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ПРИМЕНЕНИЕ ПЛЮРИПОТЕНТНЫХ СТВОЛОВЫХ КЛЕТОК В МЕДИЦИНЕ

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Резюме:
Такие свойства человеческих плюрипотентных стволовых клеток (чПСК) как способность к неограниченному размножению и образованию всех типов клеток взрослого организма делают их привлекательным источником материала для регенеративной медицины. С другой стороны, множество этических и практических проблем, связанных с чПСК, ограничивают их применение в медицине. Этот литературный обзор посвящён описанию различных видов чПСК, рисков их применения и клинических испытаний, в которых чПСК служат источником клеток для лечения дегенеративных заболеваний и травм.

HUMAN PLURIPOTENT STEM CELLS IN CONTEMPORARY MEDICINE

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Abstract:
Human pluripotent stem cells (hPSCs) are capable of indefinite proliferation and can be differentiated into any cell type of the human body. Therefore, they are a promising source of cells for treatment of numerous degenerative diseases and injuries. Pluripotent stem cells are also associated with a number of ethical, safety and technological issues. In this review, we describe various types of hPSCs, safety issues that concern all or some types of hPSCs and methods of clinical-grade hPSC line development. Also, we discuss current and past clinical trials involving hPSCs, their outcomes and future perspectives of hPSC-based therapy.

Introduction

Russian scientist Alexander Maximow for the first time in the world described the phenomenon and proposed the conception of stem cells in 1908. Many types of stem cells have been characterized by now. Although they originate from various tissues of the body and exhibit different properties, it has been agreed that all stem cells share the ability to divide giving rise to cells of the same type (self-renew) and the ability to differentiate to all, many or at least one type of somatic cells (terminally differentiated cells). The latter property has been used for classification of stem cells. Thus, totipotent stem cells are able to give rise to the whole organism. An example of a totipotent cell is the zygote. Interestingly, it is not entirely clear if totipotent stem cells are able to self-renew [1]. There are evidences that some of cells in mouse 4-cell embryos are totipotent, but it is not clear if all of them are. Pluripotent stem cells have a capacity to differentiate into all types of the body, but not extraembryonic tissues. Multi- and unipotent stem cells usually reside in various organs of the body. Such cells are able to give rise to several or one type of somatic cells. Hematopoietic, mesenchymal and neural stem cells are multipotent. In this review, we discuss current practice and challenges of human pluripotent stem cell (hPSC) use in medicine.

Promises of pluripotency

There are patient conditions that are characterized by extensive loss of certain cell populations or/and permanent loss of natural cellular milieu that often leads to formation of fibrotic tissues. Diabetes, Parkinson's disease, macular degeneration and spinal cord injuries represent several examples of such conditions. Usually, in these cases treatments based on drugs are only symptomatic and not efficient. An adequate treatment warrants development of regenerative medicine strategies. One approach is allotransplantation of organs or tissues from human donors. But, the supply of donor materials is insufficient and alternative sources of cells for transplantations are required. Pluripotent stem cells are capable of indefinite self-renewal and, therefore, are able to generate as many cells as needed. One more important advantage of pluripotent cells over other types of stem cells is the ability to differentiate into any type of somatic cells. Stem cell lines for treatments of patients must be developed in compliance with very stringent rules. Certification of a stem cell line and development of a master bank of stem cells for human therapies is a very expensive and time-taking process. Therefore, it is an attractive idea to certify and develop one master stem cell bank for all the diseases there is.

Properties and types of human pluripotent stem cells

A network of transcription factors such as Oct-4, Nanog and Sox2 supports state of pluripotency in cells [2]. Those factors orchestrate expression of the downstream effector genes, enhance expression of each other and suppress expression of other transcription factors that define different kinds of cells. Although many researches define several kinds of pluripotency in human cells [3] that are slightly different in their differentiation potency, in this review we use term human pluripotent stem cells (hPSCs) to designate cellular state described by Thomson and co-workers in 1998 [4].

