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Current status of stem cell therapy: opportunities and limitations

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Abstract: Over recent years stem cells have stood out as a promising tool for regenerative medicine, providing alternative therapeutic solutions for a large number of diseases. Many clinical trials using stem cells or induced pluripotent stem cells are focused on the repair and regeneration of various tissues and organs in degenerative diseases, whose current treatment only succeeds in slowing down the progression of the disease. Although the preliminary results are interesting, further studies are required in order to evaluate the safety and benefits of stem cell therapy, considering the teratoma development and ethical considerations in embryonic stem cell cases or reprogramming-induced somatic mutations and epigenetic defects. This review summarizes several current clinical and nonclinical data on the use of embryonic stem cells, mesenchymal stem cells, and induced pluripotent stem cells in various diseases.

Key words: Regenerative medicine, stem cells, cell reprogramming

1. Introduction

Current treatments for many degenerative diseases such as Alzheimer and Parkinson disease, motor neuron disease, multiple sclerosis, diabetes, and kidney, liver, and heart diseases, as well as for many types of cancer, are mainly symptomatic, and for certain diseases, total recovery implies whole organ transplantation (Gieseck et al., 2015).

Stem cell therapy that concerns cell reprogramming and transplantation of embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) represents an interesting yet disputed research area, with exciting results for many diseases. These pluripotent/multipotent cells can be differentiated in vitro to a desired cell type and they are used for transplantation into patients with various disorders, as illustrated in the Figure. Stem cells, including ESCs and MSCs, present self-renewal ability and they also have the capacity to differentiate into one or more mature cellular lineages, being promising tools for clinical applications. In the course of mammalian development, stem cells are involved in tissue and organ formation (ESCs), and in several adult tissues they can provide regenerative capacity (adult stem cells). These properties are directed by the interaction of cell type-specific transcription factors and chromatin regulators (Klimanskaya et al., 2008; Sarkar and Hochedlinger, 2013; Mariano et al., 2015).

Gaining knowledge about stem cells has enabled the development of a new branch of medicine, called regenerative medicine. In this new branch, the therapeutic act involves the manipulation of stem cells in order to regenerate tissues and organs of an organism altered by destruction, disease, or congenital defects (Katari et al., 2014; El-Badawy and El-Badri, 2015; McNamara et al., 2015). A first use of the stem cells has been performed in bone marrow transplantation containing multipotent stem cells in patients with various forms of hematological disorders, including acute myelogenous leukemia, acute lymphoblastic leukemia, non-Hodgkin lymphoma, and myelodysplastic syndromes (Karanes et al., 2008). This review presents several current clinical and nonclinical data concerning mainly the use of ESCs, MSCs, and iPSCs in the treatment of different diseases, highlighting both the opportunities and the limitations of this therapy.

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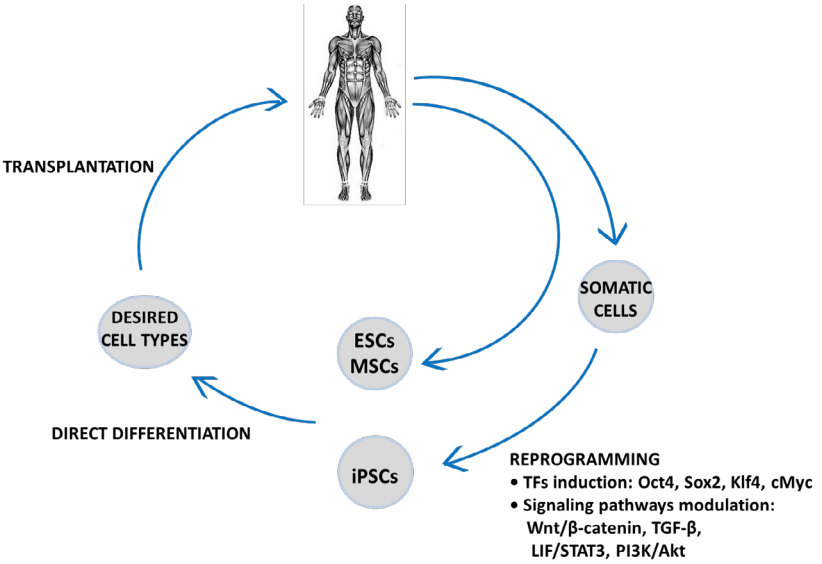

