Implications of human induced pluripotent stem cells in metabolic disorders: from drug discovery toward precision medicine

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Human induced pluripotent stem cells (hiPSCs) enable *in vitro* high-throughput pharmacological screening assays of diseased tissue. Together with recent genome-wide association studies (GWAS), hiPSCs enable the identification of key mutations for the development of effective treatments based on precise drugs. In concert with CRISPR/Cas9 systems, hiPSC technology can reveal therapeutic targets in metabolic disorders. The *ex vivo* CRISPR correction of autologous patient-derived hiPSCs has led to the development of replacement cell therapies, providing better patient prognoses.

Introduction

The incidence and prevalence of metabolic disorders such as insulin resistance, type 2 diabetes, dyslipidemia and metabolic syndrome have increased drastically in recent decades. Although several pharmacological agents are available, only a fraction of patients respond effectively to treatment regimens [1–5], owing to the genetic variability inherent in the population and the lack of specific clinical trials, which focus on individual responses and not usually to therapy – something referred to as 'precision medicine' [6]. Induced pluripotent stem cell (iPSC) technology has been widely pursued since its initial introduction in 2007 by Takahashi *et al*. [7]. The cells are defined as embryonic-like pluripotent stem cells, obtained from somatic cells by ectopic expression of pluripotency transcription factors: SOX2, NANOG, Oct3/4, among others, and can be maintained indefinitely *in vitro* [8]. This tool offers a unique opportunity to better understand the pathophysiology of diseases, facilitating the search for new therapeutic targets on which to perform drug screening, gene therapy and new therapies in the field of regenerative medicine [9,10]. Through differentiation, hiPSC technology allows somatic cells from sources such as skin fibroblasts, keratinocytes [11–13], peripheral blood cells, umbilical cord cells [14–16] and desquamated cells [17–19] to be reprogrammed to the state of stem cells. Patient genomic information is maintained during reprogramming and differentiation [20]. Alternatively, specific patient cells can be produced for autologous transplantation [21]. Because hiPSCs are derived from somatic tissues, this technology overcomes ethical problems that have overwhelmed human embryonic stem cells (hESCs), such as the destruction of human embryos [22]. Cells can be obtained in unlimited quantities, can be cultivated for prolonged periods and can be differentiated into cell types that constitute human organs [23]. However, there are challenges faced by hiPSCs, which have been noted in recent years. First, the similarity they have with cancer cells. As Knoepfler declared: the process of making iPSCs is scarily similar to the process of creating 'oncogenic foci' (OF) – a form of *in vitro* created malignant sarcoma. During OF assays, the fibroblasts are transduced with virus-encoding genes like Myc, SV40 and mutant p53 and expand the cells *in vitro*. Interestingly, if these are injected into immunodeficient mice they form malignant sarcoma tumors [24]. Second,

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the FDA and EMA regulatory issues are stringent, owing to the consequences that can arise when transferring hiPSC use to the clinic [25]. Third, if there is a reprogramming protocol based on incorporating the transcription factors there is the possibility of making epigenomic changes derived from the reprogramming process itself, regularly accompanied by incomplete or aberrant reprogramming events manifesting at the epigenomic level as unusual patterns of histone modifications [26]. Other challenges involved reflect that the process of making clinical-grade hiPSCs is time-consuming and very costly, as estimated by the majority of the published work – it takes an average of 6 months and at least US $100 000. Some of these problems are current active areas of research, designed to obtain safer hiPSCs for personalized therapy in patients and to properly model complex diseases in a dish. Here, we focus on recent progress in the modeling of metabolic pathologies, such as diabetes mellitus, and analyze modern iPSC screening techniques.

