Induced pluripotent stem cell and their applications in Drug Discovery and Precision Personalized Medicine

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ABSTRACT

The ever improving technology to generate induced pluripotent stem cells (iPSCs) has increased their potential use as novel candidates for disease modeling, drug screening, regenerative medicine and cell therapy. Scientists can derive iPSCs from the skin, blood, various other cell sources from affected patients and then convert them into the cell types involved in the patient's disease. This process could allow researchers to study the development of a disease in a petridish, with the advantage of having a potentially endless supply of new cells. Ultimately, the goal of scientists as well as pharmaceutical companies is to use these petridish models to better understand the disease process and identify novel drugs to treat the disease. The result of this promising field may be the more rapid development of drugs from which millions could benefit. Because pluripotent cells are capable of generating any type of tissue in the body, the application of iPSCs therapy to treat various diseases. This technique also offers the possibility of repairing disease-causing genetic mutations before reintroducing the new cells, an approach that has been used with adult stem cells to treat leukemia and lymphoma. Patient-specific iPSC-derived cellular transplantation should also avoid the immune response since these cells are autologous. This review focuses on the applications of iPSCs with special respect to updates of drug discovery, personalized medicine and cell therapy.

Introduction

Precision personalized medicine describing the diagnostic, prognostic and therapeutic strategies precisely tailored to an individual patient's requirements is the future of effective patient treatment. This concept was predicted in the late 1800's by Canadian physician Sir William Osler who noted in 1892 "If it were not for the great variability among individuals, medicine might as well be a science, not an art.". Understanding these genetically- and/or epigenetically-influenced disparities between individual groups may lead to better diagnostic predictors, possibly, earlier detection and hopefully the development of fine-tuned, personalized therapeutics.

All cells that exist in the body, both somatic (of all three germ layers) and germ cells, originate from the pluripotent cells of the embryo. The establishment of human embryonic stem cells (hESCs) in 1998 together with their self-renewal and pluripotential capabilities raised hopes for regenerative medicine and drug discovery [1]. But, the challenges related to ethics, safety and the less availability of hESC lines have complicated the realization of these hopes. In 2006, Kazutoshi Takahashi and Shinya Yamanaka made the induced pluripotent stem cell (iPSC) from mouse skin fibroblasts, which have the indefinite self-renewal and pluripotent differentiation capacities like ESCs [2]. Later, James Thomson & George Daley & above group succeeded to convert human fibroblasts into hiPSCs [3-5]. At present, various other somatic cell types were also been used to make iPSCs [6-11]. Various reprogramming techniques have been used to generate iPSCs, including integrating vectors, non-integrating vectors, excisable integrating vectors and non-vector systems. A minimum set of four transcription factors namely, Kruppel-like factor 4 (Klf4), Myc (cMyc), octamer-binding transcription factor 4 (Oct4) and SRY (sex determining region Y)-box 2 (Sox2) were necessary for reprogramming. Currently, iPSCs also been developed from the various other methods, which gives opportunity for exploring disease etiology, developing new drugs and also for cell therapies for various diseases.

iPS CELLS AS A DISEASE MODEL

Conventional human disease research is performed using platforms such as epidemiology, genetics, genomic and epigenomic profiling, animal models as well as various, sometimes heterologous, in vitro cell culture models. However, these approaches have several complications. Human tissue or cell samples are often difficult to obtain. Generally the isolated cells cannot be maintained with current culture conditions, except if rendered immortal. But immortalization by genetic manipulation changes the normal cell physiology. Animal models are only suitable if the physiology of the experimental species is comparable to humans. The technology of patient- or disease-specific iPSC derivation coupled with directed differentiation to appropriate target cells circumvents these difficulties. hiPSCs can be generated from patients with genetic diseases and therefore, the derived target cells thus possess the same genetic background as the patient. This is important to elicit drug response. The ability of correct genetic disease-associated lesions in patient-derived iPSCs increases their potential for eventual cell based therapies. Hanna and

colleagues demonstrated that a mouse sickle cell anemia model could be treated by transplantation of hematopoietic progenitor cells derived from "autologous" iPSCs with a repaired sickle hemoglobin allele[12]. Patient specific cells make patient specific disease modeling possible wherein the initiation and progression of this poorly understood disease can be studied. Ebert et al., generated iPS cells from skin fibroblast taken from a patient with spinal muscular atrophy[13]. This is the first study to show that human iPS cells can be used to model the specific pathology seen in a genetically inherited disease. Thus, it represents a promising resource to study disease mechanisms, screen new drug compounds and develop new therapies.

