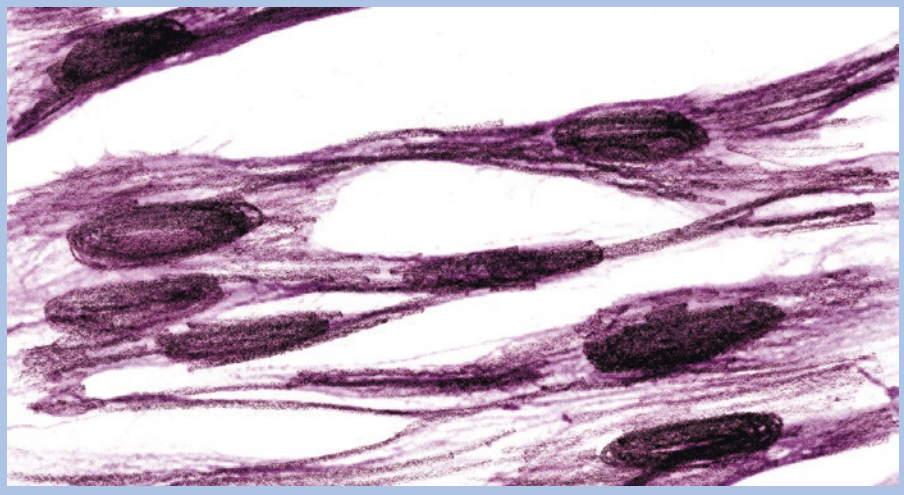
Human Induced Pluripotent Stem (hiPS) Cells: Generation and Applications

Christian Freund



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Website LUMC hiPSC core facility:
► https://www.lumc.nl/research/facilities/hipsc-core-facility/hipsc-for-lumc-researchers-and-external-parties/.

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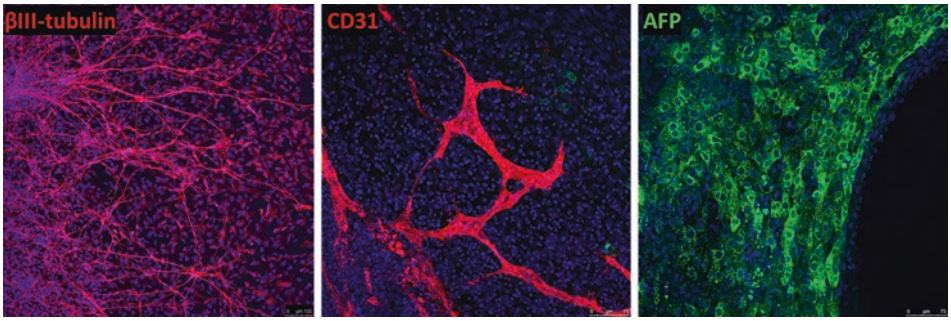
What Will You Learn in This Chapter?

In 2006/2007, it was discovered that somatic cells can be reverted to an embryonic stem cell-like state. This chapter describes how these so-called induced pluripotent stem (iPS) cells were first generated and briefly mentions a selection of historical findings which led to this groundbreaking discovery. Then, you learn how human iPS (hiPS) cells are generated from a practical point of view: what are the advantages and disadvantages of using certain somatic cell types and reprogramming vectors and what does a typical reprogramming experiment look like? Next the underlying mechanisms of reprogramming are explained briefly as well as what it takes to validate a hiPS cell. This is followed by a section on the differentiation of hiPS cells into heart muscle cells (cardiomyocytes) based on protocols developed in our own laboratory. The last part of the chapter gives examples of how differentiated derivatives of hiPS cells are currently being successfully used for toxicology screening, disease modeling, and drug screening. Finally, you learn about the potential use of hiPS cells in regenerative medicine.

4.1 Introduction

In 2007, Japanese researchers discovered that differentiated human skin fibroblasts can be reconverted to an embryonic stem cell-like state. The resulting dedifferentiated cells were referred to as "induced pluripotent stem" (iPS) cells. Human iPS (hiPS) cells have two main features which make them unique tools for research and medical applications: Firstly, hiPS cells are able to self-renew, meaning that under certain cell culture conditions these cells maintain an undifferentiated state indefinitely. Secondly, hiPS cells are pluripotent: Thus, under appropriate culture conditions, they can be differentiated into virtually all 220 somatic cell types of the human body, e.g. neurons (ectoderm), endothelial cells (mesoderm) or liver-like cells (endoderm) (◘ Fig. 4.1). hiPS cells share self-renewal and pluripotency with human embryonic stem (hES) cells, which are derived from the inner cell mass of early human embryos. However, to obtain hES cells, the embryo has to be destroyed, which is why the usage of hES cells for research is considered ethically controversial. Depending on the country, their generation and use is restricted or sometimes even banned. By contrast, hiPS cells can be generated from a small tissue biopsy (e.g. skin or blood) without any ethical constraints.



