## 690. Permanent Epigenetic Silencing of Human Genes With Artificial Transcriptional Repressors

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There are several diseases whereby the goal of gene therapy is to silence rather than replace a gene function. Paradigmatic examples are diseases caused by a dominant negative mutation or those in which silencing of a host gene confers resistance to a pathogen or compensates the function of the missing gene. Yet, gene silencing can be used to enhance efficacy of cell therapy and for biotechnological applications. Until now, two technologies have been used to silence gene expression, namely RNA interference with short harping RNAs (shRNA) and gene disruption with Artificial Nucleases (ANs). Although some promising pre-clinical and clinical data have been already obtained, the low efficiency of knock-down with shRNA and of biallelic disruption with ANs may limit efficacy of these treatments, especially when residual gene activity can exert a biological function. To overcome this issue, we have developed a novel modality of gene silencing that exploits endogenous epigenetic mechanisms to convey robust and heritable states of repression at the desired target gene. We have generated Artificial Transcriptional Repressors (ATRs), chimeric proteins containing a custom-made DNA binding domain fused to the effector domain of a chromatin-modifying enzyme involved in silencing of Endogenous RetroViruses (ERVs). By performing iterative rounds of selection in human cell lines and primary cells engineered to report for synergistic activity of candidate effector domains, we identified a combination of 3 domains that, when transiently co-assembled on the promoter of the reporter cassette, fully abrogated transgene expression in up to 90% of treated cells. Importantly, silencing was maintained for more than 250 days in cultured cell lines, was resistant to in vitro differentiation or metabolic activation of primary cells, and was confined to the reporter cassette. Silencing was associated with high levels of de novo DNA methylation at the targeted locus and was dependent on this epigenetic mark for its propagation. Finally, transient transfection of 3 ATRs targeted to the promoter region of the Beta-2-microglobulin (B2M) gene resulted in the loss of surface expression of B2M and, consequently, of the MHC-I molecules in up to 80% of treated cells. This phenotype was associated with a switch in the epigenetic and transcriptional state of the constitutively active B2M gene, which became highly decorated with DNA methylation and deprived of RNA PolII and of its transcript. Of note, silencing was resistant to IFN-γ treatment, a potent B2M inducer. Overall, these data provide the first demonstration of efficient and stable silencing of an endogenous gene upon transient delivery of ATRs. This result was made possible by repurposing the machinery involved in silencing of ERVs, which instructs self-sustaining repressive epigenetic states on the gene of interest. While silencing of B2M might be used to generate universally transplantable allogeneic cells, our hit-and-run strategy provides a powerful new alternative to conventional gene silencing for the treatment of several diseases. (LN & AL co-authorship)

## 691. A Light-Inducible CRISPR/Cas9 System for Control of Endogenous Gene Activation

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Optogenetic systems enable precise spatiotemporal control of gene expression and cellular behavior that is easily reversible and repeatable. Optogenetic technologies that facilitate dynamic control of endogenous genes would facilitate the systematic analysis of the relationship of gene expression patterns and cell differentiation or disease treatment. We engineered a light-activated CRISPR/Cas9 effector (LACE) system that induces transcription of endogenous genes in the presence of gene-specific guide RNAs (gRNAs) and blue light. This system is based on the plant proteins CRY2 and CIB1 from Arabidopsis thaliana that heterodimerize in response to blue light. The full-length CRY2 was fused to the N-terminus of the transcriptional activator VP64 (CRY2FL-VP64), and an N-terminal fragment of CIB1 was fused to the N- and C-terminus of the catalytically inactive form of Cas9 (CIBN-dCas9-CIBN). When these fusion proteins are expressed with a gRNA, CIBN-dCas9-CIBN localizes to the gRNA target. In the presence of blue light, CRY2FL binds to CIBN, which translocates CRY2FL-VP64 to the gene target and activates transcription. Unlike other optogenetic systems, the LACE system can be targeted to new endogenous loci by solely manipulating the specificity of the gRNA without having to re-engineer the light-inducible proteins.

Light-dependent activation of the IL1RN, HBG, or ASCL1 genes was achieved by delivery of the LACE system and four gene-specific gRNAs per promoter region. Illuminated cells in which IL1RN or HBG1/2 was targeted demonstrated significantly greater mRNA levels in the light compared to the dark ($p<0.0001$ and 0.005, respectively), as well as equivalent activation levels to dCas9-VP64 ($p=0.17$ and 0.35, respectively). Significant light-dependent activation was also observed when the ASCL1 locus was targeted with the LACE system ($p<0.0001$). However, in this case mRNA levels were not activated to the same extent as cells that received dCas9-VP64 and the same four ASCL1-targeting gRNAs. In all instances, transfected cells incubated in the dark maintained levels of the targeted gene that did not significantly differ from mock-transfected cells. Importantly, the fusion of CIBN to both N- and C-termini of dCas9 yielded 10- to 100-fold greater gene activation than when CIBN was fused to only one terminus.

Endogenous gene expression could be controlled in a reversible and repeatable fashion by modulating the duration of blue light exposure. Spatial patterning of gene expression was also achieved using an eGFP reporter. Cells illuminated though a photomask containing slits of varying width resulted in a corresponding pattern of eGFP-expressing cells. This versatile LACE system is a valuable tool for studying dynamic gene expression patterns in synthetic biology, basic science, and tissue engineering.