Cardiac disease model

Itzhaki et al., obtained dermal fibroblasts from a patient with Congenital long QT syndrome type 2 (LQTS2) harboring the potassium voltage-gated channel subfamily H (eag-related) member 2 (KCNH2) gene mutation and showed that action potential duration was prolonged and repolarization velocity reduced in LQTS2 iPS cells derived cardiomyocytes (iPS-CMs)compared with normal cardiomyocytes[14]. They showed that Ikr was reduced in iPS-CMs derived from LQTS2. They also tested the efficacy of nifedipine and the ATP-sensitive K+ channel (KATP) opener pinacidil and demonstrated that they shortened the action potential duration and abolished early after depolarization. Matsa et al., successfully generated iPS-CMs from a patient with LQTS2 with a known KCNH2 mutation[15]. iPS-CMs with LQTS2 displayed prolonged action potential durations on patch clamp analysis and prolonged corrected field potential durations on microelectrode array mapping. Furthermore, they demonstrated that the KATP channel opener nicorandil and PD- 118057, a type 2 IKr channel enhancer attenuate channel closing. Malan et al. generated disease-specific iPS cells from a mouse model of a human LQTS3. Patch-clamp measurements of LQTS 3-specific cardiomyocytes showed the biophysical effects of the mutation on the Na+ current, withfaster recovery from inactivation and larger late currents than observed in normal control cells[16]. Moreover, LQTS3- specific cardiomyocytes had prolonged action potential durations and early after depolarizations at low pacing rates, both of which are classic features of the LQTS3 mutation.

Blood related disease model

Ye et al. demonstrated that human iPS cells derived from periphery blood CD34+ cells of patients with myeloproliferative disorders, have the JAK2-V617F mutation in blood cells[17]. Though the derived iPS cells contained the mutation, they appeared normal in phenotypes, karyotype, and pluripotency. After hematopoietic differentiation, the iPS cell-derived hematopoietic progenitor (CD34+/CD45+) cells showed the increased erythropoiesis and expression of specific genes, recapitulating features of the primary CD34+ cells of the corresponding patient from whom the iPS cells were derived. This study highlights that iPS cells reprogrammed from somatic cells from patients with blood disease provide a prospective hematopoiesis model for investigating myeloproliferative disorders. Raya et al., reported that somatic cells from Fanconi anaemia patients can be reprogrammed to pluripotency after correction of the genetic defect[18]. They

demonstrated that corrected Fanconi-anaemia specific iPS cells can give rise to haematopoietic progenitors of the myeloid and erythroid lineages that are phenotypically normal.

Other disease models

Maehr et al., demonstrated that human iPS cells can be generated from patients with Type 1 Diabetes Mellitus (T1DM) by reprogramming their adult fibroblasts[19]. These cells are pluripotent and differentiate into insulin producing cells. These cells provide a platform to assess the interaction between â cells and immunocytes in vitro in T1DM. This will lead to better understanding of the mechanism of T1DM and developing the best cell therapeutic strategy. Lee et al., reported the derivation of human iPS cells from patient with Familial Dysautonomia, an inherited disorder that affects the development and function of nerves throughout the body[20]. However gene expression analysis demonstrated tissue specific missplicing of inhibitor of kappa light polypeptide gene enhancer in B-cells (IKBKAP) in vitro, while neural crest precursors showed low levels of normal IKBKAP transcript. Transcriptome analysis and cell-based assays revealed marked defects in neurogenic differentiation and migration behavior. All these recaptured familial Dysautonomia pathogenesis, suggesting disease specificity of the with familial Dysautonomia human iPS cells. Furthermore, they validated specific drugs in reversing and ameliorating neuronal differentiation and migration. Dimos et al., generated iPS cells from a patient with a familial form of amyotrophic lateral sclerosis[21]. Zhang et al., derived iPS cells from fibroblasts of patient with Huntington's disease[22]. They demonstrated that striatal neurons and neuronal precursors derived from these iPS cells contained the same CAG repeat expansion as the mutation in the patient from whom the iPS cell line was established. This suggests that neuronal progenitor cells derived from Huntington's disease cell model have endogenous CAG repeat expansion that is suitable for mechanistic studies and drug screenings. Further, disease specific iPS cells can be used for drug screening, which may correct the genetic defects of disease specific iPS cells. Recent studies have reported the derivation and differentiation of disease-specific human iPS cells, including autosomal recessive disease (spinal muscular atrophy), cardiac disease, blood disorders, diabetes, neurodegenerative diseases (amyotrophic lateral sclerosis, Huntington's disease, and autonomic nervous system disorder (Familial Dysautonomia).