◘ **Fig. 4.1** Differentiated derivatives of hiPS cells. Confocal images showing hiPS cell-derived neurons (left), endothelial cells (middle) and hepatocyte-like cells (right) after staining with fluorescent dye-coupled antibodies against β-III tubulin, CD31 (Pecam), and α-fetoprotein (AFP), respectively. Nuclei were stained with DAPI (blue)

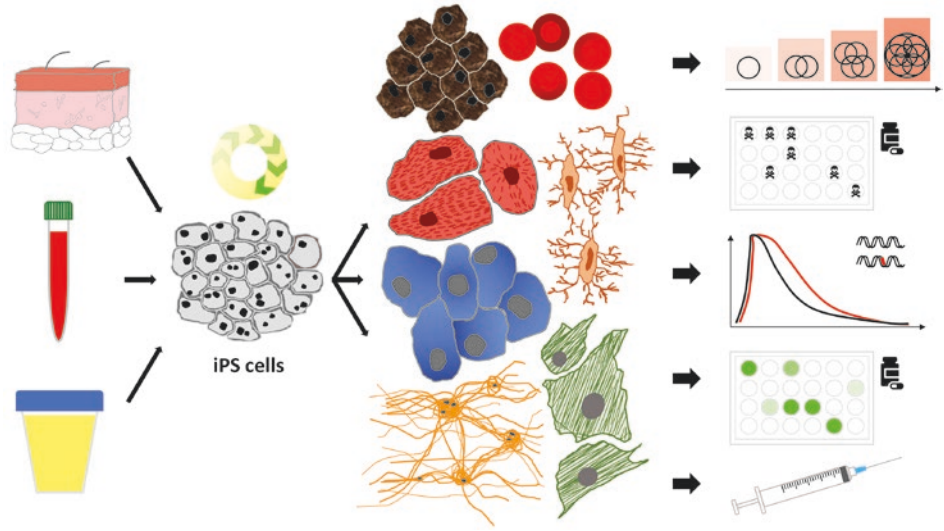


Fig. 4.2 hiPS cells: From generation to application. hiPS cells can be generated from various tissue sources such as skin, blood, and urine (left). The main features of hiPS cells are self-renewal and their capacity to differentiate into derivatives of all three germ layers (pluripotency), e.g., retinal pigment epithelial cells (dark brown), blood cells (red), cardiomyocytes (light red), liver cells (blue), bone cells (light brown), smooth muscle cells (green), and neurons (orange). Differentiated cell types from healthy individuals or patients with a genetic disease can be used for studying human development, toxicology, and drug testing as well as for clinical applications (right)

Following biopsy, the somatic cells are converted ('reprogrammed') into hiPS cells by simple overexpression of four transcription factors known to control pluripotency.

Differentiation of healthy hiPS cells toward somatic lineages is a unique tool to study human developmental processes (Fig. 4.2). Furthermore, hiPS cell derivatives can be used for toxicology tests. For example, hiPS cell-derived cardiomyocytes are utilized to analyze the side effects of chemotherapeutic drugs. Importantly, as the genotype of a hiPS cell is identical to the corresponding donor, these cells are great tools for investigating human genetic diseases in vitro, such as cardiac arrhythmias caused by mutations in a cardiac ion channel gene. hiPS cell-based disease models do not only contribute to a better understanding of disease mechanisms but may also significantly improve the screening and validation of drugs. In the past, disease modeling and initial phases of drug testing heavily relied on animal experiments, especially with genetically modified rodents. However, some important differences between humans and mice exist for example in terms of heart physiology (500 heartbeats per minute versus 80) or in the cellular composition of their organs. Furthermore, certain genetic disease variants and the associated phenotypes observed in humans are absent in rodents. Therefore, hiPS cell-based disease modeling and drug testing represent a significant advancement. Finally, in the future, somatic cells differentiated from hiPS cells are expected to play an important role in regenerative medicine, either by replenishing cells lost due to aging or injury (e.g., after macular degeneration or cardiac infarction) or in the case of certain genetic diseases by replacing genetically defective cells with healthy counterparts after genetic repair of the mutation in hiPS cells.