Figure. The main strategies for generating desired cell types for subsequent transplantation into patients involve direct differentiation of embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) derived from somatic cells by manipulation of several transcription factors (TF) or by modulation of signaling pathways.

2. Embryonic stem cells

ESCs are stem cells derived from an early stage of embryo development or from the inner cell mass of the developing embryo, capable of differentiating into nearly all cell types of all three germ layers (ectoderm, mesoderm, and endoderm). Pluripotency characterizes especially the early stage of embryo development (Mizuno et al., 2012; Chuang et al., 2015). The ESC capacity of proliferation and transformation in all somatic cells made these cells, initially, the main source of stem cells. The use of ESCs in therapy, although extremely promising, raises diverse issues such as rejection of ESCs, which requires immunosuppressive treatment, and the possibility to induce tumor cells, presenting legal and ethical limitations (Kfoury, 2007; Ramos-Zuniga et al., 2012; Schwartz et al., 2012). Thus, in vivo experiments conducted in the areas of tissue destruction showed that ESC administration to immunodeficient mice, by injection of these cells into the wound, did not lead to the restoration of the tissue but rather to the formation of tumors called teratoma, with a low malignancy potential, containing structures derived from all three germ layers. This type of experiment has shown that the therapeutic use of ESCs necessitates isolation, cultivation, and forced differentiation in order for them to derive into the desired cell types. Only these differentiated cells may be safely injected into the recipient (Hentze et al., 2009).

Limitations of ESC use are also caused by their immunological rejection phenomena; therefore, appropriate immunosuppressive strategies are required (Stuckey and Shah, 2014). Clinical trials using ESCs have focused on several disease treatments, including neurological (Ambasudhan et al., 2014), cardiac (Ban et al., 2014), and pancreatic (Wu et al., 2011) disorders, highlighting the regenerative potential of ESCs for damaged structures.

In a study that used human ESC-derived cardiomyocytes in a nonhuman primate model of myocardial ischaemia, it was shown that the infarcted monkey heart presented extensive remuscularization after treatment, with nonfatal ventricular arrhythmias as a secondary effect (Chong et al., 2014). Hepatocyte-like cells derived from human ESCs and engrafted into mice sustained proliferation of host hepatocytes and revascularization of injured host liver tissue by providing trophic factors involved in liver regeneration, while human ESC-derived pancreatic progenitor cells can differentiate in vivo into functional islets in diabetic mice (Rezania et al., 2012; Woo et al., 2012).

In the United States, two prospective phase 1/2 studies evaluated the safety and tolerability of subretinal transplantation of human ESC-derived retinal pigment epithelium in patients with Stargardt macular dystrophy and atrophic age-related macular degeneration. The preliminary results suggested that no adverse proliferation, rejection, or safety issues were associated with the treatment. Moreover, improvements in subretinal pigmentation visual acuity have been observed (Schwartz et al., 2015).

