Engineering Glucose-Responsive Non-Viral Gene Therapy for Diabetes Mellitus Control

An Nisaa Nurzak[1], Hoda M. Eltaher[1], Madeleine V King[2], James E. Dixon[1]

[1] School of Pharmacy, Division of Regenerative Medicine and Cellular Therapies, Biodiscovery Institute, University of Nottingham, United Kingdom, NG7 2RD
[2]School of Life Sciences, Medical School, Queen's Medical Centre,The University of Nottingham, Nottingham NG7 2UH, United Kingdom

Background: Insulin replacement therapy is the standard of care for patients with Type 1 or Type 2 Diabetes (DM), and this has been transformed with the engineering of recombinant insulins that are long-acting "basal" and short-acting "rapid" for the tightest control of blood glucose levels. Unfortunately, even with this improvement dosing cannot be matched to every patient's condition, with glucose levels not achieving normal control and patients are still at high risk of hypoglycaemia. Generating a responsive system, directly to the level of systemic glucose and maintaining stability and balance despite external changes, is ideal. Previous studies have shown that viral vectors delivering insulin transgene expression but that are responsive transcriptionally to fluctuating blood glucose levels, have positive effects on glucose levels, with Insulin levels upregulated during hyperglycemia and downregulated during euglycemia.
Non-viral gene delivery vectors are often seen as less efficient than viral vectors. However, they are more simplistic, safe, and viable for a strategy of glucose control with insulin gene therapy. Here we are starting to demonstrate that a glucose-lowering insulin gene (INS) can be engineered to be glucose responsive in vitro, transfected using our patented technology called Glycosaminoglycan-(GAG)-binding-enhanced-transduction (GET) system. This is based on a cell-penetrating peptide (CPP), which has been shown to improve nucleic acid delivery and have low cytotoxicity compared to other transfection agents in vivo. We have previously exploited this technology to successfully control the blood glucose levels by oral delivery in streptozotocin-induced diabetic mice by complexing and delivering recombinant insulin. For the design of our vectors, we have built on previous work which inserted glucose-responsive elements (GREs) into liver-specific promoters delivered by adeno-associated viral (AAV) vectors, which were shown to significantly reduce blood sugar levels in treated diabetic mice. In this work, we have engineered a suite of ubiquitous promoters with GREs to aspire to glucose-regulated insulin gene therapy via intramuscular delivery to restore native body homeostasis in the management of DM.

Methods: In this study, we examined the effect of the glucose-responsive element (R3G) and compared it with the original promoters using a model Gaussia-luciferase (GLuc) reporter plasmid. Using NIH-3t3 mouse fibroblasts grown in DMEM with different glucose concentrations (0, 3, 5.5, 11, and 25 mM), the transfection efficiency of glucose-responsive plasmids versus counterparts with no R3G elements was demonstrated. The transfection efficiency was normalized to the expression of the secreted embryonic alkaline phosphatase (SEAP) gene simultaneously co-transfected using GET technology. The respective cell viability was measured using PrestoBlue viability assay.

Results: R3G effectively transformed the designated promoter to be glucose-responsive depending on the sequences used. We demonstrated a notable elevation in gene expression levels at glucose concentrations of 11 mM and 25 mM (hypoglycemic), in comparison to glucose levels of 3.5 mM and 5.5 mM (normoglycemic). Moreover, the R3G promoter demonstrated significant enhancements in gene induction fold, particularly at higher glucose concentrations, when compared with those lacking R3G elements (without R3G).

Conclusions: We have engineered glucose-responsive promoters and utilized a non-viral vector that may allow for more precise regulation of gene expression, thereby facilitating molecular-level homeostasis with the expression of glucose-responsive insulin transgenes. This approach can be employed to attain adequate insulin expression in vitro. Subsequent research will involve refining the vector design to ensure enhanced glucose-responsive gene expression in cells relevant to intramuscular delivery and to demonstrate glucose control in animal models.