



Novel mouse model expands potential human α -cell research

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ABSTRACT

A glucagon knock-out mouse with preserved GLP-1 and GLP-2 secretion allows for the improved study of transplanted human islets and glucagon responses— providing an unprecedented resource in human α -cell and diabetes research

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In ancient Chinese philosophy there exists the concept of “Yin and Yang”: The balancing of contrary forces that are interconnected, and complementary to each other.¹ Similarly, in the regulation of blood glucose there exists a Yin and Yang between the blood glucose lowering hormone insulin, and blood glucose raising hormone glucagon. Insulin and glucagon are produced and secreted by β and α -cells respectfully, which reside together in the pancreas in heterogeneous cell clusters known as islets. The opposing action of these hormones function to maintain blood glucose homeostasis to meet cellular energy demands without causing long-term glucose-linked tissue damage.

A familiar disease that disrupts blood glucose balance is diabetes, which is projected to reach 700 million global cases by 2045.² One approach to study human diabetes, is to transplant human islets into an immunocompromised (i.e. transplant tolerant) mouse.^{3,4} This allows human islets to be studied in the context of living animals, as opposed to petri-dishes. Human and mouse insulin are distinguishable from each other, which allows insulin responses from transplanted human islets to be studied without being confounded by endogenous mouse insulin. Unfortunately, mouse and human glucagon are indistinguishable; thus, making the analogous study of human alpha cells and glucagon responses in a mouse model complicated.

As an attempt to overcome the complication, researchers previously deleted the mouse glucagon gene. The major issue of this approach is that depending on which cell type produces it, the premature form of glucagon (proglucagon) can mature into several products that play roles in metabolic homeostasis: Glucagon, oxyntomodulin, glicentin, glicentin-related pancreatic polypeptide (GRPP), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide 2 (GLP-2)⁵ (Figure 1). Therefore, deletion of the glucagon gene results in the unnecessary ablation of all six products. Consequently, the specific study of human α -cells and glucagon in a transplant model has been limited.

Tellez and colleagues sought to rectify the limitation by developing a glucagon deficient mouse with minimal loss to the five other products.⁶ Utilizing the gene editing technique of CRISPR/Cas9 in transplant tolerant mice (NSG), they removed the region responsible for amino acids 2–29 of mature glucagon (GKO-NSG mice). This led to the deletion of glucagon, oxyntomodulin, GRPP, and glicentin, but preserved GLP-1 and GLP-2. As intended, GKO-NSG mice secreted undetectable levels of glucagon and GLP-1 secretion was maintained albeit at higher levels. Intriguingly, juvenile GKO-NSG mice were underweight compared to NSG littermates but only up to 8 weeks of age, suggesting an unknown role of glucagon in juvenile