4.2 Historical Background and Generation of the First iPS Cells

The discovery by Shinya Yamanaka and Kazutoshi Takahashi in 2006/2007 that differentiated unipotent cells with a restricted lifespan can be reprogrammed into pluripotent, immortal stem cells [1, 2] by overexpression of four transcription factors is based on seminal findings made by various researchers in earlier decades. In the 1960s, John Gurdon showed that upon transplantation into an enucleated oocyte, a nucleus from a differentiated tadpole cell gave rise to an entire frog (somatic cell nuclear transfer, SCNT, or cloning; [3]). The first mammal cloned from an adult somatic cell was the sheep Dolly [4]. These results indicated that the nucleus from a differentiated cell contained all necessary information to give rise to a whole organism and that the identity of a differentiated cell was determined by reversible epigenetic modifications rather than by irreversible changes in the DNA sequence. The fact, that the fate of a differentiated cell was not necessarily permanent, was shown by Davis et al. in 1987: Overexpression of skeletal muscle transcription factor MyoD in fibroblasts led to the formation of myoblasts [5]. Similarly, primary B cells could be converted into macrophages by overexpression of the myeloid transcription factor C/EBPα [6]. Evans & Kaufman and Martin achieved another milestone in 1981, when they succeeded in the isolation and culture of the first mouse embryonic stem (mES) cells [7, 8]. The first human embryonic stem (hES) cell lines were established in 1998 [9]. The generation of mouse and human ES cells triggered research on factors controlling pluripotency and differentiation and led to the development of protocols for both the maintenance of undifferentiated cells and for their differentiation into various cell types.

The knowledge about cellular plasticity, transcriptional networks regulating pluripotency, and culture conditions for maintenance of ES cells paved the way for Yamanaka's and Takahashi's discovery of cellular reprogramming by exogenous factors. In order to identify candidates which are able to transform a somatic cell into a pluripotent cell, they used genetically engineered mouse embryonic fibroblasts (MEFs) in which the ES cell-specific *Fbxo15* gene was coupled to a neomycin resistance cassette [2]. Only MEFs reconverted into pluripotent cells expressing *Fbxo15* would be resistant against the drug. Initially, 24 factors known to play a role in pluripotency were tested. After having obtained the first iPS cells with all 24 candidates, the list could be narrowed down to a core set of four transcription factors: *OCT3/4*, *SOX2*, *c-MYC*, and *KLF4*. These reprogramming factors or so-called Yamanaka-factors are sufficient to generate mouse iPS cells. A year later, Takahashi and Yamanaka generated iPS cells from adult human skin fibroblasts using the same four pivotal factors [1]. In parallel, the group of James Thomson was able to obtain hiPS cells with *NANOG* and *Lin28* replacing *c-MYC* and *KLF4* [10]. hiPS cells generated in both ways displayed the hallmarks of pluripotent stem cells: They were able to self-renew and to be differentiated into derivatives of all three germ layers. In 2012, Gurdon and Yamanaka received the Nobel Prize for their groundbreaking work on cellular reprogramming.

4.3 Reprogramming Vectors

For the generation of iPS cells, the coding sequences of all four Yamanaka factors have to be introduced into the somatic target cell. Reprogramming vectors can be divided into two major groups: integrating vectors such as retroviruses and lentivi-

ruses, which persist in the iPS cells after reprogramming is completed. Importantly, the expression of the reprogramming factors must be silenced once hiPS cells have been established. By contrast, vectors like Sendai virus (SeV), episomal plasmids, and synthetic RNA are non-integrating and only transiently remain in the target cell. For this reason, non-integrating reprogramming vectors are used preferentially, but other aspects such as production costs, storability, laboratory biosafety requirements, range of target cells, and reprogramming efficiency have implications on the choice of the vector for daily use.

For their initial experiments to generate mouse iPS cells, Takahashi and Yamanaka used four retroviruses, each carrying one of the four Yamanaka factor sequences. For human iPS cell generation, an additional lentivirus encoding the murine retroviral receptor was necessary. The resulting hiPS cell lines had a minimum of five viral integrations; however, in practice, the number of integrations was sometimes up to 20. A major drawback of retroviruses is their integration into the host genome, preferentially at sites of actively transcribed genes, which may result in an altered phenotype when using hiPS cells for disease modeling. A further problem may arise from incomplete silencing of the reprogramming factors which might compromise the differentiation capacity of the cells [11].