As mentioned above, hPSCs are able to indefinitely self-renew and to differentiate into any cellular lineage of the body. Cultured hESCs should be characterized to confirm that they are pluripotent and safe before use for development of cell lineages for therapeutic applications. There is a minimal set of tests that ensures pluripotency of cells. Thus, hPSCs should express markers of pluripotency (such as Oct-4, Nanog, Sox-2 and etc.), should not express significant amounts of markers of differentiation, should be genetically and epigenetically normal and should show an ability to generate lineages of all three germ layers in in vivo and in vitro assays. Methods of hPSC characterization have been recently reviewed in details in [5].

There are several types of hPSCs that have been described to date. Among those, human embryonic stem cells (hESCs) [4] and human induced pluripotent stem cells (hiPSCs) [6] are already used in ongoing clinical trials with enrolled patients. Parthenogenetic human embryonic stem cells (phESCs) [7] and human nuclear transfer embryonic stem cells (NT-ESCs) [8] are not yet used in medicine, but have properties that may make them useful for treatment of patients in the future. Although established lines of hESCs, hiPSCs, phESCs and human NT-ESCs look similar in culture and express same markers of pluripotency, they originate from different sources and exhibit different epigenetic (and sometimes even genetic) patterns. As of today, each type of hPSCs has advantages and disadvantages (Table 1) that are described in details below.

Human embryonic stem cells (hESCs) were first derived in 1998 [8]. They originate from the inner cell mass of human blastocysts or from single blastomeres of 8-cell embryo [9]. hESCs have been extensively studied to date and are regarded as the "gold standard" of hPSCs. One important advantage is that hESCs are usually epigenetically normal. On the other hand, derivation of new hESC lines often implies destruction of the parental ex utero embryo. This is an important ethical concern

Table 1
Advantages and disadvantages of different types of hPSCs with regard to therapeutic applications.

hPSC type	Advantages	Disadvantages
hESCs	No epigenetic aberrations	Ethically controversial unless derived from single blastomeres No information on predisposition to diseases
hiPSCs	No ethical concerns Easy method for generation of patient-specific lines	Epigenetic aberrations (especially immediately after reprogramming) * Increased number of genetic mutations immediately after reprogramming *
phESCs	Homozygous phESCs may be valuable for generation of cell banks Heterozygous phESCs are patient-specific	Epigenetically abnormal Limited information on predisposition to diseases (for homozygous phESCs)
Human NT-ESCs	No epigenetic aberrations Possibility to generate fully patient-specific cells without epigenetic aberration **	Immune rejection of patient-specific NT-ESCs *** Complicated procedure of derivation

* - both number of epigenetic and genetic aberrations in niPSCs decreases with time in culture

** - if both the oocyte and the somatic cell nucleus originate from the same donor

*** - if the oocyte and the somatic cell nucleus originate from different individuals

that limits studying and using of such cells in many countries in the world. Klimanskaya et al. [9] proposed an alternative method of new line derivation using a single blastomere acquired through a procedure that is similar to that normally carried out to obtain a single cell for preimplantation genetic diagnosis (PGD). Since the PGD procedure does not interfere with developmental potential of the embryo, the method of hESC line derivation is an important breakthrough that has addressed the ethical concerns of many. One more disadvantage of hESCs is that usually there are no living human beings with the same genome. Therefore, it is not known if the genome contains genetic factors conferring predispositions to diseases.