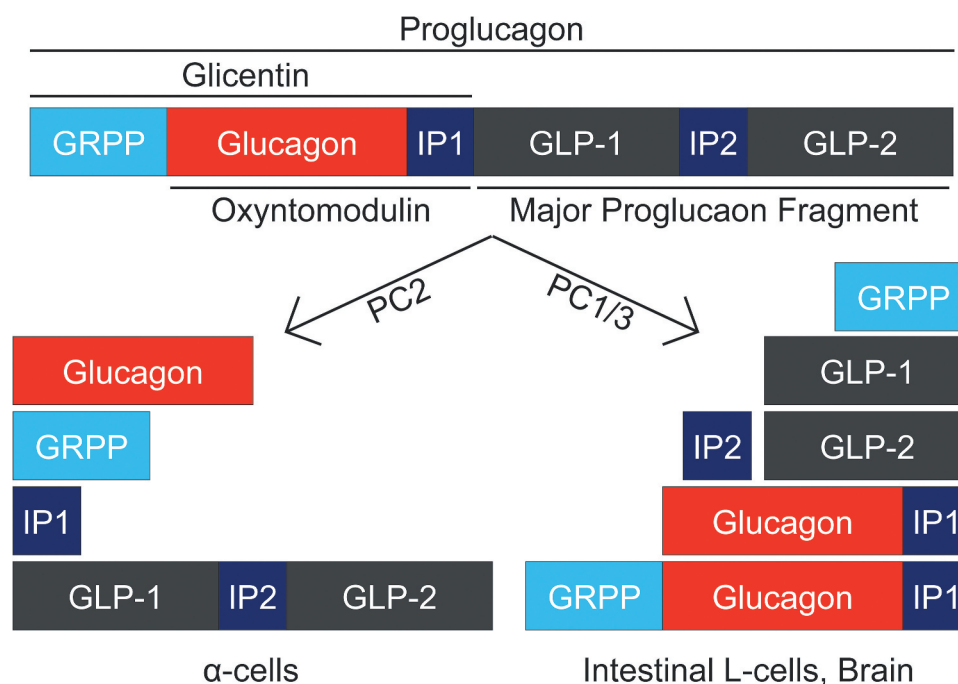


Figure 1. The pre-mature form of glucagon, known as proglucagon, can be differentially cleaved into multiple products. The type of cleavage depends on the enzyme expressed by the cells: α -cells express prohormone convertase 2 (PC2), whilst proglucagon producing intestinal L-cells and neural cells of the hypothalamus and brain stem express prohormone convertase 1/3 (PC1/3). Glucagon, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2), are separated by short intervening peptide (IP) regions, with IP1 and glucagon making up oxyntomodulin, and oxyntomodulin and glicentin-related pancreatic polypeptide (GRPP) making up glicentin. Whilst glucagon, GLP-1, GLP-2, glicentin, and oxyntomodulin's effects on metabolic homeostasis have been studied, the role of GRPP is currently unknown.

development, and an adaptation that allowed for developmental recovery.

The authors next transplanted human islets into the GKO-NSG mice (GKO-NSG-Tx). After four weeks, both blood glucagon levels and glucagon compensation after insulin challenge in the GKO-NSG-Tx mice were comparable to NSG mice, demonstrating a healthy physiological response from the human α -cells. Further, impairment of liver function due to lack of glucagon⁷ observed in GKO-NSG mice was absent in GKO-NSG-Tx mice.

A common phenotype of chronic low plasma glucagon is α -cell hyperplasia; a quantitative feedback mechanism where α -cell numbers elevate to compensate for increased glucagon demands.⁸ The authors unsurprisingly observed increases in cell mass, and proliferation markers ki67 and SLC385A in the endogenous α -cells of GKO-NSG mice; however, such observations were not present in the endogenous α -cells of GKO-NSG-Tx mice. Due to the lack of plasma glucagon, GKO-NSG mice also possessed low

blood levels of both glucose as well as insulin, and when injected with glucose, cleared it at a magnified rate. Contrastingly, none of these observations were present in the GKO-NSG-Tx mice, which behaved like NSG mice.

NSG-GKO mice were next transplanted with islets from either non-diabetes (ND) or type 2 diabetes (T2D) human donors. When compared to recipients of human ND islets, recipient mice of human T2D islets possessed elevated levels of blood glucagon and glucose, as well as enhanced glucagon secretion upon insulin challenge. This finding recapitulates previously observed inflated α -cell function in T2D,⁹ further demonstrating the relevance of their model in studying glucagon and α -cell responses in diabetes.

The authors' model is a definitive breakthrough in studying human α -cells and their involvement in diabetes. A future avenue worth pursuing is in the study of human islets from type 1 diabetes (T1D) donors. As T1D islets lack most β -cells due to autoimmune destruction, it would be interesting to observe how these α -cells – which existed in an