3. Adult stem cells

MSCs, the commonest type of adult stem cells, represent an attractive research field for scientists due to their potential to regenerate damaged or degenerated tissues and organs, and also because their use does not create ethical or immunological concerns. MSCs represent an accessible stem cell source since they can be isolated from adult somatic tissues, such as the bone marrow, skin, adipose tissue, umbilical cord, and intestines. These cells, together with endometrial stem cells, can differentiate into one or more lineages with mesodermal and ectodermal origin (hepatocytes, osteoblasts, smooth muscle, cardiomyocytes, cartilage, adipocytes, pancreatic cells, neuronal cells, dopaminergic neurons), displaying a high proliferative potential, clonogenicity, or colony forming unit activity (Ghobadi et al., 2015; Goodarzi et al., 2015). MSCs are able to manage the repair response by recruiting other cells and by secreting several bioactive molecules, including growth factors and matrix proteins capable of stimulating the recovery of damaged cells and inhibiting inflammation. These cells are also characterized by weak immunogenicity and, at the same time, by the ability to perform immunomodulatory functions. Although primary MSCs are found in a limited number in human tissues, these stem cells can be expanded in long-term culture systems, allowing the obtainment of a large-scale production of MSCs for clinical application (Wang et al., 2011; Wang et al., 2012).

Based on MSCs' unique properties, such as tissue repair and major histocompatibility complex (MHC)-unmatched immunosuppression, these cells have been used for graft-versus-host disease (GVHD) treatment, a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation. A combination therapy with MSCs and Treg cells, for example, seems to increase the immunomodulatory activity of MSCs (Kitazawa et al., 2012; Lim et al., 2014). Using in vitro and in vivo experiments, Jang et al. demonstrated that placenta-derived MSCs transplanted into mice can control GVHD after hematopoietic stem cell transplantation (Jang et al., 2013). Clinical data suggest a benefit in approximately two-thirds of the patients with steroid-resistant acute GVHD (Tolar et al., 2011; Resnick et al., 2013). In systemic sclerosis, a chronic disease characterized by early activation of the immune system, MSCs manifest immunomodulatory functions, such as an immunosuppressive effect on lymphocyte proliferation (Cipriani et al., 2013). Furthermore, preclinical and clinical studies have focused on MSC-based therapy in Crohn disease, a major inflammatory bowel disease characterized by pathological immune responses to different antigens (Forte et al., 2015). Clinical studies demonstrated that, when administered locally by injection, MSCs represent a harmless therapy that can sustain the therapeutic response in patients with Crohn disease (Ciccocioppo et al., 2015).

MSCs have been efficiently developed as a promising tool for clinical applications in digestive tract defects (Sirbu-Boeti et al., 2009), as well as in musculoskeletal diseases, including bone fractures, bone defects, focal chondral lesions, osteoarthritis, spinal diseases, and tendon injuries (Wei et al., 2014). Recent studies in the equine industry demonstrated that MSCs have the capacity to differentiate into osteoblasts by manipulation of several transcription factors, such as runt-related transcription factor 2 (Runx2) and osterix (Osx); this therapy can improve fracture healing and reduce the incidence of reinjury (Govoni, 2015). In secondary osteoporosis, systemic transplantation of human bone marrow MSCs and stem cells from exfoliated deciduous teeth in murine models recovered the reduction of bone density through IL-17 suppression (Ma et al., 2015).

Due to the important role of MSCs in bone tissue repair or regeneration, numerous studies have focused on testing pharmacological molecules able to promote MSC homing or to mobilize bone marrow MSCs in the peripheral blood for enhancing the recruitment of MSCs to the injured bone tissues (Zhou et al., 2015).

There are also several studies showing that MSCs can be considered therapeutic tools for patients with neurodegenerative diseases, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, Huntington disease, and multiple sclerosis, due to their capacity to transdifferentiate into neural cells and their neuroprotective and immunomodulatory effects (Tanna and Sachan, 2014). Genetically modified MSCs were used in neurodegenerative diseases as vehicles for transporting or releasing neurotrophic factors, such as glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), able to protect and to sustain regeneration of damaged tissue (Wyse et al., 2014). Even if initially adult neural stem cells (aNSCs) were considered a promising source for stem cells in neurodegenerative disorders due to their capacity to differentiate easily into neuronal lineages, experiments showed that these stem cells are vulnerable to immune responses following transplantation. The results obtained by an in vivo study demonstrated that transplantation of combined MSCs and aNSCs resulted in increased survival of the transplanted aNSCs as well as a longer-term behavioral benefit in a transgenic rat model of Huntington disease, mainly because MSCs are less vulnerable to rejection following transplantation and also due to the fact that MSCs might ensure a more favorable environment for aNSCs' survival (Rossignol et al., 2014). In a rat model of Parkinson disease, MSC transplantation resulted in upregulation of peripheral antiinflammatory

cytokines, increased neurogenesis, and improved memory functioning, with modulatory effects on the hippocampus (Schwerk et al., 2015).

