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Stem Cell Models as a Tool to Investigate the Genetics of Diabetes

Diabetes is a complex metabolic disease that encompasses a spectrum of disorders with multifactorial causes. Although it has historically been classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) based on clinical and etiological characteristics, this division does not fully reflect the diversity of its forms. Less common types, such as monogenic diabetes, are also recognized. These are caused by mutations in a single gene and represent between 1% and 5% of cases. Unlike T1DM and T2DM, which are considered polygenic diseases with genetic risk arising from the interaction of multiple variants in different genes, monogenic diabetes results from alterations in a single gene. However, advances in human genetics have revealed that the distinction between these categories is not absolute. Rare genetic variants can influence the risk of developing polygenic diabetes (1), while the set of common genetic variants in an individual (known as the polygenic background) can modify the likelihood of a monogenic mutation manifesting clinically. This suggests a more dynamic connection between both ends of the genetic spectrum.

Monogenic diabetes, typically diagnosed before the age of 30, exhibits significant heterogeneity depending on the affected gene and the functional impact of the mutation. These are divided into three main categories: neonatal diabetes, syndromic diabetes, and MODY (Maturity Onset Diabetes of the Young).

Neonatal diabetes presents with severe hyperglycemia before 6 months of age and can be transient or permanent. Among the genes most frequently associated with this form are ABCC8, KCNJ11, and INS. On the other hand, syndromic diabetes occurs as part of more complex multiple organ syndromes. Examples include Wolcott-Rallison syndrome, caused by mutations in EIF2AK3, or congenital pancreatic agenesis, associated with alterations in genes such as PDX1 or PTF1A. Both neonatal and syndromic forms are very rare.

In contrast, MODY diabetes accounts for more than 90% of monogenic diabetes cases. Within this category, more than 50% of cases involve mutations in the GCK and HNF1A genes. Of note, many subtypes of monogenic diabetes are ideal candidates for personalized therapeutic interventions, which could significantly improve the management of these conditions. However, most patients with monogenic diabetes remain undiagnosed or are misdiagnosed as type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM) (2), leading to missed opportunities for implementing specific treatments tailored to their needs.

THE KEY ROLE OF BETA CELLS AND THE DEVELOPMENT OF THE ENDOCRINE PANCREAS

The beta cell, responsible for secreting insulin in response to glucose, is central to the pathophysiology of all types of diabetes. A high percentage of monogenic forms of the disease are associated with mutations in genes essential for the embryonic development of the endocrine pancreas, the differentiation, or the function of mature beta cells (3). These genetic alterations can disrupt key stages of pancreatic development, such as the expansion of pancreatic progenitors or the differentiation and maturation of functional endocrine cells ***(Figure 1)***. Therefore, understanding these processes at human embryonic development level is critical for unraveling the mechanisms of many forms of monogenic diabetes.

EXPERIMENTAL MODELS IN DIABETES RESEARCH

Diabetes research relies on experimental models that allow us to understand its origin, progression, and underlying molecular mechanisms. Throughout history, animal models have been fundamental in this field. From the pioneering experiments of Banting »