Type 1 diabetes mellitus

Diabetes mellitus type 1 (T1DM) is an autoimmune disease characterized by the destruction of β cells in the pancreas, which results in severe insulin deficiency, the persistence of high levels of glucose in blood and, consequently, a lifelong dependence on insulin-replacement therapy [27,28]. Restoration of normal pancreatic function could be achieved by replacing pancreatic cells obtained from reprogrammed somatic cells of patients with T1DM, in a similar way to what was obtained in some works where the differentiation of the ESCs was carried out [29,30]. The successful generation of functional β cells derived from iPSCs obtained from human fibroblasts was first reported by Tateishi and co-workers who developed functional cluster-type islets composed of a mixture of C-peptide + glucagon + cells. The analysis of molecular markers through quantitative reverse-transcriptase PCR (qRT-PCR) coupled with immunizations during differentiation of insulin-producing islets demonstrated the positive expression of markers, such as genes and stage-dependent antigens, in each development stage: definitive endoderm cells [Forkhead box A2 (FOXA2) and SRY-box17 (SOX17)], pancreatic endoderm cells [pancreatic and duodenal homeobox 1 (PDX1)], endocrine and exocrine cells [NK6 homeobox 1 (NKX6.1), pancreas-associated transcription factor (PTF)1, insulin] and insulin-producing cells (insulin, C-peptide, glucagon), were produced in a manner similar to hESCs [31].

This technology has also been used to evaluate factors such as PDX1 (insulin promotor factor 1) and NKX6.1 (required to develop β cells) that determine the maturation of β cells [31,32]. Differentiated hiPSCs simultaneously produced insulin, glucagon and somatostatin [31,32], resemble to the work performed by Bruin *et al.* in the obtainment of polyhormonal insulin-producing cells derived from ESCs [33]. On the other hand, β cells derived from hiPSCs (SC-β cells) behave similarly to the cells obtained from cadaveric islets, during sequential glucose challenges *in vitro* [34]. A high glucose environment was associated with an increase in intracellular calcium levels and the presence of genes for mature β cells in SC-β (NKX6.1 and PDX1) was evident in a global genetic profile. Although an identical genetic profile was not obtained for cadaveric β cells, the SC-β cells derived by Pagliuca *et al.* represent the most functional in the search for new therapies [34]. SC-β cells developed by Pagliuca et al. showed a sustained monohormonal production of insulin when liver capsules were transplanted in immunocompromised mice [34], and glucose-stimulated insulin secretion similar to that of human islet cells was observed 2 weeks posttransplant. In murine diabetes mellitus models, hyperglycemia was controlled using SC-β cells in the same way as it is controlled with cadaveric islet transplantation; SC-β cells presented lower fasting blood glucose compared with that of transplanted polyhormone cells in murine models and control mice. SC-β-cell-transplanted mice sustained insulin secretion up to week 18 and exhibited a decrease in morbidity and mortality compared with control mice [34,35].

By contrast, embryonic stem cells (ESCs) can be differentiated toward the endoderm germ layer and into insulin-producing cells [36,37]. Recently, Robert and collaborators generated devices that encapsulate hESC-derived pancreatic endoderm (hES-PE) and determined the biologic characteristics with which implants establish metabolic control during a 50-week follow-up in immunecompromised mice. A metabolically adequate functional beta mass was achieved by (i) formation of a sufficient β cell number (>0.3 × 106/mouse) at >50% endocrine purity and (ii) their maturation to a functional state comparable with human pancreatic β cells, as judged by their secretory responses during perfusion; their content in typical secretory vesicles and their nuclear NKX6.1-PDX1-MAFA co-expression were also obtained [30]. By contrast, they concluded that the glucose-induced C-peptide release was not sufficient to stipulate that the functionality as mature β cells had been formed [30,38].

Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a disease characterized by subnormal insulin secretion from pancreatic β cells as a result of genetic, epigenetic, environmental or lifestyle risk factors [39,40]. Currently, >415 million people worldwide suffer from T2DM [39]. The pathogenesis of diabetes is not well understood and there is no effective treatment for this condition [40]. *In vitro* modeling of patient hiPSCs might identify the pathogenesis of the disease and suggest therapeutic targets and cell therapy. One therapeutic alternative is the transplantation of hESC grafts to complement differentiation of mature β cells, as well as the transplantation of pancreatic progenitors (DE cells) [41,42]. Co-transplantation of undifferentiated hESCs in conjunction with mouse embryonic dorsal pancreatic cells resulted in the generation of undifferentiated insulin-producing functional cells in mice [42]. However, transplantation of hESCs in mouse embryonic liver tissue did not produce insulin. Many signals in the pancreatic microenvironment that involve cell differentiation have not yet been elucidated and such signals could have a central role in the differentiation and development of β cell function [35,43]; hiPSC technology is a promising alternative to evaluate the regulatory pathways in pancreatic development.