Probably the most commonly used type of hPSCs in research laboratories is human induced pluripotent stem cells [6]. Earlier, Gurdon et al. [10] and others [11] showed that frog somatic cell nuclei could be reprogrammed (pushed back in development) to totipotent state again by transplantation into enucleated oocytes. In 2007, Prof Yamanaka's group [6] reported a set of defined factors that can reprogram human somatic (fully differentiated) cells into pluripotent stem cells, which are called hiPSCs now. Generation of hiPSCs (reprogramming) is achieved by ectopic expression or direct delivery of certain proteins and/or small molecules into somatic cells. Induced pluripotent cells are free from major ethical concerns and autologous to the somatic cell donor. Early reprogramming methods relied on lenti- and adenoviruses for the ectopic expression of the proteins. Since lenti- and adenoviruses integrate into the host genomes causing random mutagenesis, such hiPSCs were useless for therapeutic applications. Contemporary methods that are based on sendai-virus or direct delivery of mRNAs or proteins, generate much safer cells [12, 13]. In general, hiPSCs are a useful tool for disease modeling, since they can be generated from the actual patients with the diseases of interest and later be used in drug testing or scientific studies. Development of cell lineages for therapeutic applications is complicated by imperfections of even contemporary reprogramming methods. Many reports suggest that hiPSCs retain partial epigenetic memory of the initial somatic cell lineage [14–16] and even transmit those epigenetic markers to their differentiated progeny [17]. Sometimes hiPSCs, but not hESCs, give rise to only partially functional differentiated cells, e.g. cardiomyocytes [18]. Since it is too expensive and time-taking to generate hiPSCs for only one patient, it is widely accepted that, similar to hESCs, future iPSC-based therapies are going to rely on collection of hiPSC line banks for allotransplantations. Nobel Prize winning Laureate Prof Yamanaka has acknowledged that [19]. Nevertheless, the Gurdon's experiments suggest that it is possible to reprogram somatic cells into genetically and epigenetically normal pluripotent stem cells. More studies on mechanisms of reprogramming methods are warranted to achieve this goal.

One more interesting type of hPSCs is parthenogenetic human embryonic stem cells (phESCs). Parthenogenesis is a form of reproduction in which development of embryos occur without fertilization. While lower vertebrates are able to produce healthy parthenogenetic offspring, mammalian parthenogenetic embryos are incapable of full-term development probably due to genomic imprinting. Nevertheless, parthenogenetic embryos may reach blastocyst stage of development in most mammalian species. Revazova et al. [7, 20] for the first time reported two slightly different protocols that allow human oocyte activation, development into blastocysts and derivation of phESCs from them. Depending on the activation procedure, the resulting cells can be either patient-specific and autologous [7] or homozygous [20]. The former cells support autologous transplantations because of immune compatibility. The latter cells are homozygous for major histocompatibility complex (MHC, in humans also called human leukocyte antigens, HLAs) and may be useful for allotrasplantaions into unrelated patients (see below). Since parthenogenetic embryos are not fertilized, some of the ethical concerns that are associated with hESCs are not applicable to phESCs. Still, procurement of unfertilized oocytes may raise certain ethical issues [21]. Also, phESCs are epigenetically abnormal because of the aberrant genomic imprinting that may complicate applications of the cells in regenerative medicine. Transplantation of HLA-homozygous cells into heterozygous hosts may induce NK-cell immune response even without HLA-mismatch (see in details below). Therefore, each approach has its pitfalls and more studies are warranted to understand the potential of phESCs for use in medicine.

Experiments with cloning of frogs showed that transplantation of single somatic cell nuclei into enucleated oocytes could give rise to healthy animals. The procedure that is called somatic cell nuclear transfer (SCNT) can be also used to generate human blastocysts and, subsequently, derive pluripotent stem cells from them (human NT-ESCs). Because of many technical difficulties, successful SCNT in human cells was reported for the first time only in 2013 [8]. Similar to parthenogenetic, SCNT embryos are not fertilized, but the procurement of unfertilized human oocytes may be controversial [21]. Although NT-ESCs are MHC-matched with the donor of the somatic nucleus, allogeneic mitochondria, which mainly originate from the oocyte, may still trigger an alloimmune response [22]. One intriguing future possibility is generation of human NT-ESCs from an oocyte and a somatic cell nucleus taken from same individual. Such cells should be fully autologous to the donor, epigenetically normal and may be useful for treatment of her family members because close relatives have higher chances for HLA compatibility. Therefore, donation of oocytes may become beneficial reducing ethical concerns.