Neural crest cells represent a multipotent and migratory cell population able to generate a variety of cell and tissue types, such as craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, glia, melanocytes, or connective tissue (Achilleos and Trainor, 2012). Neural crest progenitors persist in adult life in differentiated tissues, including the enteric nervous system of the gut, the hair follicles of the facial skin, etc. (Teng and Labosky, 2006). Although differentiated, the neural crest-derived cells possess phenotypic plasticity. Dupin et al., using in vitro experiments, showed that epidermal pigment cells and Schwann cells from peripheral nerves have the capacity to reverse into multipotent neural crest-like progenitors with a self-renewal property. It seems that various neural crest progenitors can express stem cell properties; at the same time, differentiated cells of neural crest origin can reacquire these functions, raising the possibility of using them as a promising tool for regeneration (Dupin et al., 2007). Human epidermal neural crest stem cells from the bulge of hair follicles are also suitable candidates for cell-based therapies, disease modeling, and drug discovery (Sieber-Blum, 2014).

Recently, a rare population of very small embryonic-like stem cells (VSELs), still under scientific debate, was identified in a quiescent state in bone marrow and other adult tissues. They are smaller than red blood cells, express several markers characteristic for pluripotent stem cells (Oct 4, Nanong, SSEA), and could be mobilized in peripheral blood under stress conditions. They are supposed to be a link between early development stages and adult stem cell compartments. VSELs hold the promise of a new source for developing regenerative therapies to repair complex tissue damaged by trauma or degenerative conditions, such as osteoporosis (Ratajczak et al., 2012; Havens et al., 2013; Ratajczak et al., 2014).

Recent but also disputed studies have identified stem cells in adult mammalian ovaries that can be involved in oocyte renewal. These ovarian germline stem cells are well characterized in nonmammalian model organisms and the perspective of isolation and growth of human ovarian stem cells could offer new opportunities for the treatment of women's infertility (Hanna and Hennebold, 2014). In vivo studies demonstrated that transplantation of a small proportion of human and mouse cells from ovarian epithelium and cortical tissue can generate immature oocytes into ovaries of immunodeficient mice (Gheorghisan-Galateanu et al., 2014).

Nasal stem cells are multipotent stem cells localized in the olfactory mucosa, being considered as an attractive source for autologous stem cell-based therapies due to their accessibility. These stem cells are involved in adult neurogenesis and tissue regeneration after injury; therefore, several studies focused on the use of nasal stem cells for biomarker identification in brain disorders or for repairing processes in the pathological/traumatized nervous system (Fletcher et al., 2011; Feron et al., 2013; Stamegna et al., 2014).

Hematopoietic stem cells (HSCs) symbolize the classical stem cell of the organism. The first clinical use was in hematological malignancy therapy, aiming to restore normal hematopoiesis. Nowadays, HSC transplantation is utilized with increased success rates in various malignant and nonmalignant conditions. HSCs can be isolated from bone marrow and umbilical cord blood and they have the capacity to repopulate the entire hematopoietic system. HSCs also have the capacity to sustain the regeneration of nonhematopoietic tissue such as that of the liver, heart, and brain. More than 2000 clinical studies regarding the use of HSCs in the treatment of numerous diseases (e.g., cancers, leukemia, lymphoma, cardiac failure, neural disorders, autoimmune diseases, immunodeficiency, and metabolic or genetic disorders) are currently being conducted (Chivu-Economescu and Rubach, 2015; Porada et al., 2015).