Owing to the intricate etiopathogenesis of T2DM, there are few studies in the literature that obtained hiPSCs derived from patients for the purpose of modeling the disease. Ohmine *et al.* first demonstrated the iPSC derivation from epidermal keratinocytes in elderly patients with T2DM. They observed a reestablishment of the rejuvenated state that shows elongated telomeres and suppressed senescence-related p15INK4b/p16INK4a gene expression and oxidative stress signaling. Then, they differentiated the hiPSCs into insulin-producing islet-like progeny, which expressed insulin, glucagon, somatostatin and GLUT2 [43]. Hence, a robust platform

based on hiPSC-T2DM for drug screening was established, leading to regenerative medicine applications.

Diabetes has a negative effect on the cardiac muscle, independently of the other muscular influences such as coronary disease; this condition is known as diabetic heart disease [44]. Drawnel *et al.* [45] used hiPSC models to induce a phenotypic surrogate of diabetic cardiomyopathy, observing structural and functional disarray. Interestingly, the cardiomyopathic phenotype recapitulated the patient-specific original clinical status. Later, they established an *in vitro* screening platform to identify small molecules that rescue the function of cardiomyocytes (cardiac muscle cells) from diabetic stress [46].

Obesity

Obesity is a chronic, complex, multifactorial disease that usually manifests in childhood or adolescence, its origin is a genetic and environmental imbalance between energy intake and expenditure. Obesity is a serious international health problem; >2.1 billion adults are overweight and 400 million overweight adults are considered to be obese worldwide [47]. Obesity is a risk factor for the development of insulin resistance, diabetes, metabolic syndrome, cancer and osteoarthritis, thus decreasing life quality and life expectancy [48,49]. Endothelial cells derived from hESCs (hESC-EC) have been evaluated in models of myocardial infarction and ischemia of the hind limbs and are used in angiogenesis and neovascularization treatment [50–52]. However, because ethical implications restrict the use of hESC-EC, hiPSC technology is a less contentious option for the treatment of vascular ischemia [52,53].

There is little information available about hiPSCs obtained from endothelial cells (hiPSC-EC) of overweight individuals. In addition, the molecular mechanism that causes the endothelial dysfunction or impaired vascular function in the native ESCs remains unknown. Several research groups have evaluated the derivation of hiPSC-EC to examine the molecular pathways involved. Gu *et al.* showed that hiPSC-EC derived from mice with diet-induced obesity (DIO) exhibited decreased vascular function *in vitro* and reduced function and vascular incorporation in the host *in vivo* [53]. Histological evaluation showed muscular atrophy and an increased infiltration of inflammatory cells in the hindlimbs of mice that received hiPSC-EC from mice with DIO. EC dysfunction was associated with apoptosis and inflammation, whereas oxidative stress was due to a decreased production of nitric oxide (NO). Another finding was that coadministration of low doses of hiPSC-EC from mice with DIO and pravastatin reversed EC dysfunction *in vivo* and *in vitro* in a murine ischemia model [53]. These results could lead to new discoveries in patient-specific hiPSC-EC therapies in a prediabetic population or in obese patients with peripheral vascular disease [53]. Paneni *et al.* pointed out that a central finding by Gu *et al.* was that epigenetic markers related to DIO are apparent on hiPSC-EC despite cellular reprogramming with transcription factors, suggesting that hiPSC-EC preserve a memory of the disease [54]. It has also been shown that epigenetic modifications derived from environmental stimuli can promote cardiometabolic disturbances [55]. Possible dysregulation before reprogramming could be an important inconvenience when carrying out cellular transplantations. Such problems are currently being eradicated through clustered regularly interspaced short palindromic repeat-associated Cas9 (CRISPR/Cas9) gene editing, enabling cells to be transplanted, eliminating their original genetic pathological conditions (Fig. 1).

