Exploiting endogenous microRNA regulation for controlling transgene expression and improving gene and cell therapy

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MicroRNAs (miRNA) are an abundant class of small non-coding RNAs which can suppress expression of a gene with sufficient sequence complementarity. Although much remains unknown about miRNA regulation, molecular analysis has shown that miRNAs have distinct expression profiles in different tissues, and, together with functional studies, indicate important roles for miRNAs in establishing cell identity.

We recently reported that endogenous miRNA regulation could be exploited for controlling transgene expression from a lentiviral vector (LV). By inserting a sequence complementary to a hematopoietic-specific miRNA, miR-142-3p, into our vector, transgene expression was completely prevented in all hematopoietic cells, while expression in non-hematopoietic cells remained unaffected (Brown et al. Nat Med 2006). This work provided the first demonstration that endogenous miRNA regulation could be used to effectively de-target expression of a transgene from a particular cellular lineage, and thus forms the basis for a new means of controlling vector expression.

To develop a better framework for designing miRNA-regulated vector systems, we set out to determine the factors that influence the effectiveness of endogenous miRNA regulation. To monitor miRNA activity, we developed a novel LV reporter, which utilizes the bidirectional activity of a single promoter to coordinately express two transgenes in opposite orientation. A panel of miRNA target sequences were cloned into one of the two transgenes within the vector, and miRNA activity was evaluated by monitoring changes in transgene expression. Studies were carried out in cell lines, as well as in primary human cells. Using this approach, we provide the first evidence that endogenous miRNA activity is dependent on a threshold level of miRNA expression within the cell. We also uncover new findings which reveal that endogenous miRNA regulation is highly robust, but can be saturated at high target concentration. These results have important implications for our understanding of miRNA biology and for further development of miRNA-regulated vectors. We then exploited these data to generate vectors that rapidly and effectively adjust transgene expression in response to changes in miRNA expression levels coupled to the differentiation state of the cell. These vectors sharply segregated transgene expression between closely related cellular states in cells highly relevant for therapeutic applications, including dendritic cells, and hematopoietic and human embryonic stem cells, as well as their differentiated progeny.

The effectiveness of endogenous miRNA regulation in controlling vector expression suggested that it may be possible to take advantage of this pathway for improving hemophilia B gene therapy. One of the major barriers to delivering the coagulation factor IX cDNA (F.IX), which is mutated in hemophilia B patients, is the development of anti-F.IX immunity. This occurs because of vector transduction and expression in antigen presenting cells (APCs), which leads to T cell priming, and a subsequent immune response.
To overcome this problem, we modified an LV vector encoding F.IX to contain a target sequence for the hematopoietic-specific microRNA, mir-142-3p. As we previously showed, inclusion of this element prevents transgene expression in hematopoietic cells, and thus, may serve as a means for preventing antigen presentation in APCs, which are of hematopoietic origin. Indeed, treatment of hemophilia B mice with our miRNA-regulated LV enabled sustained F.IX gene transfer in hemophilia B mice for >280 days post-injection. Treated mice had >10% of normal F.IX activity, and no detectable anti-F.IX antibodies. Importantly, the mice were able to survive tail-clip challenge, thus demonstrating phenotypic correction of their bleeding diathesis. This work, which is amongst the first applications to exploit the microRNA regulatory pathway, provides the basis for a promising new therapy for the treatment of hemophilia B.