4. Human induced pluripotent stem cells

In 2006, a seminal paper (Takahashi and Yamanaka, 2006) showed, using mouse fibroblasts, that adult somatic cells could be converted to stem cells. These cells share similar features to ESCs (morphology, stem cell markers, stem cell gene expression, self-renewal, and differentiation potential) and they were designated as iPSCs. The technology employed by Yamanaka's team to reprogram adult cells required simultaneous introduction of four transcription factor genes (called reprogramming factors), Oct3/4, Sox2, c-Myc, and Klf4, by means of a retroviral system. A year later, 2 research groups managed to independently generate human induced pluripotent stem cells (hiPSCs) from somatic cells using either the same approach as in mouse cells –Oct4, Sox2, c-Myc, and Klf4 with a retroviral vector (Takahashi et al., 2007) – or OCT4, SOX2, NANOG, and LIN28 with a lentiviral system (Yu et al., 2007).

Although the precise process by which somatic cells are converted to iPSCs is not fully understood, recent studies have revealed that the reprogramming factors Oct3/4, Sox2, and Nanog inhibit genes involved in cell differentiation, and together with c-Myc determine epigenetic changes (methylation of DNA, chromatin modification) that lead to the generation of iPSCs (Loh et al., 2006; Kim et al., 2008). Moreover, similarly to ESCs, the promoter regions of Nanog, Oct3/4, and Fbx15 were demethylated in iPSCs (Takahashi and Yamanaka, 2006; Miyazaki et al., 2012).

Since their first description, hiPSCs have received huge interest not only among scientists but also in the general population due to their tremendous potential applications in regenerative medicine, drug development, toxicity tests, and disease modeling. They overcome the ethical aspects related to ESC production and represent an endless source for patient-specific stem cells.

Besides dermal fibroblasts, commonly used as source for hiPSC generation (Takahashi et al., 2007; Yu et al., 2007; Raab et al., 2014), a wide variety of cells were employed: hepatocytes (Liu et al., 2010), keratinocytes (Aasen et al., 2008), melanocytes (Utikal et al., 2009), astrocytes (Ruiz et al., 2010), dental pulp cells (Yoo et al., 2013), umbilical vein endothelial cells (Haile et al., 2015), cord blood (Haase et al., 2009), peripheral blood mononuclear cells (Fuerstenau-Sharp et al., 2015), kidney mesangial cells (Song et al., 2011), exfoliated renal epithelial cells present in urine (Zhou et al., 2012), etc.

However, though a promising tool for future therapies, hiPSC technology implies certain challenging hurdles in terms of safety and efficacy: the risk of insertional mutagenesis, as well as tumor formation, a low efficiency rate of conversion, and incomplete reprogramming. In order to avoid the integration of vector and transgene sequences into the cell genome, alternate vectors were used: adenoviral vectors (Zhou and Freed, 2009), Sendai virus (Fusaki et al., 2009; Chichagova et al., 2016), episomes (Fontes et al., 2013), plasmids (Okita et al., 2008), transposons (Kaji et al., 2009; Woltjen et al., 2009), synthesized mRNAs (Warren et al., 2010), and protein (Kim et al., 2009).

Direct transfection of human somatic cells with specific mature microRNA molecules (such as a combination of mir-200c, -302s, and -369s families (Miyoshi et al., 2011) or the miR302/367 cluster (Anokye-Danso et al., 2011)) is capable of reprogramming human somatic cells to pluripotency (Okano et al., 2013).