Currently, lentiviruses are commonly used for the generation of hiPS cells destined for in vitro applications. Their production is easy and cost-effective, virus stocks can be stored frozen, and they require a lower biosafety level than retroviruses. A lentivirus generated by Warlich et al. carries the sequences of all four Yamanaka factors attached to a red fluorescent dye [12]. The latter enables monitoring of infection efficiency of somatic cells as well as silencing of the reprogramming factors in established hiPS cells. Furthermore, a single viral integration is sufficient for successful reprogramming, and if required, the transgenes can be removed by using the Flp enzyme, as Flp target sequences have been introduced at the 5′ and 3′ end.

Non-integrating vectors currently used for generation of hiPS cells include episomal vectors, SeV, and synthetic RNA. Episomal vectors are easily produced in bacteria and can efficiently generate hiPS cells [13]. However, they may occasionally integrate into the host genome, and therefore extensive screening for integration-free hiPS cell lines is obligatory prior to application in humans [14].

SeV can infect a wide range of human target cells [15]. These viruses remain exclusively in the cytoplasm of the infected cell and are therefore completely non-integrating (‘zero footprint’). However, SeV vectors are relatively difficult to produce, and commercially available SeVs are expensive. In addition, in the Netherlands, hiPS cells generated with SeVs require a higher biosafety level, unless the absence of the vector has been proven at RNA and protein level.

Synthetic RNA is another non-integrating reprogramming vector, yet it normally is rapidly degraded by ribonucleases in the transfected cells. Initial protocols therefore required daily transfections in a two-week period, making synthetic RNA reprogramming laborious, expensive, and prone to error. A major improvement was achieved by Yoshioka et al. whose reprogramming method with modified synthetic RNA only requires a single transfection together with blocking RNA degradation [16]. Nevertheless, RNA reprogramming remains expensive and might not reprogram all somatic cell types with sufficient efficiency.

At our hiPS cell core facility lentiviruses, episomal vectors, SeV, and synthetic RNA are routinely used for generation of research-grade hiPS cells derived from various tissue sources [17, 18].

Various other reprogramming methods have been proposed as alternatives. One approach aims at the complete replacement of exogenous reprogramming factors using chemical compounds. Generation of iPS cells with small molecules in the absence of transgenes has been achieved using mouse somatic cells [19]. For reprogramming human somatic cells, Sox2 can be replaced by a small molecule targeting the TGF-β pathway [20], but efficient reprogramming solely with chemical compounds has not been reported yet. Finally, the directed DNA binding capacity of CRISPR/Cas9 has recently been employed to successfully reprogram human cells by activating transcription of endogenous pluripotency genes [21]. Although potentially interesting, for widespread application, the use of CRISPR/Cas9 for reprogramming cells requires further verification. In conclusion, various satisfactory reprogramming systems have been developed, but an efficient method that combines cost-efficiency, low biosafety requirements, a wide range of target cells, absence of alteration of the host genome, and high reprogramming efficiency is still pending.

4.4 Somatic Tissue Sources for Reprogramming

In theory, any somatic cell type can be used for generation of hiPS cells, even neural stem cells from brain [22]. However, in practice, the choice of human donor tissue is strongly influenced by the accessibility/invasiveness of the biopsy taken. Additional criteria include the possibility to store tissue material prior to cell isolation, the feasibility, and cost-effectiveness of cell culture, the proliferation capacity of the isolated somatic cells and their ability to be reprogrammed with common vector systems. The vast majority of hiPS cells are therefore generated from skin, blood, or urine.

Skin fibroblasts can easily be isolated and expanded from 4 mm punch biopsies. We previously found out that skin biopsies can be stored in saline buffer at 4 °C for up to 2 weeks prior to fibroblast isolation which would allow long-distance shipment of rare donor material [17]. Importantly, skin fibroblasts can be reprogrammed with any of the standard reprogramming vector systems (e.g. lentivirus, Sendai virus, episomal vectors, synthetic RNA). However, punch biopsies are painful and at least in the Netherlands the procedure is not applicable to minors. By contrast, milk teeth from children constitute a completely noninvasive tissue source, and dental pulp cells are readily reprogrammed into hiPS cells [17]. The disadvantage of using teeth lies in a higher risk of cell culture contamination with bacteria and fungi which is why they have to be processed immediately.