Steps in disease modeling

The first step in hiPSC-based disease modeling is selecting a disease. In general, genetic disorders are easier to model than those caused by predominant environmental or epigenetic factors. Monogenetic disorders with a clear disease phenotype are easier to model than complex genetic disorders. The same holds true, for familial or congenic diseases compared to sporadic diseases. With approval by review board, suitable patients are recruited, informed about the process and study, their consent obtained, and finally blood, skin or other samples are collected. Once the tissue or cell samples are collected, they need to be expanded. For example, separation of the buffy coat fraction from blood, followed by

expansion of T-cells in the presence of IL-2 and activating antibodies CD3 and CD28 for 4-6 days. One fraction of these cells are used for quality control assays, another fraction banked for future DNA finger printing studies and one fraction used for iPSC generation. Fibroblasts, on the other hand, can routinely be grown from skin biopsies, expanded using standard tissue culture conditions, and then subjected to similar quality control and banking procedures as described for blood cells. Reprogramming can be achieved using the various methods. The non-integrating Sendai virus or Stemgent's latest synthetic mRNA/microRNA transfectionis are methods. Reprogramming of blood-derived cells is currently only feasible using Sendai virus. hiPSCs derived using Sendai virus need to be selected for absence of the virus, which can take up to 8-10 passages or 6-10 weeks. If patient-specific iPSCs or their derivatives are considered for cell replacement then the derivation process needs to be performed under Good Manufacturing Practice conditions.

Then proper passaging and expansion techniques were need to maintain reprogrammed clones and differentiation. Selection of accurately reprogrammed, "true" hiPSC lines may be facilitated by "live"-staining for pluripotency-associated cell surface markers such as TRA-1-60 or TRA-1-81. Established hiPSC lines need to be DNA fingerprinted to ensure that they originate from the donor cell source and are not contaminated by other cell lines. In addition, the newly generated hiPSC lines need to fulfill a set of criteria to confirm complete reprogramming and pluripotency. Currently there is a need for a consensus minimal set of criteria that can be used in multiple studies. This will enable rigorous cross laboratory comparisons. Currently, many groups perform in vivo teratoma assays in immunodeficient mice to assess pluripotency. This is costly and labor intensive and clearly, cannot be routinely performed for numerous hiPSC lines. This as well as other criteria are currently the subject of lively discussions. In addition, selection of high-quality patient specific iPSC lines would be dramatically improved by identification of molecular markers that would predict hiPSC differentiation potential. Established hiPSC lines those meet to the high quality need to be expanded for banking. Before cryopreservation, expanded lines should be tested for a normal karyotype. Then, the characterization of iPSC lines may also be studied.

Disease modeling, drug discovery and future cell replacement therapies rely on efficient differentiation of hiPSCs to appropriate specialized cells. Many years of work have defined protocols for differentiation of hESCs and more recently hiPSCs to distinct cell types, including neurons, cardiomyocytes and hepatocytes, mimicking pathways of human development. Protocols for differentiation to more specialized cell subsets, such as nodal, atrial, ventricular or Purkinje fiber cardiomyocytes, have not yet been established but will be critical for modeling specific disorders and for drug discovery.

iPSCs and Drug Development

The use of hiPSC in screening of drug could decrease the number of animals sacrificed during drug testing. Tanaka and colleagues differentiated hiPSC to cardiomyocytes expressing cardiac markers including NKX2-5, GATA-4 and atrial naturetic peptide as well as cardiac specific forms