Recent studies were pursued to generate iPSCs using combinations of small molecules that could replace either partially or completely the transcription factors and improve the efficiency of reprogramming. These small chemical compounds are epigenetic modifiers, WNT signal modulators, moderators of cell senescence, modulators of metabolism, and regulators of MET (Lin and Wu, 2015). In 2013, Hou et al. described for the first time a cocktail of small molecules (forskolin, valproic acid, CHIR99021, 616452, tranylcypromine, 3-deazaneplanocin) that are able to reprogram differentiated cells into iPSCs (Hou et al., 2013).

iPSCs offer an attractive application in disease-modeling and drug discovery. To date, many patient-specific iPSC lines have been established and used to create disease models, and they are expected to facilitate studies on rare diseases (Bellin et al., 2012). An international collaborative project, StemBANCC, started in 2012, aims to generate and characterize 1500 hiPSC lines. The project, conducted by the University of Oxford and gathering 10 pharmaceutical companies and 23 universities, will offer researchers valuable resources for a better understanding of the disease pathogenic mechanisms and the chance to develop new treatments (http://stembancc.org/).

The ability to differentiate into many cell types, the relatively easy accessibility, the renewable capacity, and the possibility to use individual autologous and allogeneic cells contribute to the therapeutic potential of hiPSCs in regenerative medicine. In diseases previously considered incurable, such as neurodegenerative disorders (Ross and Akimov, 2014; Brandl et al., 2015), spinal cord injury (Kobayashi et al., 2012; Nakamura and Okano, 2013), heart failure (Fujita et al., 2012; Hsiao et al., 2013; Khan et al., 2015), diabetes (Abdelalim et al., 2014; Bose et al., 2014; Holditch et al., 2014), and retinal disease (Mead et al., 2015), hiPSC replacement-based therapies represent a promising approach (Seki and Fukuda, 2015).

The damaged or degenerated tissue is repaired by means of iPSCs obtained from the patient's somatic cells, differentiated in vitro and then transplanted in the affected tissue. In the case of diseases caused by a genetic mutation, it is possible to correct the genetic defect by obtaining mutation-free iPSCs, further to be differentiated to specific cell types. In a very recent paper, using reprogramming and editing genome technologies, Crane et al. managed to correct CFTR gene mutations responsible for cystic fibrosis in patient-derived iPSCs and restored gene function (Crane et al., 2015). Another group reported gene correction of alpha 1 anti-trypsin (A1AT) deficiency, one of the common genetic disorders associated with liver disease that might progress to cirrhosis and hepatocellular carcinoma, as well as with pulmonary emphysema. Hepatocyte-like cells obtained through differentiation of iPSCs presented the genetic and phenotypic correction of the Z mutation, one of the most common mutant forms of A1AT (Choi et al., 2013).

In 2013, Takebe et al. reported the generation of a functional human organ from pluripotent stem cells for the first time. They managed to create a three-dimensional vascularized and functional human liver using a coculture of hepatic endoderm cells derived from human iPSCs, human umbilical vein endothelial cells, and human MSCs. iPSC-derived liver buds transplanted into mice generated a vascularized and functional human liver. Although there are many steps to be done before their application in clinical medicine, these findings open a promising path in regenerative medicine (Takebe et al., 2013).

The first clinical trial using hiPSCs received the approval of the Japanese Ministry of Health and started

in Japan in 2014; the study addresses people suffering from wet age-related macular degeneration, a disease that may lead to blindness in aged people. The study employs autologous iPSCs derived from the patient's skin that are differentiated to retinal pigment epithelial cells and transplanted into the affected retina (Reardon and Cyranoski, 2014). Nevertheless, several studies have demonstrated that during in vitro culture, reprogrammed cells can accumulate genomic instability and genomic abnormalities, including de novo mutations that are not identified in human ESCs. Although the consequences of these epigenetic and genetic alterations are not well known, the affected cells seem to acquire premalignant properties, such as decreased differentiation and increased proliferative capacity. These observations support the necessity of controlling the hiPSCs' genome integrity before any clinical application (Puri and Nagy, 2012; Nguyen et al., 2013).