Challenges of pluripotency

The major safety risk of clinical treatments involving hPSCs is tumorigenicity of pluripotent cells [23]. Biochemical signaling networks that are responsible for pluripotency and oncogenesis are partially overlapping. As a result of that, pluripotent and tumor cells share expression of certain genes, exhibit glycolytic metabolism, high proliferation rate and capacity, DNA repair checkpoint uncoupling, etc. Injection of hPSCs into immunodeficient mice leads to formation of benign tumors [24], a property that is used to confirm pluripotency of the cells. Since existing

differentiation protocols are not absolutely efficient and sometimes yield a mixture of differentiated and residual undifferentiated cells, a hPSC-derived cell population aimed for transplantation into patients should be treated to contain no residual pluripotent cells. This goal can be achieved by purification of desired differentiated cell populations [25], by removing of residual pluripotent cells using cell sorting [26] or by selective elimination of residual hPSCs using cytotoxic agents that are specific to pluripotent stem cells [27, 28].

High proliferation rate and DNA checkpoint uncoupling are probably responsible for accumulation of adaptive genetic changes in hPSCs during prolonged culturing in vitro. It has been shown that propensity to undergo genetic changes is attributed to the nature of hPSCs themselves rather than to a certain set of culture conditions [29, 30]. The genetic abnormalities commonly affect chromosomes 12, 17, 20 and X and, sometimes, show similarity to those found in tumors increasing the risks of tumorigenecity. Importantly, some of them are too small to be detected by karyotyping prompting development of methods with higher resolution for routine testing of hPSCs [30]. At the moment, a combination of karyotyping and whole genome genotyping array analysis with resolution about 50 Kb performed every 10 passages (2 months) are regarded as sufficient to monitor genetic integrity of hPSCs cultured in vitro. In close future, the genotyping will probably be replaced by whole genome sequencing. Freezing down a master bank of hPSCs as early after derivation (at low passages) as possible is one more approach for reducing the risk of adaptive genetic abnormalities in pluripotent stem cells.

Apart from common for all types of hPSCs risks mentioned above, hiPSCs and phESCs may be associated with additional tumorigenic potential. It has been shown that reprogramming of somatic cells into hiPSCs leads to generation of increased number of genomic aberration that are often deletions of tumor-suppressor genes [31]. Unlike genetic mutations caused by cultural adaptations, de novo mutations that appear during the reprogramming process render the cells into selective disadvantage and affect only part of the cell population. Expansion of cells in vitro selects against the affected cells and, eventually, genetic state of hiPSCs starts resembling that of hESCs. Nevertheless, increased number of cancer-related mutations soon after reprogramming [32] complicates banking of hiPSCs at low passages.

Generation of homozygous phESCs may be useful for development of hPSCs banks (see below). But, loss of heterozygosity may be associated with additional risk of tumorigenicity [33]. Many cancer-related mutations in human genomes are compensated by expression of normal second alleles. Therefore, absence of the second allele may be detrimental for homozygous phESCs and, at a lesser extent, even for heterozygous phESCs.

Epigenetic imperfections are one more feature of some types of hPSCs that may complicate their use in medicine. Epigenetic marks, such as DNA methylation and chemical modifications of histone proteins, do not change DNA sequence, but dramatically alter expression of associated genes. Majority of cells in the human body contain same genetic information and epigenetic pattern distinguish one cell type from the others. Parthenogenetic pluripotent stem cells are epigenetically abnormal by definition due to aberrant genomic imprinting in parthenotes. Although number of imprinted genes in humans is comparably low (around 100), their aberrant expression prevents normal development of human parthenogenetic embryos. More studies are warranted to understand how crucial those aberrations for therapies involving hpESCs.

