome is a vast repository of genetic information that can be mined to better control the expression of genes for therapeutic purposes. As we begin to better understand how the genome regulates itself to generate the diverse populations of cells that make up complex higher organisms, we can also apply that knowledge to identify regulatory elements that are functional in particular cell types or that are only active in certain environments.

Synthetic promoters are DNA sequences that do not exist in nature and which are designed to regulate the activity of genes, controlling a gene's ability to produce its own uniquely

encoded protein. Currently, within the biotech industry, naturally occurring promoters are largely used to drive protein production. However, natural promoters have evolved for biological functions within the context of the organism in question, and as such they were not purpose-designed for applications within the biotech industry. Depending on the promoter and the specific application, natural promoters are not always able to drive a high level of gene expression and may also be lacking in the desired specificity.

Synthetic promoters are constructed by assembling core promoter and enhancer elements derived from the genome in novel combinations to form new unique gene regulatory sequences that don't exist in nature[2]. It is possible to identify individual enhancer regions that are tissue, or cell-type specific, by examining the gene expression profile of the target cell and by identifying differentially expressed genes using microarray or NGS technology. One can then use various bioinformatics resources to identify the enhancer elements that control the expression of those genes.

At Synpromics, we integrate data from a diverse array of sources in order to identify these enhancer regions and feed that data into the customised genome browser that we have developed. This allows us to integrate data from a vast array of sources, either publicly available or from functional genomics data we generate in house. We then use novel library screening technology to resolve the precise enhancer sequences that control transcription and use these sequences as parts to build synthetic promoters using a rational engineering biology approach. The pipeline generates novel promoters that tightly control gene expression in the particular cell type and condition of interest, and comprises a sequence, constituting a novel combination of enhancer elements that does not exist in nature and thus can be patented.

The resultant synthetic promoters are exceptionally useful in the design of novel cell and gene therapies, as they can be used to build an expression cassette that regulates the therapeutic gene in a highly selective and efficacious manner. The optimal synthetic promoter that drives the precise level of therapeutic protein can be selected, which is only active in the target cell type and which mediates the required level of transcriptional targeting to achieve a potent therapeutic effect.

We and others have been successful in identifying and isolating enhancers from the human genome and incorporating them into the design of cell-type selective synthetic promoters. The genome also comprises other gene regulatory elements that function at the transcriptional and post-transcriptional levels, and that can be used to further bolster levels of therapeutic protein expression from viral vectors *in vivo*. For instance, canonical and non-canonical introns can be used to either improve translation by facilitating nuclear export of the transcribed mRNA, or by enabling inducible expression of the therapeutic protein in response to certain external stimuli.

Untranslated regions located both 5' and 3' to the coding sequence are abundant in the genome and act to stabilise the mRNA, and thus result in an increase in translation and subsequently therapeutic protein levels. Indeed, miRNA when inserted into the 3'UTR of the expression cassette can be used to destabilise mRNA levels in antigen presenting cells and hence prolong expression of the therapeutic protein *in vivo*[3]. Therefore, it is essential that the entire expression cassette is optimised when designing any gene transfer vector for therapeutic purposes, so that multiple different levels of control are engineered into the final vector construct.

Table 1: AAV Serotypes and Tissue Tropism

Serotype	Tissue Tropism
AAV1	CNS, Heart, Skeletal Muscle, Retinal Pigment Epithelium
AAV2	CNS, Kidney, Photoreceptors, Retinal Pigment Epithelium
AAV3	
AAV4	CNS, Lung, Retinal Pigment Epithelium
AAV5	CNS, Lung, Photoreceptors, Retinal Pigment Epithelium
AAV6	Lung, Skeletal Muscle
AAV7	Liver, Skeletal Muscle
AAV8	CNS, Heart, Liver, Pancreas, Photoreceptors, Retinal Pigment Epithelium, Skeletal Muscle
AAV9	CNS, Heart, Liver, Lung, Skeletal Muscle

Conclusion

When developing the optimal gene therapy vector based on the AAV vectors, there are multiple things that can be done to target the correct tissue or cell type, reduce immunogenicity and improve selectivity of expression, thereby achieving a therapeutic response. If repeat dosing is required one can envisage a scenario where multiple vector administrations are possible without inducing significant immunity, through the serial use of different serotypes, or rationally designed hybrid capsids. Furthermore, engineering selective transduction and transcription into gene therapy treatments increases the safety profile and potency of the gene delivery vector. This results in a reduced viral load in non-target cells, which results in reduced immune response, and further allows increased control of the therapeutic transgene, where the correct therapeutic dose can be achieved through the design of a suitable synthetic promoter. As the cell and gene therapy field begins to mature, the tools are finally being developed that allow safer and more effective therapeutic options.

References:

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This article was written by Michael L Roberts, PhD, founder and chief scientific officer of Synpromics Ltd in the UK.