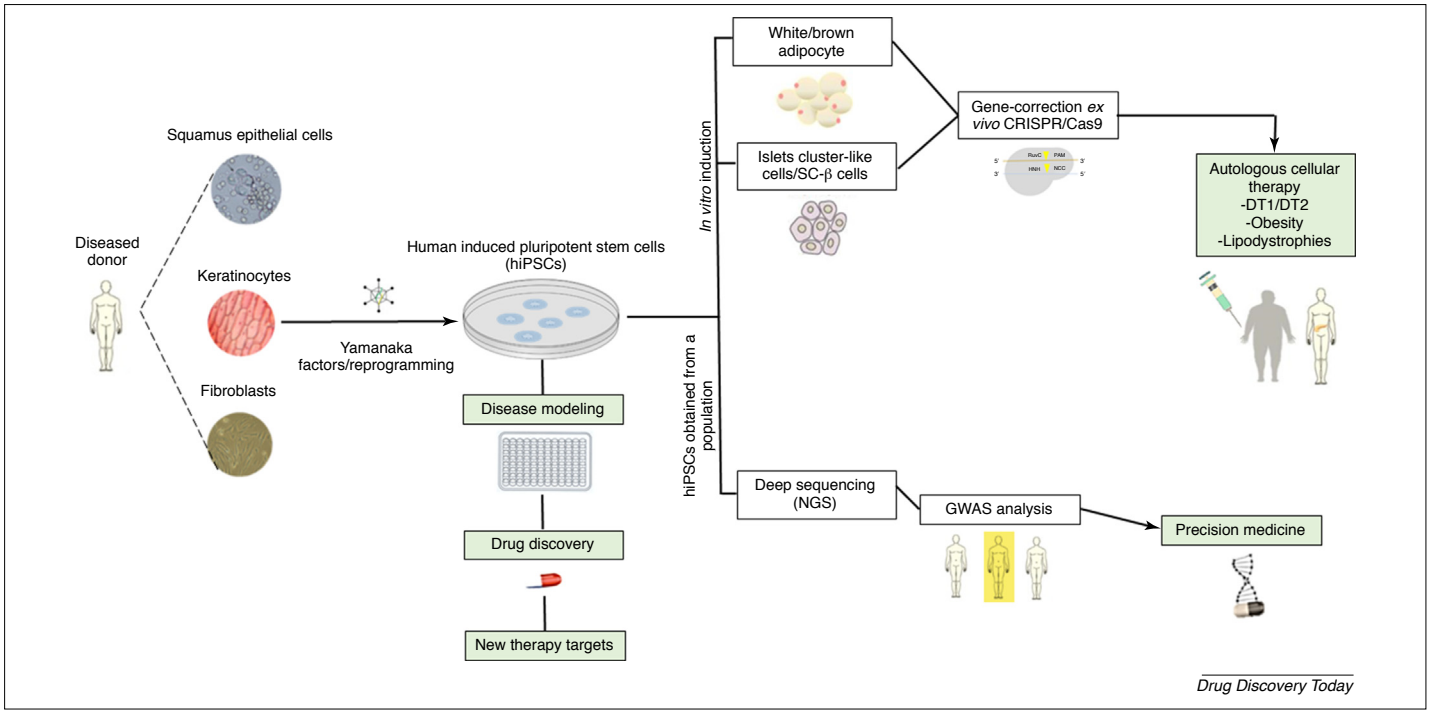

FIGURE 1

Applications of induced pluripotent stem cell (iPSC) technology in metabolic disorders. The reprogramming of human cells from healthy or diseased patients has allowed *in vitro* reprogramming for β cells and functional islets, as well as differentiation into brown and white tissue adipocytes, coupled with the CRISPR/Cas9 editing system and new bioinformatics analyses, allowing the search for customized therapeutic targets and on-site cellular therapy.

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is a prevalent (30% in Western countries) metabolic disorder characterized by a broad spectrum of liver damage ranging from simple steatosis (i.e., excessive hepatic fat accumulation) to steatohepatitis (i.e., steatosis with associated inflammation), advanced fibrosis and cirrhosis. Fatty livers are a common occurrence in the general population, mainly owing to sedentary lifestyles and high-fat diets, and fatty livers can progress to cirrhosis and liver failure [56]. NAFLD is associated with insulin resistance, T2DM and obesity. Genome-wide association studies (GWAS) have identified polymorphisms in several genes: patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), apolipoprotein 3 (APOC3), neurocan (NCAN) and protein phosphatase 1 regulatory subunit 3B (PPP1R3B), that confer susceptibility to NAFLD [43]. Hepatocyte-like cells (HLCs) derived from hiPSCs (hiPSC-HLC) could be used to study NAFLD. Different nutritional conditions can be mimicked using *in vitro* cultures of hiPSC-HLC to control specific effects on metabolism and lactate dehydrogenase (LD) incorporation. In contrast to primary hepatocytes, the hiPSC-HLC number is not limited, enabling the development of HTS studies; hiPSC-HLC also remain longer in culture than primary hepatocytes before they become detached, which extends the time available for analysis [57].