of sodium, potassium and calcium channels[23]. Using multi-electrode arrays (MEAs), they measured changes in the electrophysiological properties of these hiPSC-derived cardiomyocytes in response to different ion channel inhibitors namely, quinidine, verapamil, and a potassium channel blocker. The pharmaceutical compound dantrolene was found to rescue the arrhythmogenic defect in a patient-specific iPSC model of catecholaminergic polymorphic ventricular tachycardia 1 (CVPT1) caused by the S406L mutation in the cardiac ryanodine receptor type 2 (RYR2) gene. This receptor mutation alters the frequency and duration of elementary Ca2+ release from the sarcoplasmic reticulum upon catecholaminergic stress and, thus, causes elevated diastolic Ca2+ concentrations, a reduced sarcoplasmic reticulum Ca2+ content and an increased susceptibility to delayed after-depolarizations and arrhythmia when compared to control cardiomyocytes. They postulated correctly that dantrolene, which is believed to stabilize the interaction between the N-terminal and central domains of RYR2 could suppress the effect of the S406L mutation, which is positioned at the interface between these two regions. Treatment of S406L RYR2 CVPT1 hiPSC derived cardiomyocytes with dantrolene indeed restored normal Ca2+ sparks and fluxes.

Lee and colleagues modeled familial dysautonomia (FD), a genetic disease of dysfunction of the autonomic and sensory nervous systems due to the incomplete development of and survival of sensory, sympathetic and some parasympathetic neurons caused by mutations in IKBKAP encoding for IkB kinase complex associated protein, using iPSC technology[20]. Treatment of differentiating FD-iPSCs but not derived neural crest precursors with kinetin, a plant hormone, significantly increased the number of developing neurons and the expression of key peripheral neuron markers but not the neural crest cell migration defect. Treatment of the patient-specific iPSC derived neural cells with valproic acid and tobramycin increased survival motor neuron protein levels suggesting that drug screening was possible in this system. In another study, by Marchetto and colleagues, hiPSC derived from a patient with Rett Syndrome, exhibited reduced spine density and neurons having smaller cell bodies[24]. Neurons derived from these hiPSCs were employed in a drug screen that identified IFG1 and gentamycin as being able to rescue synaptic defects. Alzheimer's disease patient specific hiPSCs were differentiated into neuronal cells expressing the forebrain marker, FOXG1 and neocortical markers, CUX1, SATB2, CTIP2, and TBR1 as well as amyloid precursor protein, â-secretase and ã-secretase components. Differentiated cells were capable of secreting Aâ into the conditioned medium. ãâ production was inhibited by â-secretase and ã-secretase inhibitors, as well as sulindac sulfide, a non-steroidal anti-inflammatory drug. Cooper and colleagues derived hiPSC from patients with Parkinson's disease with a mutation in PINK1 (PTEN-induced putative kinase 1) and LRRK2 (leucine-rich repeat kinase 2) genes. These hiPSCs were differentiated to neural cells and analyzed for mitochondrial function, including production of reactive oxygen species, mitochondrial respiration, proton leakage, and mitochondrial movement. Mitochondrial dysfunction in hiPSC-derived neural cells from familial Parkinson's disease patients and at-risk

individuals could be rescued with coenzyme Q(10), rapamycin, and the LRRK2 kinase inhibitor GW5074 suggesting that pharmacological rescue of mitochondrial deficits in these cells may result in a viable treatment option.

iPS Cells for Cardiac differentiation

A few studies have demonstrated the regenerative potential of iPS cells for three cardiac cells: cardiomyocytes, endothelial cells, and smooth muscle cells in vitro and in vivo. The ability of mouse and human iPS cells to differentiate into functional cardiomyocytes in vitro through embryonic body formation[25, 26]. Rufaihah et al. derived endothelial cells from human iPS cells, and showed that transplantation of these endothelial cells resulted in increased capillary density in a mouse model of peripheral arterial disease[27]. Nelson et al. demonstrated for the first time the efficacy of iPS cells to treat acute myocardial infarction[28]. They showed that iPS cells derived from MEF could restore post ischemic contractile performance, ventricular wall thickness, and electrical stability while achieving in situ regeneration of cardiac, smooth muscle, and endothelial tissue. Ahmed et al. demonstrated that beating cardiomyocyte-like cells can be differentiated from iPS cells in vitro[29]. Dubois et al. demonstrated that signal-regulatory protein alpha (SIRPA) was a marker specifically expressed on cardiomyocytes derived from human ES cells and human iPS cells[30]. Cell sorting with an antibody against SIRPA could enrich cardiac precursors and cardiomyocytes up to 98% troponin T+ cells from human ESC or iPS cell differentiation cultures.

iPS Cells for Diabetes Mellitus

Regeneration of functional â cells from human stem cells represents the most promising approach for treatment of type 1 diabetes mellitus (T1DM). Stem cell based approaches could also be used for modulation of the immune system in T1DM, or to address the problems of obesity and insulin resistance in T2DM. Tateishi et al., demonstrated that insulin-producing islet-like clusters (ILCs) can be generated from the human iPS cells under feeder-free conditions[31]. The iPS cell derived ILCs not only contain C-peptide positive and glucagonpositive cells but also release C-peptide upon glucose stimulation. Zhang et al., reported an efficient approach to induce human ES and iPS cells to differentiate into mature insulin-producing cells in a chemical defined culture system[32]. Most of these cells coexpressed mature â cell-specific markers such as NKX6-1 and PDX1, indicating a similar gene expression pattern to adult islet beta cells in vivo.

