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Improving AAV Gene Therapy: Graduating From Transgene Expression "Everywhere, All The Time" To "Disease-Specific"

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Introduction

Adeno-associated virus (AAV) is now one of the top gene delivery/gene therapy vectors in use today [1-6]. The hallmark goal of gene therapy is to deliver a therapeutic gene which then counteracts a negative phenotype or disease within the patient or animal model. While there are many types of diseases to treat, each disease is somewhat different and there are a variety of delivery/expression strategies which can be undertaken. AAV capsid type and tropism clearly play a valuable role in gene therapy; however the strategy for expressing the transgene is even more important. Here we discuss the three most prominent types of gene expression approaches, that is, which transcriptional promoter should be chosen for expressing the therapeutic gene. The issue is that many, perhaps all, therapeutic genes will likely have consequences, adverse reactions, if expressed at high levels. Yet the gene therapy agents must be safe, and not induce widespread unintended damage.

The first major expression approach is the "constitutive" approach, such as using the cytomegalovirus (CMV) immediate early promoter (pr) (Figure 1).

However there are a number of competitors in this category [8]. The treatment of genetic syndromes might be the most appropriate for this approach. Genetic syndromes result from a faulty protein which is important within a tissue or organ for normal function. For genetic syndromes the goal of gene therapy is simple; get that therapeutic gene and its protein delivered and expressed into as many cells of the patient, organ or tissue as possible to give back normal function. The strategy then is usually for maximum gene delivery and gene expression. A similar approach might also be needed for the treatment of cancer which is a malignant tissue and requires maximum gene delivery of the cancer-killing or cancer-altering gene.

Many, perhaps most, therapeutic genes actually have a down-side because of their inherent function and give adverse reactions when expressed at high levels. An example of this is in the treatment of inflammatory diseases, such as cardiovascular disease. Therapeutic genes that we have used in the treatment of atherosclerosis, in particular interleukin 10 (IL10), which, while strongly immunosuppressive (needed for its efficacy against atherosclerosis), is also associated with a number of adverse reactions which are manifested in the clinical setting and in some animal models. These adverse reactions include increased bacterial, fungal and viral infections, cancer, headache, and anemia [9-16]. Thus, as a generalization, the strongest therapeutic genes are also likely to be the most dangerous and they must be tightly regulated. We call this strong constitutive transcriptional promoter use, introducing and expressing the gene in many cell types, as the "everywhere, all the time" approach. We consider this approach to be somewhat dangerous. Only in the treatment of genetic syndromes does this strategy seem appropriate.

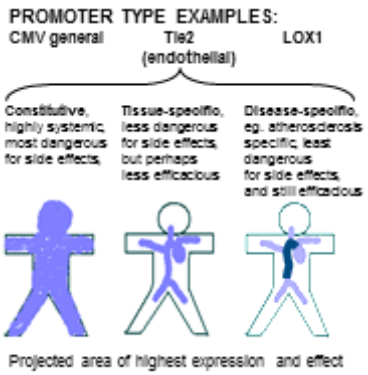

Figure 1: The three general approaches in therapeutic gene delivery. CMVpr represents the constitutive "everywhere, all the time" approach. It is dangerous. Tie2pr represents the somewhat better tissue specific approach. The LOX1pr represents the atherosclerosis-specific, disease-specific approach. It is the safest approach, yet still gives efficacy [7]. This proposal intends to further verify and make further advances in the latter, the disease-specific approach. This cartoon is not intended to be completely accurate in showing specific expression patterns, but rather to show the differences between the approaches.

A major refinement of the constitutive approach is the "tissue-specific" approach, the second general approach. In this scheme the delivered transgene is to be expressed only within a specific cell type, thereby limiting its overall expression [17]. Clearly this is an improvement over the constitutive approach, yet we consider this approach as only a modified version of the constitutive approach, limited to a specific cell type. This is still rather dangerous as one still changes, wholesale, an entire tissue. This expression strategy may be, in fact, superior for most genetic syndromes than the constitutive approach as usually one tissue is overly affected and limited expression to only that one tissue can give efficacy without the chance of

unintended consequences in other tissues. However, the gene-modified tissue possibly then becomes essentially a new tissue type, with perhaps a new, still abnormal, phenotype.

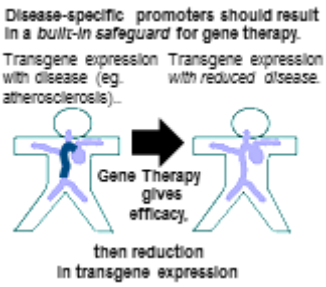

Figure 2: The disease-specific expression approach provides a built-in safeguard for the gene therapy. As planned and envisioned the use of the disease-specific transcriptional promoter within the AAV vector will give targeted expression at the site of disease, and little expression elsewhere. Additionally, when the level of disease is reduced then so too should the expression of the therapeutic transgene. This should result in a built-in safeguard for the gene therapy and prevent the appearance of adverse reactions due to transgene overexpression

Here, however, we wish to highlight the third approach, the "disease-specific" approach [4]. The goal of the disease-specific approach is to limit the expression of the therapeutic transgene to the site of disease, and in the cells which are changing towards the disease-associated phenotype. We view this disease-specific approach as highly desirable in particular for diseases which are related to inflammation, which encompasses a long list of major diseases which increase in prevalence as we age (atherosclerosis, dementia, arthritis and so on). The disease-specific approach gives the gene therapy an important safety feature against adverse reactions from the over expression of a powerful therapeutic transgene such as IL10. The prototype disease-specific promoter which we have recently used to express IL10 for the treatment of atherosclerosis in a mouse model is the LOX1 promoter (LOX1pr) [4]. A variety of stimuli up- and down-regulate LOX1pr, so it is a broadly responsive promoter which is also active in a variety of cell types [17]. Our recent study on the use of the LOX1pr to drive expression of the IL10 cytokine gene within the AAV vector to inhibit atherogenesis in the low density lipoprotein receptor knockout mouse on high cholesterol diet (LDLR KO-HCD) demonstrates that this disease-specific strategy is viable [4]. While mice do not readily demonstrate adverse reactions for IL10 expression as has been documented in humans [9-16], it was observed that the overall level of human IL10 gene expression was much lower when the LOX1pr was used compared to that when the CMVpr was used. In spite of this lower overall expression of hIL10 the disease-specific approach (using LOX1pr) resulted in aortic measurements which were statistically not significant , therapeutically the same, as those resulting from the use of the constitutive approach (CMVpr)[4]. Thus, the disease-specific nature of promoters such as LOX1 should minimize the overall transgene expression, and thereby should limit adverse reactions. Importantly, the therapeutic gene is expressed where it is needed most, at the site of disease. Moreover, if the disease-stimulus is eliminated or reduced (after the disease-specific gene therapy treatment is giving a reduced level of disease), then, in response, the transgene expression, from the disease-specific promoter, itself, should be down-regulated. That promoters should function as envisioned within the AAV vector can be expected due to the lack of strong promoter elements within the AAV inverted terminal repeats [17,18].

In conclusion, the disease-specific approach, using promoters which respond to disease-stimuli, is an under-studied area of gene therapy. Yet we consider the disease-specific approach to be critical to the success of the field of gene therapy. We believe that the disease-specific approach will be grow in use and popularity because of its advantages, most importantly because it provides a built-in safeguard against the dangers of transgene overexpression (Figure 2).

Now the quest starts for the identification of appropriate disease-specific promoters for use in AAV vectorology.

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