Progress has now been made related hiPSC in the treatment of nonalcoholic fatty liver disease [58]. Graffmann *et al*. developed an *in vitro* model of a nonalcoholic fatty liver (NAFLD/steatosis) based on ESCs and hiPSCs. They used ESC line H1 and iPSCs derived from fetal foreskin fibroblasts to compare two distinct hPSC lines [59]. Morphological changes were monitored at every step of the differentiation process. They observed that ESCs and iPSCs behaved similarly during differentiation and maturation. Hepatocyte markers were positive for albumin, HNF4a and E-caderin in hiPSC-HLC and ESC-HLC. At this point of the study, the authors demonstrated the resemblance of the hiPSCs with the ESCs as a potential source of hepatocyte-like cells for disease modeling. Furthermore, steatosis was induced in the hiPSCs throughout oleic acid (OA) and a significant increase was observed in the regulation of lipid coating, liquid droplet coating perilipin 2 (PLIN2) and numerous genes in the peroxisome proliferator-activated receptor (PPAR) pathway. Interference with PLIN2 and PPARα resulted in significant alterations in gene expression, especially affecting the metabolism of lipids, glucose and purines. Remarkably, the hiPSC-HLC model reflected many metabolic changes typical of NAFLD, enabling the dissection of molecular pathways associated with metabolic diseases involving lipid metabolism and the correlation of disease progress and patient genetic backgrounds [59]. Cayo *et al*. generated hiPSC-HLC using iPSCs obtained from patients with homozygous familial hypercholesterolemia (hoFH) to search for drugs that could reduce low-density lipoprotein cholesterol (LDL-C) levels in serum [60]. It was found that cardiac glycosides reduced the production of apolipoprotein B (apoB) in human hepatocytes *in vitro* and in the serum of avatar mice carrying humanized livers, through the pharmacological mechanism of increased protein turnover (i.e., balance between synthesis and protein degradation) of apoB. It was also observed that patients treated with cardiac glycosides had reduced serum levels of LDL-C. The authors concluded that the use of hiPSC-HLC in the identification of drugs to treat congenital liver metabolism disease had a cost benefit, and that hiPSC-HLC could be used to discover new molecules that can cope with diseases in the liver and the cardiovascular system [60].

Discovery of new drugs associated with hiPSCs in metabolic disorders

The discovery and conventional development of drugs is based on traditional animal models such as mice, rats, pigs and monkeys. However, such models rarely reflect the pathological mechanism of human disease. For example, mice are at least ten times more tolerant to 37% of drugs than humans, whereas the tolerance to concentrations of various chemical compounds is between 4.5 and 100 times higher in rats and dogs than in humans. The most notorious example of a drug that was considered safe after animal testing but had significant devastating effects in humans was thalidomide, which had no effect on prenatal development in rodents but caused serious developmental defects in human children whose mothers took it during pregnancy to avoid nausea [60]. Although animal models can provide clues to the human response to a chemical, differences between animals and humans in reactions to a drug must be considered along with other parameters [61]. Such factors prolong the testing and raise the cost of drug development [62]; the average time to develop and launch a new drug to the market is 10 to 15 years, and the cost is ~US$5 billion.

Interestingly, as Han *et al*. declared, current cell and animal models might not predict whether a drug candidate is likely to modify disease progression or improve patient behavior. Added to the lack of transparency in communicating and sharing data for negative outcomes in clinical trials and other studies, this hinders the possibility of finding a common solution [63]. In contrast to the present *in vivo* models, emerging drug screening in hiPSCs represents a dynamic platform for high-throughput drug discovery studies, owing to the patient fidelity that cells retain, toxicity and dose–response assays are more accurate. Another crucial aspect is the validation of the targets; the evaluation of the hit drug candidates *in vitro* must be tested in the metabolic disorder patient-derived hiPSCs [63] mentioned above as SC-β-hiPSC/CM-hiPSC/HLC-hiPSC to analyze the phenotype changes in all the cell lines. Therefore, a robust data platform should increase predictive response to personalized refined drugs, selected before the evaluation in patients. One example of this approach is the collaboration of the groups that develop the MNI iPSC/CRISPR platform (https://www.mcgill.ca/ipsquebec-mni/) [62]. However, some characteristics of hiPSCs must be refined hereafter as the heterogeneity of the patient-derived hiPSC lines and the epigenetic memory for the sake of a safer technology (Fig. 2).