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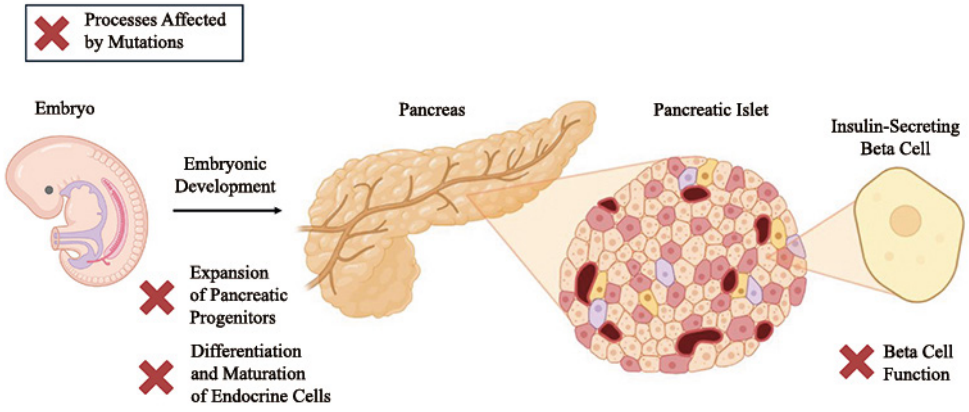
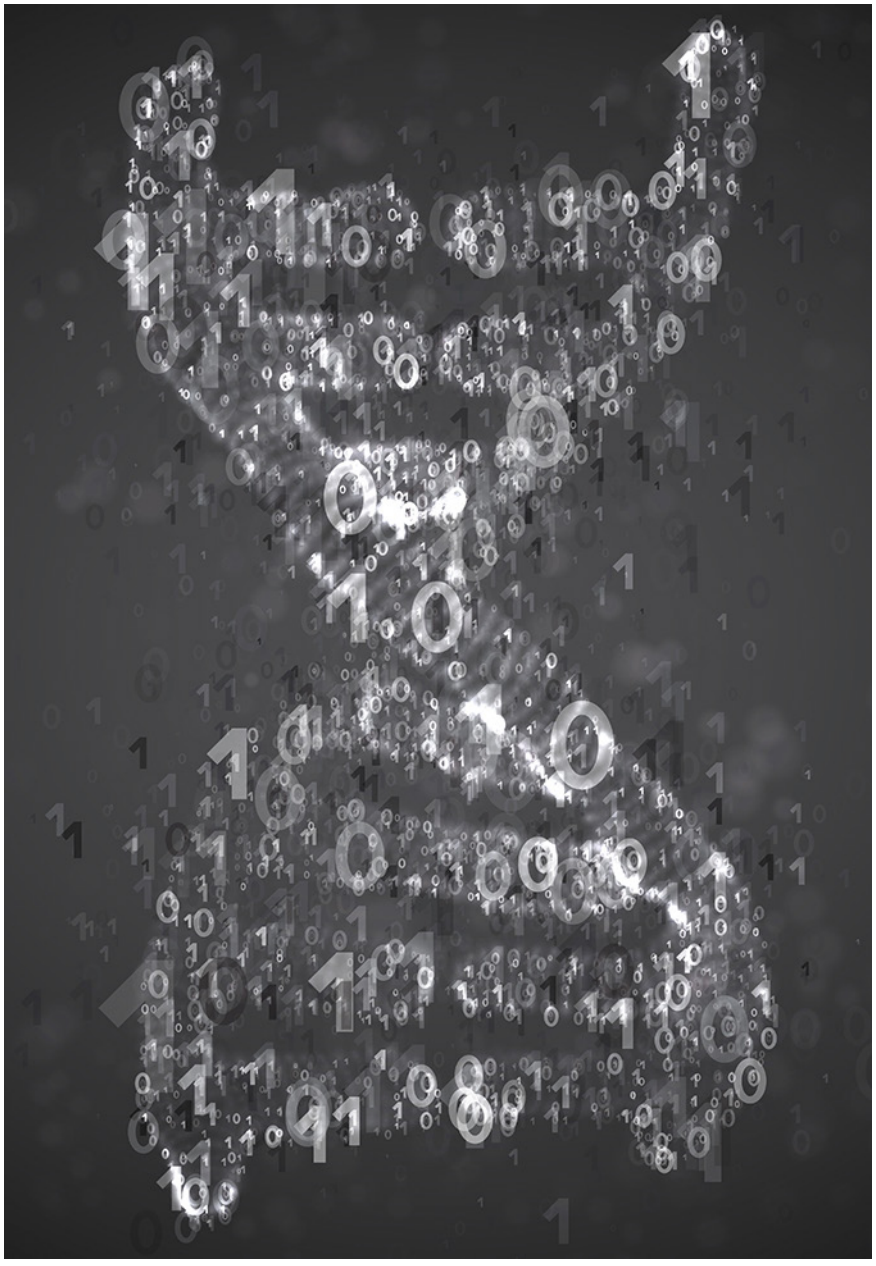


FIGURE 1. Processes affected by mutations leading to monogenic diabetes. These mutations can impair both embryonic development and the function of mature beta cells.

GENETIC EDITING TOOLS SUCH AS CRISPR/CAS9 ALLOW US TO INTRODUCE SPECIFIC MUTATIONS INTO STEM CELLS TO GENERATE MODELS THAT REPLICATE THE GENETIC CHANGES ASSOCIATED WITH MONOGENIC DIABETES