Alipo et al. used mouse skin derived iPS cells for differentiation into â-like cells that were similar to the endogenous insulin-secreting cells in mice[33]. These â-like cells were able to secrete insulin in response to glucose and to correct a hyperglycemic phenotype in mouse models of both T1DM and T2DM after iPS cell transplant. It is noted in several studies that the general efficiency of in vitro iPS cell differentiation into functional insulin-producing â-like cells is low. Thus, it is highly essential to develop a safe, efficient, and easily scalable differentiation protocol before its clinical application.

Neural Cells

The first studies on iPSC therapy was accomplished by Wernig and colleagues using a rat model of Parkinson's disease. Fibroblasts were reprogrammed into iPSCs and then differentiated into neuronal precursors and injected into adult rats suffering from drug induced Parkinson's disease. The transplanted cells were shown to engraft into the adult rat brain and form axonal connections with native neurons. Furthermore, some functional dopaminergic neuronal recovery was observed in rats injected with iPSC-derived dopaminergic cell. Chambers et al. demonstrated that the synergistic action of Noggin and SB431542 is sufficient to induce rapid and complete neural conversion of human ES and iPS cells under adherent culture conditions[34]. The average time required for generating purified human NSC precursors will be 2–5 weeks. Wernig et al., showed that iPS cells can give rise to neuronal and glial cell types in culture. Upon transplantation into the fetal mouse brain, the cells differentiate into glia and neurons, including glutamatergic, GABAergic, and catecholaminergic subtypes[35]. Hargus et al., demonstrated the survival and functional effects of neurons derived from iPS cells reprogrammed from patients with PD[36]. iPS cells from patients with Parkinson's disease were differentiated into dopaminergic neurons that could be transplanted without signs of neuro-degeneration into the adult rodent striatum. The functional recovery of spinal cord injury was demonstrated by Tsuji and colleagues using normal iPSC-derived neurospheres that were transplanted into the brain of a spinal cord injury mouse model. The mice exhibited functional re-myelination and axonal re-growth as well as differentiation of neurospheres into all three neuronal cell types including neurons, astrocytes and oligodendrocytes and axonal regrowth of host 5HT+ serotonergic fibers, promoting locomotor function recovery without forming teratomas.

iPSCs for Blood related disorders

In a study by Xu and colleagues injected the livers of irradiated hemophilia A mice with iPSC-derived endothelial cells that produced wild-type factor VIII (mutated in Hemophilia A patients)[37]. As mentioned previously, correction of a disease-causing mutation has been demonstrated by Hanna and colleagues, who employed a humanized sickle cell anemia mouse model where iPSC bearing the sickle mutation were corrected by gene specific targeting[12]. Mice transplanted with hematopoietic progenitors derived from the corrected iPSCs exhibited restored hemoglobin function.

Clinical trails

The world's first pilot clinical traial to use patient-derived iPSCs for retinal regeneration was officially announced in Japan on July 30, 2013. This study, which is currently recruiting six patients over the next two to 3 years, targets age-related macular degeneration (AMD) in the exudative form, commonly called wet AMD [38, 39].

Limitations & Risks

Despite these potential advances, some limitations regarding the use of iPSCs in the clinic still remain, including the immunogenicity of iPSCs and their derivatives, which is currently under debate in the field. The retroviruses used in the generation of

iPSc are associated with cancer because they insert DNA anywhere in a cell's genome, which could potentially trigger the expression of cancer causing genes (like c-Myc).