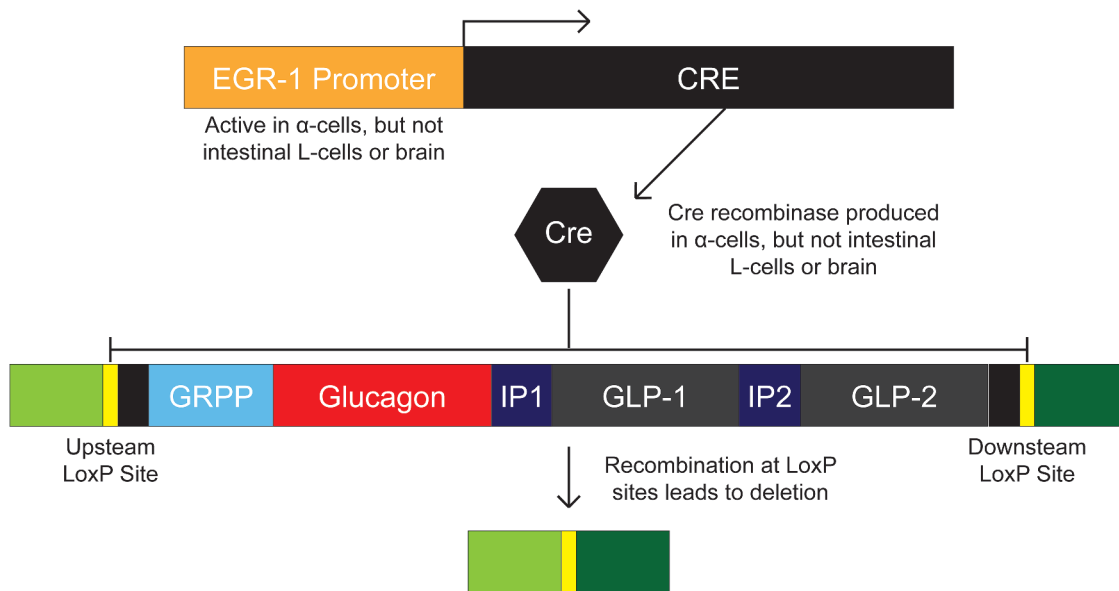


Figure 2. The utilization of the Cre/LoxP system could potentially allow for the specific deletion of glucagon exclusively in α -cells. Expression of the Cre recombinase is under the control of an α -cell specific promoter such as EGR-1 – the promoter of PC2, which is not expressed in proglucagon producing intestinal L-cells and neural cells of the hypothalamus and brain stem. Cre’s enzyme function is activated in the presence of tamoxifen, which not only makes this system specific to α -cells, but also inducible, allowing the deletion to take place in developed adult mice, instead of in a developing embryo.

environment lacking normal insulin responses – behave post-transplantation in the presence of functioning mouse β -cells. The findings would be particularly beneficial to the research focused on β -cell transplants as a treatment for T1D, which is primarily focused on optimizing the responses of the transplanted β -cells,¹⁰ and not on the T1D host’s α -cell response.

Whilst a clear improvement from previous models, a deficiency in Tellez’s model was the loss of oxyntomodulin, GRPP, and glicentin. A possible rectification would be to utilize promoter-specific gene editing tools, such as the Cre/LoxP system.¹¹ By having the expression of Cre under the control of EGR-1 – the promoter for PC2¹² – one could specifically knock out the glucagon gene in α -cells, whilst maintaining GLP-1, GLP-2, GRPP, oxyntomodulin, and glicentin production in the proglucagon producing intestinal L-cells and neural cells of the hypothalamus and brainstem⁵ since these cells lack PC2 expression (Figure 2). Additionally, immune cells play roles in maintaining islet endocrine homeostasis, which is particularly true for islet

resident macrophages that exert benefits on β -cell function.¹³ Therefore, it would be apt to study transplanted human α -cells in the context of endogenous β -cell insulin responses in an immune-competent mouse; however, with immune competency comes rejection of the foreign human islet transplant. A possible solution would be to place the human islet transplant into an immune privileged site to circumvent the rejection, and the anterior chamber of the eye (ACE) is a prime candidate. In addition to being an optimal immune privileged site for transplantation, a major benefit of the ACE is that it allows for intravital imaging through the cornea, providing researchers with an *in vivo* methodology to visually assess the transplanted islets without having to sacrifice the animal.¹⁴

Glucose control is regulated by the Yin and Yang relationship of β -cell insulin and α -cell glucagon, yet diabetes research tends to be weighted to the former. Tellez and colleague’s excellent mouse transplant model is a step in the right direction, and will provide multiple avenues to study human glucagon and α -cell

responses; thus, establishing balance to human islet and diabetes research.

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