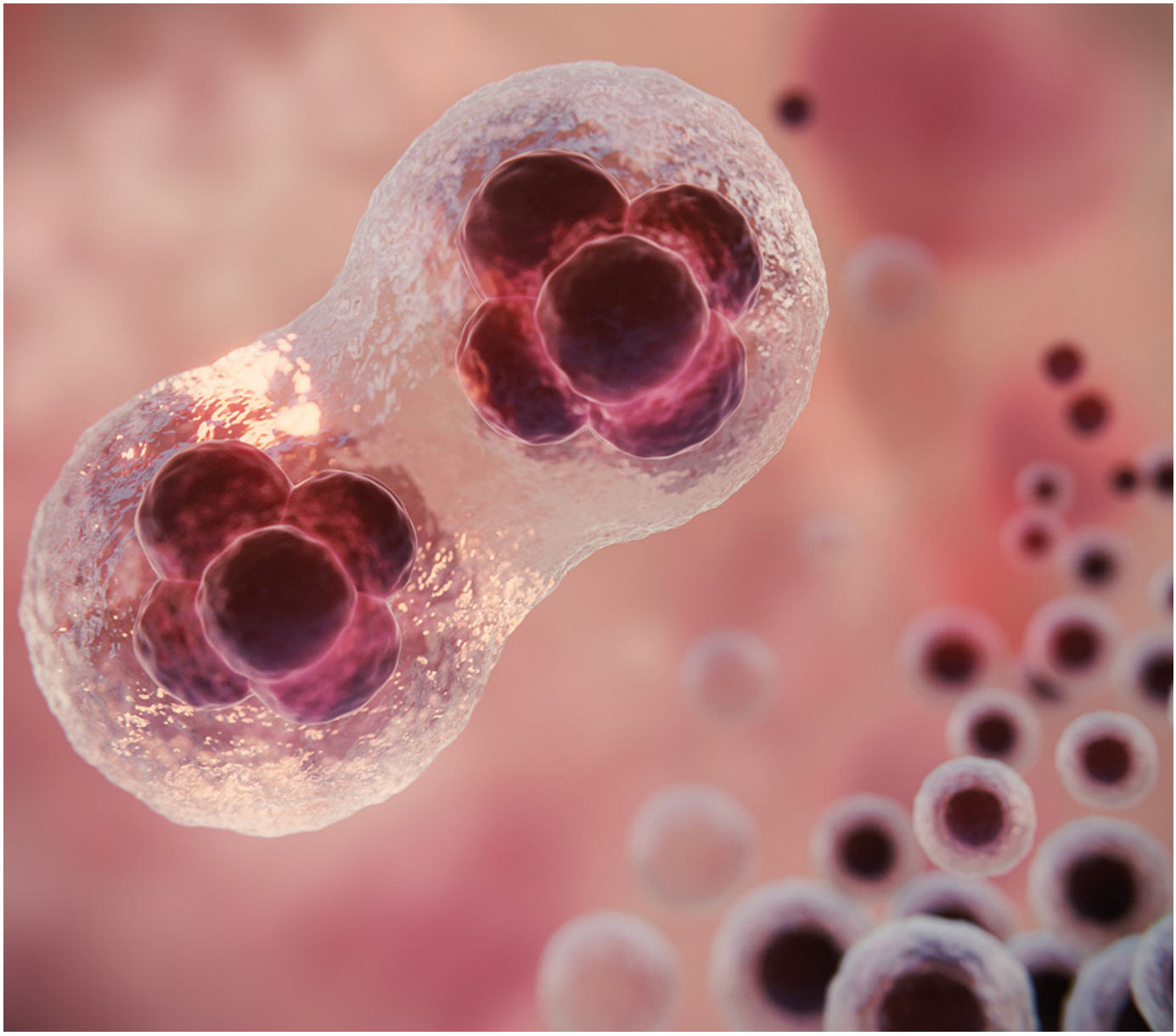
» et al., who introduced insulin therapy over a century ago, to the development of modern therapies such as GLP-1 receptor agonists, these models have been crucial for advances in diabetes treatment.

However, animal models have significant limitations, especially for studying monogenic forms of diabetes. Although rodents and humans share many signaling pathways involved in pancreatic development, the differences are considerable and prevent the translation of many advances made in animal models. A paradigmatic example is the recently described role of the ZNF808 gene (4), which has no equivalent in the genomes of rodents or other experimental animals and whose pathogenic variants have been associated with pancreatic agenesis and neonatal monogenic diabetes in humans. Even in the case of shared genes, such as HNF1A, animal models do not adequately reproduce the clinical characteristics of the human disease (5).

Alternative models, such as human pancreatic islets obtained from donors, offer greater proximity to the human context. However, these models also have their own limitations: their availability is scarce, and it is unfeasible to use them as a model for monogenic diabetes due to the rarity of cases. Additionally, adult islets do not allow the study of embryonic development, a critical aspect in many monogenic forms of diabetes. These restrictions underscore the need to develop more advanced models to better understand these diseases.

HUMAN PLURIPOTENT STEM CELLS AS AN ALTERNATIVE MODEL

Human pluripotent stem cells have become »



a revolutionary tool for modeling human diseases. These cells can self-renew indefinitely and differentiate into any cell type, including endocrine islets containing beta cells. This process can be reproduced in the laboratory using growth factors and other molecules that act as agonists or inhibitors of certain cellular signaling pathways, guiding stem cells through the different stages of pancreatic development to generate endocrine aggregates containing beta cells. This allows the reproduction of both embryonic development and the effects of genetic mutations in a human context. Moreover, their use is more accessible and cost-effective vs animal models or obtaining islets from donors.