Reprogramming of somatic cells into iPSCs involves major reconfiguration of epigenome. Contemporary methods of reprogramming generate hiPSCs that have aberrant DNA methylation and aberrant expression levels of some genes [14,17, 15]. It is not entirely clear whether those changes are associated with the reprogramming methods themselves or represent epigenetic memory of the parental somatic cells, but they may be transmitted to differentiated progeny of hiPSCs [17]. A direct comparison of genetically matched hESCs, human NT-ESCs and hiPSCs reported by Ma et al. [34] has revealed a significantly higher incidence of genetic aberrations in the latter cells. This or other reasons sometimes lead to incomplete differentiation of hiPSCs yielding only partially functional cells{Foldes, 2014 #7}. It is important to note that number of epigenetic errors in hiPSCs decreases with time in culture and, eventually, epigenetic state of hiPSCs starts resembling that of hESCs. Nevertheless, at the moment clinical-grade hiPSC lines may demand more rigorous testing than hESC lines [23].

One important issue that hinders clinical applications of hPSCs is ethical issues associated with some types of them. Only hiPSCs are completely free of ethical concerns. In this respect, hESCs are probably the most problematic. In vitro fertilization (IVF) clinics collect and fertilize many oocytes for almost each couple that needs infertility treatment. Only some of them are used in the actual treatment. Some of the embryos are not qualified for the infertility treatment due to aberrations in their development. Only such embryos with informed consent of both parents are used for derivation of new hESC lines. Nevertheless, destruction of fertilized embryos is ethically controversial and forbidden in many countries in the world. Klimanskaya et al. [9] reported derivation of new hESC lines without destruction of the parental embryo that is based on a method resembling PGD biopsy of 8-cell embryo. PGD biopsies are a standard IVF clinic routine for patients with certain genetic background. This method has addressed the ethical concerns of many at certain extent. Generation of human NT-ESCs and phESCs is also associated with certain ethical issues because of procurement of unfertilized human oocytes [21]. Standard routines for such procedures include superovulation of a donor that is distressful and is associated with some side effects. One way to circumvent the problem is to make the generation of pluripotent stem cells beneficial for the donor of the oocytes. In this respect, generation of human NT-ESCs from somatic cells and oocytes of the same donor may be of particular interest (see above).

Complex legislation regarding studying, patenting and using of hPSCs in medicine is yet one more hurdle for biomedical research in the world. The regulations differ not only in different countries, but also in different regions of the same county. Thus, California is the world-leading place for hESC research and therapies involving hESCs,

but in some other states in the US there is a complete ban on hESC research and use in medicine. Moreover, the legislation sometimes unexpectedly and abruptly changes with time. Thus, the European Court of Justice banned patent protection for hESC lines in 2011 jeopardizing future of hPSC-related regenerative medicine in Europe. Indeed, current clinical studies involving hPSCs are performed in the US and in Japan only. A group of leading stem cell scientists proposed the establishment of an international authority that would develop and harmonize all technical, ethical, legal and regulatory aspects of hPSC-based therapies [35]. Such authority may greatly facilitate hPSC-based research and regenerative medicine in the world.

General risks of cell-based treatments

There are general risks of cell-based treatments that are applicable to hPSCs-based therapies. Development of cells aimed at therapeutic applications should be done in compliance with current good manufacturing practices (cGMPs), which is a set of minimal requirements to meet for production of therapeutic agents and devices. National authorities such as the US Food and Drug Administration (FDA) or the World Health Organization define the requirements in different countries. Although different in details, cGMPs follow same line in majority of the countries in the world. The aim of cGMPs is to minimize risks for the patients and if problems appear to be able to trace the source of them. All stages of a therapeutic agent development should comply with cGMP. In case of therapies involving hPSCs, many steps of the final cell product development should comply with cGMP, for instance derivation of hPSC lines, culturing of hPSCs, freezing/thawing of hPSCs, development of a master bank of pluripotent cells, thawing of cells for differentiation, differentiation procedures, testing of differentiated progeny before transplantations, etc. Therefore, all the reagents that are in contact with stem cells should be traceable and, preferably, chemically defined. Ideally, derivation, culturing and storing of hPSCs should be done in chemically defined conditions without contact with components of animal origin. The latter is an important issue, because a contact with components of animal origin may induce immunogenicity of hPSCs and their differentiated progeny [36]. To date, methods of derivation, culturing and freezing/thawing under xeno-free (devoid of components of animal origin) and chemically defined conditions have been reported for hESCs [37–42] and hiPSCs [43, 44]. The methods comply with the most stringent rules and it will be easy to receive an approval of regulatory agencies for treatments based on them. Current clinical trials involving hPSCs rely on obsolete technologies (see below) of derivation and culturing and it took a lot of efforts and time to earn the approval for them.