Blood samples can generally be stored for a maximum of 24 hours at room temperature before processing. Peripheral blood contains various cell types suitable for reprogramming. In the beginning, we focused on blood outgrowth endothelial cells (BOECs). However, these are rare and thus require high blood volumes (80 ml), which in the Netherlands is contraindicated for children. To obtain sufficient numbers of BOECs for reprogramming may take weeks and the reprogramming efficiency of this cell type with lentivirus is low [17]. By contrast, as little as 10 ml peripheral blood is needed to isolate sufficient peripheral blood mononuclear cells (PBMCs) by a simple density gradient centrifugation. PBMCs are a mixture of various cell types

such as T and B cells, macrophages, and erythroid progenitor cells. The latter are rare but can be easily expanded and reprogrammed with episomal vectors or lentivirus. In our hiPS cell core facility, we now routinely use erythroblasts for reprogramming.

Interestingly also urine is a source of somatic cells for reprogramming: About 7000 renal epithelial (RE) cells are excreted daily via the urinary tract [23]. RE cells can be expanded in a specific cell culture media and are readily transformed into hiPS cells with various vector systems. Collecting urine samples is truly noninvasive, but they have to be processed immediately after collection.

When patients undergo surgical procedures, tissue material that normally is not accessible with regular biopsies may be obtained for reprogramming. For example, we were able to isolate fibroblasts and generated hiPS cells from nasal epithelium removed from patients suffering from recurrent nosebleeds due to a defect in TGF-β signaling (hereditary hemorrhagic telangiectasia). Furthermore, we isolated and reprogrammed chondrocytes from cartilage tissue leftover from hip replacement surgery.

It has been reported that hiPS cells retain epigenetic marks that are specific of the somatic cell type they were derived from ('epigenetic memory' [24]). As a consequence, hiPS cells may be differentiated more easily into their cell type of origin which is advantageous for cell types without efficient differentiation protocols. Furthermore, certain internal organs might be better cell sources for reprogramming than skin: Exposure to UV radiation from sunlight is known to cause DNA damage and skin cells may acquire more spontaneous mutations than blood cells. Finally, for disease modeling, the choice of donor material also depends on whether a disease-causing mutation is present in all tissues and cells or not. In so-called mosaic patients, for instance, only certain somatic cell types carry the mutation, whereas the healthy gene is present in others. Here, mutated and normal hiPS cells can be obtained from the same individual and, thus, have the same genetic background (isogenic), which certainly represents an ideal situation for studying the effect of the disease-specific mutation.

In conclusion, various somatic cell types from skin, blood, and urine can be readily used for reprogramming, but the choice is often limited due to the invasiveness of biopsy-taking, the feasibility of cell culture, and the reprogramming efficiency. Of note, all tissue biopsies, with the exception of leftover surgical material, must be taken with a proper informed consent in which the donor agrees with the use of the tissue material for reprogramming and downstream applications.

4.5 Generation and Validation of hiPS Cells and Mechanisms of Reprogramming

For a typical reprogramming experiment, $1 \times 10^5 - 5 \times 10^5$ somatic cells, e.g., skin fibroblasts are infected with virus or transfected with plasmids or RNA and are allowed to expand in somatic cell media for 1 week (Fig. 4.3). They are then plated on irradiated (cell cycle arrested) mouse embryonic feeder cells in culture medium containing fetal calf serum or serum replacement. Alternatively, defined conditions such as recombinant extracellular matrix proteins and animal component-free media can be used [25]. First colonies of hiPS cells emerge after 3 weeks and are selected ('picking') and expanded separately to establish various clonal hiPS cell lines from the same donor.