Well-established protocols exist for differentiating stem cells into functional beta cells, mimicking the stages of pancreatic development: from definitive endoderm to pancreatic and endocrine progenitors, culminating in the generation of beta cells capable of synthesizing and secreting insulin. Although initially these protocols generated immature cells, recent optimization allows the production of cellular aggregates that partially emulate the architecture of human pancreatic islets (6), including essential cellular interactions. These cells can achieve a level

WELL-ESTABLISHED PROTOCOLS EXIST FOR DIFFERENTIATING HUMAN PLURIPOTENT STEM CELLS INTO FUNCTIONAL BETA CELLS, MIMICKING THE STAGES OF PANCREATIC DEVELOPMENT

of functional maturity nearly equivalent to that of native islets, positioning them as a promising option for both research and therapeutic applications.

Indeed, stem cell-derived beta cell therapies are emerging as an innovative solution for treating diabetes, particularly T1DM (7). These therapies aim to restore insulin production by replacing destroyed beta cells with functional stem cell-derived beta cells. Beyond their therapeutic potential, these cells also constitute an unprecedented model for investigating the effects of genetic variants in human development, overcoming the limitations of animal models and opening new possibilities in understanding the genetic basis of diabetes.

GENETIC EDITING AND APPLICATION IN MONOGENIC DIABETES

Genetic editing using tools such as CRISPR/Cas9 allows the introduction of specific mutations into stem cells in a relatively straightforward manner, enabling the generation of models that faithfully replicate the genetic alterations associated with monogenic diabetes found in patients. Lines with variants in genes such as PDX1, HNF1A, or RFX6, among others, have been generated to study their effects on pancreatic development (8). These models allow the investigation of all stages of pancreatic development, from the initial phases to the formation of endocrine aggregates containing mature beta cells. With them, it is possible

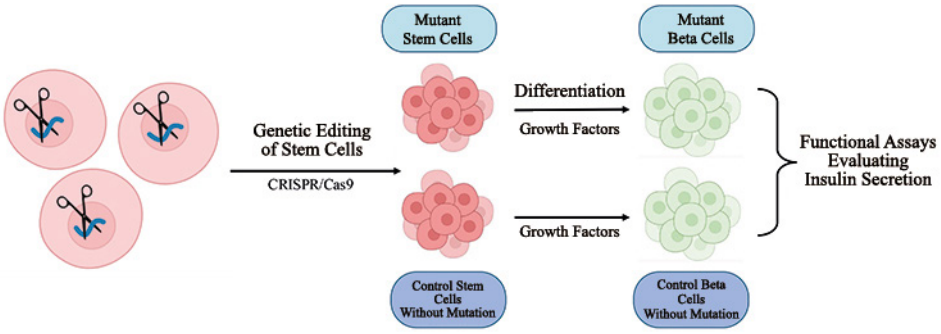


FIGURE 2. Schematic representation of the use of human pluripotent stem cells in the study of monogenic diabetes. Stem cells are genetically edited using tools such as CRISPR/Cas9 to generate stem cell lines with variants in specific genes. Following certain differentiation protocols, beta cells can be obtained from stem cells. By comparing beta cells with and without mutations through functional assays, the effect of the variant on beta cell function is determined.

» to conduct functional assays to evaluate whether insulin secretion is normal or altered, comparing it with controls without pathogenic mutations ***(Figure 2)***.

In addition to providing a detailed molecular understanding of the causes of monogenic diabetes, these models constitute an essential tool for testing therapies designed to improve insulin secretory function in the context of mutations associated with these forms of diabetes. Genetic editing not only allows the study of alterations but also their correction, opening the door to future therapeutic strategies. For example, in cases of permanent neonatal diabetes caused by mutations in the insulin gene, it would be possible to treat patients with transplants of stem cell-derived beta cells with the corrected mutation (9). This represents a potentially curative approach for some forms of monogenic diabetes.

However, pluripotent stem cells also have limitations. Although they are a powerful tool for modeling diseases, they do not replicate the complexity of a complete organism, where interactions with other tissues and systems play a critical role in the disease. Additionally, the costs associated with maintaining these lines and differentiation experiments remain high, which also impacts the economic viability of stem cell-derived therapies (10). D

CONCLUSIONS

Human pluripotent stem cells are a revolutionary tool for investigating the genetic basis of diabetes and modeling the disease in a human context. Their ability to reproduce pancreatic development and associated genetic mutations offers new opportunities to unravel the molecular mechanisms of the disease and advance toward personalized therapies. Despite technical and cost challenges, their potential to transform both research and the clinical management of diabetes is undeniable.

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