A key problem of regenerative medicine is immune rejection of allografts. In case of a transplantation of organs or cells from non-identical individuals, they prompt immune reaction in a recipient. Immunological rejections can be caused by incompatibility of ABO blood group, major histocompatibility complex (MHC, in humans also called HLA) and minor histocompatibility complex antigens. Generation of autologous hPSCs is an obvious solution to this issue. Thus, hiPSCs, hpESCs and human NT-ESCs allow generation of autologous stem cell lines. If derived from a single cell PGD biopsy [9, 41], hESCs may also be autologous to the individual that is born from the parental embryo. But, generation of hPSCs for one patient is too time-taking and expensive to be used as a standard approach in the close future.

Use of immune privileged sites of the body for allotrasplantations is the most common approach for prevention of immune rejections in current clinical trials involving hPSCs. The eye, testes and central nervous system are regions of the body that may protect allografts from immune rejection for significant periods of time. Another approach involves using of artificial devices that protects allografts from the immune rejection, but allows oxygen, nutrients and some (small) proteins transport via a semi-permeable non-immunogenic membrane. The devices can be implanted under skin generating an artificial “immune privileged” site. Although already used in practice, this approach is limited to a subset of patient conditions only.

There is a wide consensus among stem cell scientists that future treatments involving pluripotent cells will rely on banks of hPSC lines that HLA-match a target population [45, 19]. To achieve an essential level of immunocompatibility, HLAs of transplanted cells should match HLA-A, HLA-B, HLA-C and HLA-DR loci of a recipient individual. Since HLA-homozygous cells contain identical alleles of HLAs, they facilitate finding an HLA-match for recipient individuals and it is an attractive idea to use the cell lines for development of hPSC banks. Indeed, it has been calculated that as little as 50 HLA-homozygous hPSC lines can provide cells that HLA-match 80–90% of the Japanese population{Nakatsuji, 2008 #60}. Homozygous individuals are rear and, therefore, generation of HLA-homozygous phESCs is of interest for generation of hPSC cell banks. Nevertheless, it is not clear if even HLA-compatible homozygous cells would not induce NK cell toxicity in heterozygous recipient individuals. NK cells are capable of detecting and reacting to levels of antigen expression and missing HLA-alleles may induce the immune response (reviewed in [33]). Although preliminary experiments in mice are encouraging [46], this issue should be thoroughly studied before development of clinical-grade HLA-homozygous hPSCs banks. As of today, banks of clinical-grade heterogeneous hESC and hiPSC lines are under development in the UK and Japan, respectively.

There are several other methods for reduction of immune rejection. Systematic immunosuppression is the most commonly used method in current medicine, but it is associated with serious side effects. Other methods have shown encouraging preclinical data, but they are not tested in clinical trials. The methods include simultaneous transplantation of the graft and hematopoietic cells derived from same hPSCs [47], disruption the co-stimulatory blockade required for T-cell activation [48] and genetic manipulation of hPSCs for reduction of HLA-expression in them.