Physiologic fidelity and epigenetic memory of patient-derived hiPSCs

Recent evidence shows that hiPSCs retain significant epigenetic memory of the somatic state of donor cells, causing a shift in the cell differentiation spectrum; that is, the hiPSCs differentiate into somatic cells of the initial type [64]. Epigenome high-resolution studies provide a clearer picture of the remodeling

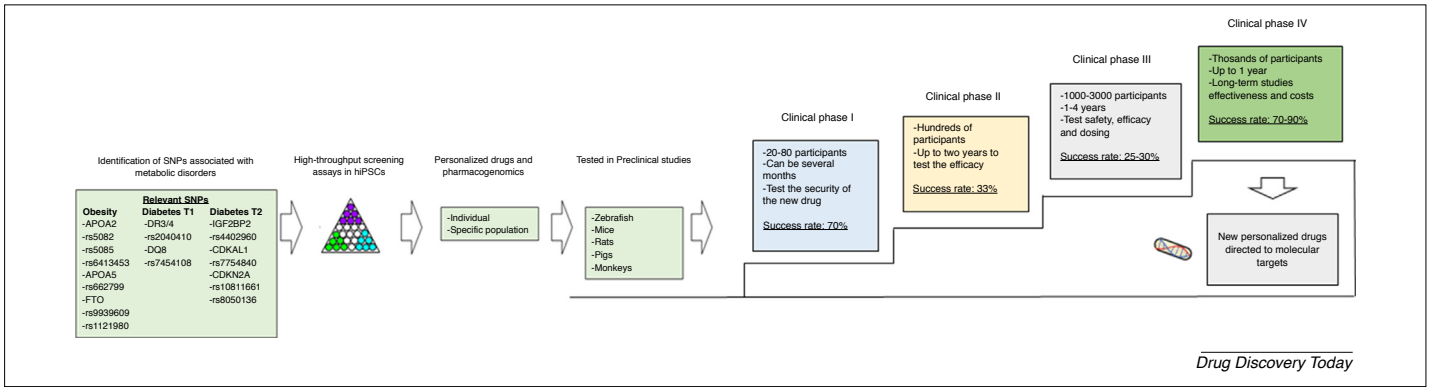

FIGURE 2

Workflow of discovery and development of new drugs in metabolic disorders. First, the most relevant single nucleotide polymorphisms (SNPs) associated with diabetes T1/2 and obesity were identified, then a reprogramming of patient cells was performed for an HTS in the induced pluripotent stem cells (iPSCs) of >1000 molecules to point out novel therapeutic targets. Then, personalized drugs (pharmacogenomics) were obtained for an individual or a specific population. Subsequently, the drugs were evaluated in preclinical models and, if remarkable results were obtained, clinical trials were started, passing Phases I to IV. If this succeeds, they will begin to produce iPSCs on an industrial scale, bringing to market new drugs based on precision medicine.

brought about by the reprogramming process in the epigenetic hiPSC landscape and most recently used methyl cytosine (CpG, where C = cytosine, p = phosphate and G = guanine) sequencing analysis, which allows the methylation of cytosine to be determined in the complete genome, with a level of nucleotide resolution even in non-CpG methylation regions (most methylcytosines are found upstream to a guanine) [26]. Lister *et al.* reported the whole genome profile of DNA methylation in human iPSC lines: (i) iPSCs derived from adipocytes using transduction by retroviruses, (ii) iPSCs obtained using lentivirus, (iii) iPSCs obtained from IMr90 lung fibroblasts, and (iv) three iPSC lines obtained from foreskin fibroblasts using vectors of non-integrative episomes. The findings indicated the existence of 'hot-spots' lacking epigenetic reprogramming, probing the principle that aberrant methylation sites exist in some parts of the genome after the process; furthermore, iPSCs share megabase-scale differentially methylated regions, proximal to centromeres and telomeres that display incomplete reprogramming of non-CG methylation, and differences in CG methylation and histone modifications. To conclude, differentiation of iPSCs into trophoblast cells revealed that errors in reprogramming CG methylation are transmitted, therefore a broad study of functionality is necessary [26].