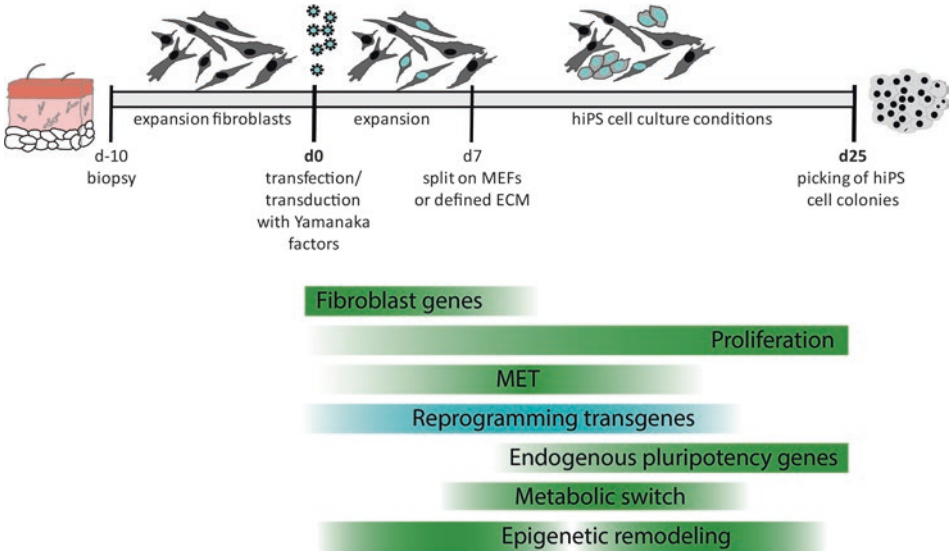


Fig. 4.3 Schematic of a reprogramming experiment. Timecourse in days (d), starting with the isolation of fibroblasts from a skin biopsy, infection of fibroblasts with viral vectors encoding the Yamanaka factors up to picking of hiPS cell colonies. Events during reprogramming are shown below. MET: mesenchymal-to-epithelial transition

Recently, reprogramming was demonstrated in a miniaturized cell culture system: Gagliano and colleagues used a microfluidic chamber allowing for a significant downscaling of somatic cell numbers and reprogramming compounds as well as the generation of multiple hiPS cell lines simultaneously [26].

Although the mechanisms of reprogramming are still not fully understood, gene expression analysis of intermediate stages has shed some light on the underlying processes [27, 28] (Fig. 4.3). Whereas transgenic *c-MYC* plays a role in the early reprogramming phase, transgenic *OCT3/4* and *SOX2* are thought to act mainly at later stages of reprogramming. When skin fibroblasts are used as somatic cell source, the expression of the Yamanaka factors initially leads to the suppression of genes controlling fibroblast identity. Transforming fibroblasts increase their proliferation rate and transition from a mesenchymal (migratory, loose cell–cell contacts) to an epithelial state (stationary, tight intercellular contacts). This process is known as mesenchymal-to-epithelial transition (MET). In addition, a metabolic switch from oxidative phosphorylation to glycolysis for cellular energy production occurs. Importantly the whole reprogramming process is accompanied by a massive epigenetic remodeling. Furthermore, the cellular morphology changes drastically: The large spindle-like fibroblast is converted into a small rounded hiPS cell of which the cytoplasm is almost completely occupied by a nucleus that contains large nucleoli (Fig. 4.4). The expression of endogenous pluripotency genes such as *OCT3/4* and *SOX2* maintains the emerging hiPS cells in an undifferentiated state, whereas the transgenic Yamanaka factors become transcriptionally silenced or are eliminated.

Although a hiPS cell colony can be clearly identified solely based on morphology, for each hiPS cell line, complete reprogramming and hES cell properties, such as self-renewal and pluripotency, have to be verified. For mouse iPS cells, the most stringent

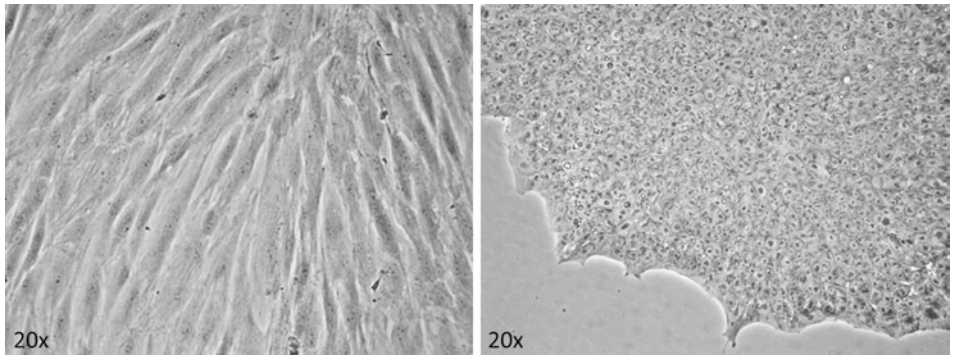


Fig. 4.4 Cellular morphologies: Skin fibroblasts (left) and hiPS cells generated from skin fibroblasts (right) at 20× magnification