CONCLUSION

iPS cells appear to have the greatest promise without ethical and immunologic concerns incurred by the use of human ES cells. Furthermore, human iPS cells have the potential to generate all tissues of the human body and provide researchers with patient and disease specific cells, which can recapitulate the disease in vitro. The combination of established clinical pathophysiological indexes, GWAS, patient-specific iPSC disease modeling, drug evaluation, discovery and development, as well as hiPSC-based pharmacogenomics has the potential to unravel the causative or predisposing genetic loci of diseases ultimately resulting in improved diagnostic predictors, earlier detection and finally the development of individual patient-tailored therapeutics. Long-term safety data must be obtained to use human iPS cell based cell therapy for treatment of disease. This is true precision personalized medicine.

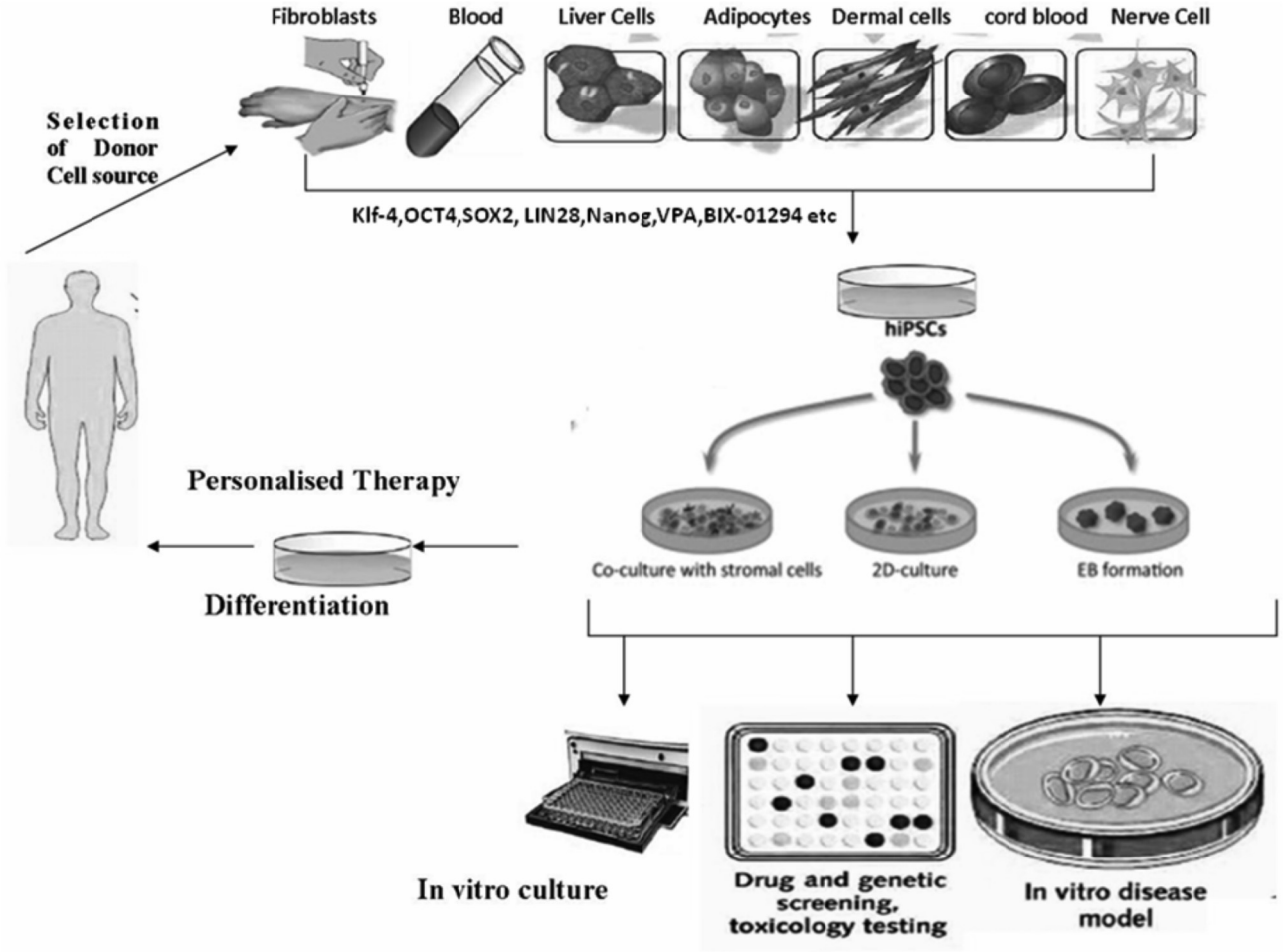

Figure 1. Overview of iPSC generation differentiation and applications towards cell therapy and drug development.

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