In another study, a particular feature of epigenetic memory was found in iPSCs derived from elderly patients [65]. Epigenetic signatures were evidenced throughout the episomal/Yamanaka reprogramming pathway in peripheral blood cells of 16 donors aged 26–100 years. By 450 k array methylation assay, they found a reduction in the methylation profile over extended passages in the cell lines [66], concordant with the results of Nishino *et al.* [67] Cells from older donors were significantly different from cells from younger donors at the point of passage 8 in the methylation assay. These changes in the methyloma could be reduced or eliminated with the extension of the number of passages in the culture. Therefore, one line was expanded per donor for 20 additional passages. All but six of the 34 persistent changes in methylation were restored to levels similar to those of younger donors [65]. Metabolic disorders tend to increase with age and it has been shown that the efficiency of reprogramming can be affected [67]. Therefore, methylation changes in the DNA of older donors could have a deleterious effect on regenerative medication and cell therapy and must be considered in autologous cell therapy based on hiPSCs. Fortunately, it has been shown that such changes could be reduced by extending the reprogramming. In parallel, new cell reprogramming technologies can generate lines free of constitutive integration or exogenous material (so called 'footprint-free') [68,69]. Continuous transfection of free virus type vector-plasmids [70], lentiviruses, minicircles of polycistronic vectors [71], modified synthetic mRNA [72], polyethyleneimine (PEI) nanoparticles [73] and small synthetic molecules such as valproic acid and butyrate [74] can successfully express the necessary transcriptional factors to generate pluripotency in somatic cells. Moreover, it has been shown that the use of chemical platforms as Lin *et al.* may dramatically increase the reprogramming process by 200 fold [75].

Impact of relevant single nucleotide polymorphisms in pharmacogenomics

Teo *et al.* classified candidate genes and single nucleotide polymorphisms (SNPs) associated with diabetes mellitus (DM) and obesity in disorders such as monogenic diabetes, neonatal diabetes, mitochondrial diabetes and syndromes of insulin resistance [76]. They found many associated genes and up to three related SNPs were described for some genes. Dissections of the metabolic pathways involved in conjunction with nucleation-based editing tools allowed the relevance of these genetic variations to be determined. SNPs exhibited only modest effects and 85% of the variants were assigned to noncoding regions that employed enhancers or regulatory elements [76]. GWAS have found that diseases can involve the direct participation of genes. A clear example of the genotype–pathogenesis association has been observed in DM, where it was demonstrated by a complete genome association analysis that loss of function of NKX6.1 led to dysregulation in the synthesis, insulin secretion and proliferation of β

cells, culminating in diabetes [77]. In other studies, the differentiation of hiPSC-T1DM in T lymphocytes and pancreatic β cells has been demonstrated to form cocultures. Because the central focus is the resistance to insulin of different tissues, hiPSC-T1DM has been used to derive myocytes from skeletal muscle, adipocytes, hepatocytes and pancreatic β cells to dissect and elucidate signaling pathways related to cell death, dedifferentiation and altered secretory function in T2DM [78,79,80].

Rich and Cefalu examined how personalized medicine must be based on genetic diagnosis (pharmacogenomics) to prescribe appropriate drugs for individual patients with T1DM or T2DM [81]. Because the TBC1D4 mutation exclusively increases postprandial glucose in the blood, Manousaki *et al*. performed exome sequencing on 114 Canadian Inuit samples to detect the variant TBC1D4 and performed specific sequencing in 1027 Alaskan Inuit samples. The presence of the TBC1D4 mutation was determined in 27% of the Inuit from Canada and Alaska, and two copies of the mutation were associated with biochemical changes such as increased levels of glucose and insulin in the blood 2 h after ingesting glucose. Thus, precision medicine can aid the treatment of diabetes in this population [82]. In another example of precision treatment, 833 T2DM patients with genetic variants in the SLCO1B1 drug transporter gene and the CYP2C8 metabolizing enzyme gene received treatment with pioglitazone or rosiglitazone (tiazolidinedione derivatives), which have been used in combination with other oral agents for the treatment of T2DM acting as peripheral insulin sensitizers by activating the nuclear PPARγ [82,83]. The CYP2C8 variant was associated with a reduced glycemic response to rosiglitazone and less weight gain, and the SLCO1B1 variant was associated with an enhanced glycemic response to rosiglitazone. Neither of the variants had a significant impact on the response to pioglitazone [84,85].