assay to prove pluripotency is injection into blastocysts. Upon transplantation of the chimeric blastocysts into a surrogate mother, the organs of the embryo forming will partially contain cells that originate from the injected pluripotent stem cells (chimera). In case of injecting miPS cells into a tetraploid blastocyst which can only give rise to extraembryonic tissues, the entire embryo evolves from the iPS cells [29]. In most countries, analogous experiments injecting human iPS cells into mouse blastocysts are considered unethical and are thus prohibited. Therefore, the most stringent assay to assess the pluripotency of hiPS cells is injection under the skin of adult mice, where they spontaneously form differentiated benign tumors ('teratomas') [30]. These teratomas consist of derivatives of all three germ layers, e.g., neuroectoderm, endodermal gut epithelium, and mesodermal cartilage. As the teratoma assay is non-quantitative, time-consuming and animal-dependent alternative assays such as the analysis of global gene expression in undifferentiated hiPS cells in combination with directed in vitro differentiation into derivatives of the three germ layers have been proposed [30]. Finally, it should also be confirmed that hiPS cells have a normal karyotype.

4.6 Differentiation of hiPS Cells

A prerequisite to use hiPS cells for various applications is the efficient differentiation into the cell type(s) of interest. For example, hiPS cell-derived heart muscle cells (cardiomyocytes) can be used for testing potential cardiotoxic side effects of drugs or for modeling arrhythmias caused by a mutated gene encoding for a cardiac ion channel. Originally we differentiated human ES cells into cardiomyocytes by coculture with visceral-endoderm-like (END-2) cells [31]. Since then, significant progress has been made and now cytokines and small molecules are being used to induce a sequence of developmental events leading to cardiac differentiation: First the formation of mesoderm is induced by a combination of Activin A, bone morphogenic protein (BMP) 4, and a glycogen synthase kinase (GSK-3) small molecule inhibitor that activates the Wnt/β-catenin pathway. Further specification into cardiac progenitors is achieved by exposure to a small molecule Wnt/β-catenin inhibitor. About 1 week after the initiation of differentiation, spontaneously contracting cardiomyo-

cytes can be observed [32]. The recent refinement of the method now enables the simultaneous generation of cardiomyocytes and endothelial cells [33]. Similar multistep protocols mimicking early developmental processes have been developed for the differentiation into many other cell types, for example, pancreatic β-cells [34], skeletal muscle precursors [35], or retinal pigment epithelial (RPE) cells [36].

4.7 Application of hiPS Cells in Medical Research and Therapy

Due to their unique features, hiPS cells are currently being used in a number of applications in medical research and therapy (Fig. 4.2).

4.7.1 Toxicology Testing

Doxorubicin was one of the first chemotherapeutic drugs and is still used for treatment in about 50% of the breast cancer patients. Its cardiotoxic side effects that lead to arrhythmia, cardiac infarction, and heart failure are well known. However, not all patients develop doxorubicin-induced side effects and at present it is impossible to predict who will be affected. Recently, Burridge et al. tested whether hiPS cell-derived cardiomyocytes were able to recapitulate the patient's doxorubicin susceptibility [37]. For this purpose, hiPS cells were generated from four healthy individuals and 8 doxorubicin-treated breast cancer patients, of which half experienced cardiotoxicity, whereas the other half was unaffected. Interestingly, hiPS cell-derived cardiomyocytes from doxorubicin-affected patients were also more susceptible to the drug in vitro: A higher degree of sarcomeric disarray was observed, arrhythmias occurred more frequently, and cell viability was significantly reduced [37]. These results nicely demonstrate the usefulness of hiPS cell derivatives for assays examining pharmacological toxicity.

4.7.2 Disease Modeling

Currently, hiPS cells are most widely used for developing models to study genetic diseases. The long-QT2 syndrome is a life-threatening cardiac disease resulting from a mutation in a cardiac ion channel gene. The defective potassium channel causes an abnormal (prolonged) repolarization of the heart after a heartbeat. Bellin et al. generated hiPS cells from a long-QT2 patient. As the genetic background influences the disease phenotype, the mutation was corrected in the long-QT hiPS cells in order to generate the matching control. In parallel, the long-QT2 mutation was introduced into a healthy hES cell line, which resulted in two isogenic pairs of mutant and normal cells. hiPS and hES cells were differentiated into cardiomyocytes and electrophysiological analysis confirmed the prolonged action potential in mutated cells when compared to controls [38].