Clinical-grade lines and banks of hPSCs

Clinical-grade hPSC lines should be established, propagated and stored in adherence to ethical standards,

regulations of international and national authorities and in compliance with cGMPs. First article reporting derivation, propagation, storage and testing of clinical-grade hESCs was published in 2007 [49]. The work was done by ES Cell International Pte Ltd., a biotechnology company from Singapore. Since xeno-free chemically defined methods had not been developed by then, the authors used human feeder cells and products derived from bovine serum for derivation and propagation of clinical-grade hESCs. Next generation of clinical-grade hESCs was derived and propagated in fully xeno-free conditions [50, 51].

Plans for development of two banks of hPSC lines have been announced to date. Thus, the UK Medical Research Council has supported derivation and banking of clinical-grade hESC lines in several research facilities around the country [52]. Several clinical-grade lines that are developed for the bank have been already described in scientific articles [51]. In Japan, generation of hiPSC lines bank has been initiated by Prof Yamanaka and supported by the Japanese Government [53]. The hiPSC lines bank should HLA-match a large proportion of the Japanese populations, although it is not clear how many clinical-grade lines have been generated by now.

The first clinical trial involving hPSCs

Geron Corporation, a biotechnology company from the US, conducted the first in the world clinical trial of a cellular product (GRNOPC1) derived from hESCs. The study was done to assess the safety of transplantation of hESC-derived GRNOPC1 into patients with spinal cord injuries. Preliminary data had indicated that injection of hESC-derived oligodendrocyte progenitor cells (OPCs) into rat spinal cord injury sites led to differentiation of the cells into terminally differentiated oligodendrocytes, enhanced remyelination and substantial improvement of locomotor ability [54]. The therapeutic effect differed significantly between injections 7 days and 10 months after injury. In the latter case, only differentiation of OPCs was detected without any improvement of locomotor ability suggesting that there was a limited therapeutic window for the treatment. The cells for the treatment were developed in contact with Matrigel that is an undefined batch-to-batch different animal-derived protein mixture. Matrigel is an extracellular matrix protein extract from Engelbreth-Holm-Swarm mouse sarcoma. The cancer-related nature of Matrigel and overall concerns about safety of hPSCs-based treatments were probably the reasons why it was very difficult for Geron to earn an approval of the trial by the US Food and Drug Administration (FDA). In the US, approval by the FDA is a necessary step for all clinical trials. Moreover, spinal cord injury is a complicated condition that affects several types of cells and is associated with formation of a scar tissue. It is not entirely clear why Geron chose that complicated disease for the first in the world clinical trial involving hPSCs.

Geron's application for the clinical trial to the FDA was very extensive containing thousands of pages. The study was finally approved in 2009, but later was halted twice by the regulator [55]. First time, it was halted because of concerns about the purity and homogeneity of GRNOPC1 and second time after Geron's report on microscopic cysts in spinal cords of rats treated during preclinical studies. Both times, additional information provided by Geron prompted the FDA to allow the study to proceed. In 2011, Geron ceased work on its stem cell-related programs because of financial reasons [56]. Only 4 out of 8 planned patients [56] were enrolled in the study (according to other reports 5 out of 10 [55]). No report on the trial results has been published. Asterias Biotherapeutics, a biotechnology company that bought Geron's stem cells therapy, continues to monitor the patients' conditions. None of the patients has suffered serious adverse effects [55]. No improvements in patients' conditions have been observed, but phase I clinical trials are designed to test for safety only. Recently, Asterias Biotherapeutics has announced that the clinical trial is going to be restarted [55].

Ongoing hPSC-based clinical trials with enrolled patients

Another the US-based company, Advanced Cell Technology, leads two connected and very well described phase I/II clinical trials aimed at assessing the safety and the efficacy of hESCs-based treatments of patients with Stargardt's Macular Dystrophy and Dry Age-Related Macular Degeneration. The FDA approval was earned in 2010 and two scientific articles describing the results have been published since then [57, 58]. Here, similar to the Geron's study, a disease affecting an immonoprivileged site was chosen to reduce immune rejection of allografts thus reducing a number of clinical-grade hPSC lines needed for the trial.