5. Cell signaling pathway modulation promotes somatic cell reprogramming

Stem cells are involved in tissue development, renewal, and regeneration, activities sustained by the 'niche', a local stem cell microenvironment. On the other hand, stem cells can generate their own niche, using several signaling pathways crucial for stem cell maintenance (Clevers et al., 2014). An important resource for regenerative medicine is the reprogramming of mature somatic cells to become pluripotent by the manipulation of several factor expressions. Trying to understand and improve the molecular mechanisms of induced pluripotency, cell signaling pathways that control the reprogramming process have also been extensively studied, including LIF/STAT3, PI3K/Akt, Wnt/β-catenin, TGFβ, and MAPK cascades (Kim et al., 2011; Hawkins et al., 2014).

In order to reprogram somatic cell, Samavarchi-Tehrani et al. used a secondary mouse embryonic fibroblast model to obtain iPSCs by manipulating Oct4, Klf4, c-Myc, and Sox2 expressions. Based on the results obtained by temporal gene expression analysis, they suggested that somatic cell reprogramming is a multistep process that includes initiation, maturation, and stabilization phases (Samavarchi-Tehrani et al., 2010).

The initiation phase of reprogramming is characterized by loss of the somatic cell program (e.g., loss of the transcription factors Snail1/2 or Zeb1/2 and gain of an epithelial signature by increased expression of CDH1, EpCAM, or the epithelial-associated miRNA family), metabolism changes, increased proliferation rate, inhibition of apoptosis and senescence, and morphologic changes (e.g., fibroblasts undergo a mesenchymal-to-epithelial transition, MET) (David and Polo, 2014). The epigenetic regulators have a significant role in the initiation phase; using mouse embryonic fibroblasts deleted in all three TET genes, Hu et al. observed that this model failed to initiate the reprogramming, mainly because TETs are involved in miR-200 activation and MET, respectively (Hu et al., 2014). The maturation phase is characterized by a major transcriptional modification, the gain of a subset of pluripotency associated genes (Nanog, Oct4, Esrrb, ICAM1), while the stabilization phase includes transgene-independent self-renewal, pluripotency, loss of epigenetic memory, x-reactivation, and telomerase elongation, presenting as specific markers Sox2, Dppa4, and PECAM (David and Polo, 2014).

Recent studies demonstrated that modulation of Wnt/β-catenin, MAPK/ERK, TGF-β, or PI3K/Akt signaling pathways enhances somatic-cell reprogramming (Sanges and Cosma, 2010).

The Wnt/β-catenin signaling pathway comprises Wnt ligands, Frizzled receptors, and a complex composed of APC (adenomatous polyposis coli), Axin1, GSK-3β (glycogen synthase kinase 3-β), and CK1 (casein kinase 1) that stabilizes β-catenin (Amado et al., 2014). Several studies demonstrated the critical role of Wnt signaling in self-renewal and maintenance of stem cells, and also in somatic-cell reprogramming. Marson et al. showed that Wnt cascade activation by soluble Wnt3a can directly sustain the induction of pluripotency, even in the absence of c-Myc transduction (Marson et al., 2008). In a recent study, Aulicino et al. demonstrated that in the early reprogramming phases of mouse embryonic fibroblasts into iPSCs, Wnt signaling must be downregulated, while in the late reprogramming phases an activation of this signaling is required in order to obtain an efficient reprogramming (Aulicino et al., 2014). These results are supported by another study showing that Wnt signaling, through its transcriptional factors Tcf1, Lef1, Tcf3, and Tcf4, can inhibit the early stage of reprogramming to iPSCs (Ho et al., 2013). An in vitro study showed that blocking nuclear localization of β-catenin by upregulation of E-cadherin (inhibitor of Wnt signaling) sustains the epiblast stem cells' reprogramming to ESCs (Murayama et al., 2015).

