

GENE VECTORS FOR USE IN GENE TRANSFER AND GENE THERAPY APPLICATIONS, AND METHODS OF PRODUCING THEM

The present invention relates to a new gene transfer vector system which exploits the microRNA post-transcriptional gene silencing machinery for regulating transgene expression. MicroRNAs are a family of small, non-coding RNAs involved in downregulating gene expression by recognizing in a sequence-specific manner target mRNAs. By developing new vectors which take advantage of endogenously expressed microRNAs for their regulation, the inventors have added a layer of control to gene delivery systems that did not previously exist. These new vectors take advantage of endogenously expressed microRNAs for their regulation. Lentiviral vectors for transgene expression for gene therapy or genetic engineering applications can in fact be engineered with microRNA target sequences in order to be recognized by endogenous cell-type specific microRNAs, thus regulating transgene expression in a subset of cells.

By utilizing this invention, improved expression and targeting of an exogenously delivered gene can be achieved. This invention introduces a new layer of control to gene delivery systems that did not previously exist.

Proof of Principle

This strategy is safe, and does not perturb natural microRNA. The inventors developed a lentiviral vector (LV) which can provide robust expression in hepatocytes and other non-hematopoietic cells, while preventing expression from hematopoietic cells. This design is particularly relevant for systemic gene therapy in which the host immune response against the transgene limits therapeutic efficacy. As a demonstration of the power of this approach, microRNA regulation can be used to achieve long-term gene transfer and correction of a mouse model of hemophilia B, following a single injection of a microRNA-regulated LV encoding coagulation factor IX, without provoking an immune response (Brown et al. 2007).

Innovative Aspects of the offer

1. This invention could be employed to prevent immune-mediated rejection of a transferred gene.
2. miRNA-regulated vector design could be used to improve the safety of cell therapy.
3. miRNA-regulated vector design could be used in the treatment of cancer. Several reports have indicated that specific miRNAs are downregulated in certain tumors.
4. The miRNA-regulated system can be used to achieve divergent expression of two transgenes from a single gene delivery system. This can be accomplished using a bidirectional promoter. In this way, expression of one of the transgenes can be prevented in a particular cell type, while the other transgene continues to be expressed.
5. miRNA-regulated design could be used in a lentiviral vector to prevent vector mobilization in transduced hematopoietic cells which become superinfected with wild-type HIV.

Main Advantages of the offer

1. This invention allows specific repression of gene expression in selected cell types and lineages.
2. This system does not reduce transgene expression in other cell types.
3. microRNA regulation can prevent the anti-transgene immune response: expression of the transgene within hematopoietic cells can in fact be detrimental to therapeutic objectives. Upon vector

administration *in vivo*, the present invention will avoid vector expression in antigen presenting cells of the immune system, and thereby prevent the initiation of an immune response against the transgene product.

4. Combinations of microRNA target sequences can be added to obtain vectors with highly specific cell expression patterns. The miRNA-mediated approach for restricting gene expression has several advantages over other strategies of regulating transgenes. In particular, it allows for strong, tissue-specific expression without the need to use large, and often weak and inexact tissue-specific promoters. Moreover, because designing a microRNA-regulated vector only requires knowledge of a 23 nucleotide sequence, vector design is extremely simple.
5. miRNA-regulated vector design could be used to improve the safety of cell therapy. For example, a suicide gene, such as thymidine kinase (TK), carrying a tissue-specific microRNA target sequence, can be introduced into undifferentiated embryonic stem cells (ESCs). ESCs could then be differentiated, and subsequently cultured in ganciclovir. Neurons, which express mir-124, would suppress expression of TK, and not be affected by ganciclovir. In contrast, undifferentiated ESCs, which can form teratomas *in vivo*, do not express mir-124, and would therefore express TK, and be killed in the presence of ganciclovir.
6. miRNA-regulated vector design could be used in the treatment of cancer. Specific miRNAs are downregulated in certain tumors. For example, mir-145 expression is often absent in a variety of solid tumors, including breast cancer. Oncolytic vectors and vectors encoding suicide genes could be constructed to contain a microRNA target sequence, such as one for mi-145. When the vector is introduced into a tissue containing a mix of normal and tumor cells, mir-145 present in normal cells will suppress the lytic activity of the vector, while in tumor cells, which do not express mir-145, the lytic vector will function normally, and selectively destroy the tumor.
7. miRNA-regulated design could be used in a lentiviral vector to prevent vector mobilization in transduced hematopoietic cells which become superinfected with wild-type HIV. This could be accomplished by including a hematopoietic-specific microRNA sequence within the vector. In this way, even if vector mobilization occurs, the vector RNA genome would be destroyed by endogenous microRNA regulation.
8. The miRNA-regulated system can be used to achieve divergent expression of two transgenes from a single gene delivery system. This can be accomplished using a bidirectional promoter, which coordinately produces two distinct mRNA transcripts. Until now, this system could only be used to coordinately express two transgenes in the same cell. Using the invention, each transgene can be designed to incorporate different target sequences for different tissue-specific microRNAs. In this way, expression of one of the transgenes can be prevented in a particular cell type, while the other transgene continues to be expressed.

Partners profile

Fondazione Telethon is seeking commercial partners interested in developing/manufacturing lentiviral gene vectors for use in:

- gene transfer, gene and cell therapy applications for the treatment of genetic diseases and cancer by avoiding immune mediated rejection of the transgene
- screening procedures
- research tools and manufacturing of viral vectors.

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