The treatment is based on differentiation of hESCs into retinal pigment epithelium (RPE) and injection of RPE into the subretinal space of one of the patients' eye. The only hESC line MA09 used in the study was derived and propagated in contact with mouse fibroblast cells. Therefore, its differentiated progeny is qualified as a xenotransplantation product [58]. Similar to the Geron's trial, the obsolete technology of hESC development raised many concerns regarding safety, complicated the FDA approval process and increased the overall price of the therapy because of additional testing applied to xenotransplantation products. The differentiation method used in the study is unreliable at certain extent and is based on arbitrary criteria. The cells are developed in contact with gelatin that is a batch-to-batch different mixture of proteins. RPE colonies are manually isolated with a glass pipett. Nevertheless, all the procedures are performed in accordance to the cGMPs and RPE cells are assessed for safety and specific attributes at various times.

By now, 18 patients have been enrolled to the studies (9 for each disease) [58]. After surgery, 13 of 18 patients had an increase in subretinal pigmentation that is consistent with transplanted RPE. There has been reported no safety issues related to the transplanted cells after a median of 22 months after transplantation suggesting medium-to-long term safety of the treatment. There has been reported a significant improvement in the eye function in 10 patients, modest or no improvement in 7 patients and a decline in the eye function in one patient. No improvement in the untreated fellow eyes function has been observed. The

results suggest medium-to-long term graft survival and possible biological activity of hESC progeny in the patients.

Rather a prove of principal study than a clinical trial involving hPSCs (particularly hiPSCs) was launched in Japan in 2014. A 70 years old woman with wet type age-related macula degeneration received a hiPSC-based treatment that was probably similar to the Advanced Cell Technology's therapy. The authors used hiPSC line that had been derived from the patient's own skin as a source of cells for differentiation into RPE. It is not clear how many patients have been enrolled to the study to date. No data on the results have been published to date and the study has not been registered in ClinicalTrials.gov.

A different approach to protection of allografts from immune rejection is used in a clinical study involving hPSCs for treatment of patients with type I diabetes that is conducted by ViaCyte, a biotechnology company from California, the US. According to the company's web page, ViaCyte has designed a semi-permeable cell containment device for immunoprotection of allografts. If implanted subcutaneously, the device allows transport of oxygen, nutrients and small proteins but contains the graft cells inside and protects them from immune rejection. The device may be useful for treatment of several diseases, but in this trial it is used to prevent rejection of hESC-derived pancreatic endoderm in patients with type I diabetes. The details of the differentiation procedure is not clear, but according to the company's web page, it is based on a four-step differentiation protocol developed by Kroon et al [59]. By now, 40 patients have been enrolled in the study. No results have been published yet.

Conclusion remarks

In spite of numerous ethical issues, safety concerns and technological challenges, hPSCs-based therapies are already used to treat patients with various diseases. Results of the Geron's and the ongoing clinical trials involving hPSCs have suggested medium-term and even long-term safety of such treatments. None of the four patients treated with hESC-derived grafts during the Geron's study 4 years ago have developed any serious complications related to the transplanted cells. Schwartz et al. have reported similar results for 18 patients 2 years after transplantations. Surprisingly, the treatment of Stargardt's Macular Dystrophy and Dry Age-Related Macular Degeneration with hESC-derived grafts have exhibited high rate of success already during Phase I/II clinical trials. The overall results are promising, but any hPSC-related complication may jeopardize the future of pluripotent stem cells in medicine.

One important threshold for new clinical studies involving hPSCs is difficulties in approval by the regulatory agencies. It was especially complicated and costly for Geron to earn the approval for the first in the world hPSC-based clinical trial. But, it was less difficult already for the second study done by Advanced Cell Technology because they knew what to expect from the FDA. Generally, it is hard to prove safety of cells that are developed in contact with xeno-components and with batch-to-batch different chemically undefined components. Recently, several scientific groups have reported methods that enable development of hESCs and hiPSCs under xeno-free and chemically defined conditions. Such method may facilitate the approval of new clinical trials involving hPSCs.

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