Recent advances in the study of stem cells include the development of organoids – *in vitro* disease models with organ-like levels of organization. In some cases, technologies can be coupled – hiPSC-CRISPR/Cas9-organoides – to more closely model a pathology and to evaluate personalized therapies. Broutier *et al*. cultivated adult stem cells from patients with metabolic disorders and established the self-renewal of these cells to form of organoids of intestine, stomach, liver and pancreas [86], providing an *in vitro* culture system for studying hepatic stem and progenitor cells to establish adequate treatments for individuals affected by these pathologies. This 3D approach enables the formation of self-renew organ-like tissue with the genomic variations (e.g., relevant SNPs) of the patients and could dramatically improve the personalized design of drugs in those affected by metabolic disorders. A breakthrough study by Dekkers and colleagues utilized patient-specific intestinal organoids (i.e., mini-guts) to identify VX-770, which successfully treated two patients with rare mutations in the CFTR gene, severely reducing the function of the CFTR protein in cystic fibrosis [87]. This personalized enforcement could be applied in the coming years to design disease-modifying drugs in mini-stomach, mini-liver or mini-pancreas and will be taken to the next level with organ-on-chip systems for next-generation drug discovery [88,89]. Interestingly, the merger of these technologies – hiPSC-organoids-CRISPR/Cas9 – could be a game-changer in the development of personalized therapies.

CRISPR/Cas9 systems for remodeling epigenetic signatures in hiPSCs

The CRISPR/Cas9 gene editing system is the most efficient technology for making rational changes in the genome [89,90]; variations in this technology allow the epigenetic landscape to be explored in specific ways. Stepper *et al*. used a programmable construct composed of methyltransferase Dnmt3a–Dnmt3L fused to the nuclease-inactivated dCas9 to introduce DNA methylation in the human genome at sites corresponding to epithelial cell adhesion molecule (EpCAM), C-X-C motif chemokine receptor 4 (CXCR4) and transferrin receptor (TFRC) gene promoters. Efficient methylation of the promoters was achieved when individual single guide RNAs (gRNAs) were directed to the locus. The authors concluded that the CRISPR/Cas9 system can be programmed to introduce DNA methylation at defined genomic-endogenous loci, leading to transcriptional silencing [91]. Similar studies using CRISPR have been successfully developed and used to regulate gene expression [92–94].

Mechanical methylation of DNA carries a unique potential to correct epimutations in disease states [91]. However, directing DNA methylation requires an understanding of the functional importance of methylation in genetic repression and control of cell differentiation, and attention to epigenetic regulatory networks. Braun *et al*. developed the FIRE–Cas9 system and enhanced the dCas9–MS2 anchor for genome targeting with Fkbp/Frb dimerizing fusion proteins to allow the rapid and reversible recruitment of endogenous chromatin complexes to any genomic locus in almost any cell type. In contrast to other methods, this endogenous recruitment complex is inducible by proximity, which links causalities between epigenetic regulators and modification of histones in a matter of minutes [95,96]. Because many enzymes responsible for writing, erasing and reading epigenetic marks are present in protein complexes that bind chromatin [96] the design of controllable systems coupled to dCas9 using these enzymes could lead to the simulation of natural processes of methylation and demethylation, enabling transcription of key genes to be controlled *in vivo*. This system could be used to assure the target in the cellular differentiation of hiPSCs obtained by reprogramming cells derived from patients. There is a current limitation in differentiation protocols of very specialized cells, such as β cells and pancreatic islet cells, decreasing the chance of obtaining functional cells *in vitro*.

Concluding remarks

The molecular study of metabolic disorders increases the possibility of developing drugs that target individuals or population sectors. The reprogramming of somatic cells to hiPSCs provides invaluable tools for *in vitro* studies. Because hiPSCs obtained from a diseased individual maintain physiological fidelity, the molecular mechanisms involved in the disease can be identified and targeted with less effort and lower cost than traditional methods. The progress of hiPSC technology lies in the direction of personalized medicine, requiring new policies and regulatory frameworks to assess the safety of this emerging approach.

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