Whereas the long-QT2 syndrome often affects young patients, other diseases have a late onset. For example, Parkinson's disease only becomes manifest in the sixth or seventh decade of life and is caused by loss of dopaminergic neurons. A major hurdle to efficiently use hiPS cell derivatives for disease modeling is the fact that differentia-

tion of hiPS cells often results in immature cells. For example, hiPS cell-derived neurons resemble primary neurons from a fetal stage [39]. Accordingly, no disease-associated phenotype was observed in dopaminergic neurons differentiated from Parkinson's hiPS cells [40]. However, neurodegeneration eventually became apparent upon overexpression of a protein inducing cellular aging: Progerin-expressing dopaminergic neurons derived from Parkinson's hiPS cells had shorter dendrites and were more prone to apoptosis than their progerin-expressing healthy counterparts [40].

Whereas analysis of regular cultures of cardiomyocytes or neurons was sufficient to reveal the disease phenotype for long-QT2-syndrome or Parkinson's disease, respectively, modeling other diseases will likely require more complex in vitro assays. For example, hereditary hemorrhagic telangiectasia (HHT) is caused by defects in TGF-β signaling and leads to leaky blood vessels due to a disturbed interaction between inner endothelial cells and pericytes lining the outside of the blood vessel wall. A model mimicking the in vivo situation with three-dimensional luminal structures formed by both cell types has recently been developed [41]. Even more complex structures containing multiple cell types ('organoids') have been generated for various organs, e.g., the kidney [42] and will likely further improve disease modeling.

4.7.3 Drug Testing

Besides gaining further insight into the mechanisms of genetic diseases, an important goal for hiPS cell-based disease models is the identification and validation of specific drug candidates. Amyotrophic lateral sclerosis (ALS) is caused by the death of neurons controlling voluntary muscles. In 2014, the disease gained worldwide media attention by the 'ALS Ice Bucket Challenge'. In vitro hiPS cell-derived motoneurons recapitulate the disease phenotype and show hyperexcitability and reduced survival [43]. Interestingly, an already approved drug normally used for treatment of epilepsy, ezogabine was able to reduce neuronal excitability and improved cell survival [43, 44]. From this finding, it took less than 2 years to initiate a clinical trial to test ezogabine for treatment of ALS patients [45]. Ezogabine is a good example for identifying new targets for already existing drugs ('repurposing'), but hiPS cell-based disease models will be equally important for identification of new compounds.

4.7.4 Regenerative Medicine

Recently, the first clinical trials using differentiated cell types derived from hiPS cells have been started. The goal of such early-stage trials is to test the feasibility and the safety of potential cellular therapies. One disease which may be treated with hiPS cell derivatives in the future is age-related macular degeneration (AMD). Patients suffer from the degradation of pigmented epithelial (RPE) cells of the retina resulting in impaired vision. Mandai et al. generated hiPS cells from tissue material of two AMD patients [14]. hiPS cells generated from one AMD patient were differentiated into a RPE cell sheet which was subsequently transplanted into the eye. After 1 year of follow-up, the patient's vision had neither improved nor worsened. The graft appeared to have survived for an additional year. Importantly no adverse effects such as tumor

formation were detected. For safety reasons, the second AMD patient did not undergo transplantation, as the DNA sequence of his hiPS cells contained small deletions in certain endogenous genes which might have altered their normal expression [14]. Future regenerative medicine based on hiPS cell derivatives may also include cardiac disease [46], diabetes, Parkinson's, and kidney disease.

In conclusion, disease models based on hiPS cell derivatives represent a significant improvement in understanding disease processes when compared to previously used animal models. In addition, hiPS cells reflect human genetic variants which influence the disease phenotype and are therefore particularly interesting for customized treatments ('personalized medicine'). hiPS cell-based models for drug testing hold a lot of promise, but need to be proven how well they can mimic the effect of a drug in vivo. The first clinical trials using hiPS cell-derived cell types are on their way. The inclusion of larger patient cohorts will prove whether such treatments are safe and efficient.

Take-Home Message

- hiPS cells can be generated from various somatic cell types. Minimally or non-invasive biopsies such as peripheral blood or urine are preferred.
- Delivery methods for the Yamanaka factors are preferentially nonintegrating, for example, RNA or SeV.
- By addition of cytokines and growth factors, hiPS cells can be differentiated into a multitude of cell types.
- Since hiPS cells capture the genotype of the donor, they are great tools for studying disease mechanisms and for drug testing. In addition, they are being used successfully for toxicology testing and developmental studies.
- hiPS cell derivatives hold great promise for future cell therapies. The first clinical trials with small patient cohorts aim at testing feasibility and safety.

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