The TGF-β cascade is another critical signaling pathway for somatic-cell reprogramming. Several studies demonstrated that Sox2, Oct4, and c-Myc factors sustain MET by suppression of Snail and TGF-β1/TGF-βR2 (epithelial-to-mesenchymal transition activators), while the Klf4 factor directly sustains MET by E-cadherin activation. Thus, TGF-β signaling inhibition has a crucial role in both early and late events of somatic cell transition to a pluripotent state (Li et al., 2010; Samavarchi-Tehrani et al., 2010; Vidal et al., 2014). Yuan et al. reported that a TGF-β receptor inhibitor, A-83-01, in combination with a protein arginine methyltransferase inhibitor, AMI-

5, sustained the reprogramming of mouse embryonic fibroblasts transduced with only Oct4 factor (Yuan et al., 2011). Primordial germ cell reprogramming into a pluripotent state is carried out by downregulation of TGF-β and ERK (extracellular signal-regulated kinase) signaling pathways, using specific inhibitors (Attari et al., 2014).

The JAK-STAT3 cascade is one of the most significant signaling pathways for the maintenance of mouse ESC pluripotency and propagation, by activation of c-Myc transcription and stabilization of c-Myc protein levels; moreover, it has been demonstrated that Klf4 expression is induced by JAK-STAT3 signaling activation, depending on LIF signaling (Tang and Tian, 2013). LIF/STAT3 signaling is crucial for the maturation phase of mouse iPSCs' reprogramming by suppression of DNMT1 (DNA methyltransferase) and histone deacetylases 2, 3, and 8, with the final result of demethylation of pluripotency-associated gene promoters (Hawkins et al., 2014).

PI3K/Akt signaling activation was identified in the initiation phase, being involved in the metabolic switch from oxidative phosphorylation to glycolysis by activation of glycolytic regulators AS1060 and PFKB2 (Chen et al., 2012; Hawkins et al., 2014). Zhu et al. demonstrated that Akt activation is capable of increasing reprogramming by upregulation of glycolytic genes (Zhu et al., 2010).

Cell signaling pathways modulated by extrinsic factors and an intrinsic transcriptional network control the somatic cell reprogramming and the use of small molecule modulators of these signaling pathways can induce reprogramming with greater efficiency, substituting for classical transcription factors (Ma et al., 2013; David and Polo, 2014).

6. Biomimetic scaffolds and stem cells

Stem cell therapy in regenerative medicine involves the use of different strategies for in vitro construction of three-dimensional tissues or organs. Usually these approaches combine diverse scaffolds and signaling systems, thus inducing the differentiation of stem cells. Biomimetic scaffolds are made of natural or synthetic polymers or natural/synthetic hybrids. Natural polymers-collagen (Ott et al., 2008), fibrin (Christman et al., 2004), alginate (Landa et al., 2008), Matrigel (Giraud et al., 2008), chitosan (Lu et al., 2009), and hyaluronic acid (Holloway et al., 2015) are biodegradable proteins or polysaccharides that have a structure similar to the native components of the extracellular matrix, making them biocompatible and less immunogenic than synthetic polymers. They also have a higher capacity for cell adhesion and influence on various cellular functions. Disadvantages of such natural polymer-based scaffolds reside in the limited mechanical properties and biodegradability.

Skin and oral mucosa were generated on a micronized acellular dermal matrix (micronized Alloderm), using a combination of keratinocytes and adipose tissue stem cells (hASCs) (Yoo and Lim, 2009), as well as on other matrixes (Fang et al., 2014). The same cells (hASCs) were used on advanced collagen scaffolds with regular architecture, generating in vivo neovascularization and adipocyte differentiation (von Heimburg et al., 2003; Hemmrich et al., 2005).

In cardiac repair, some promising results were related to the use of collagen scaffolds, reported to enhance the survival of cardiomyoblasts and improve survival of ischemic rats (Miki et al., 2012). Injection of biomimetic scaffolds at the site of myocardial infarction (eventually supplemented with bioactive molecules) is reported to decrease the amount of fibrosis and ventricular dilation and to promote angiogenesis and recruitment of native stem cells (Kutschka et al., 2006; Zamora et al., 2013).

Bone reconstruction using scaffolds like hydroxyapatite, hydroxyapatite gel, or calcium phosphate was reported in bone reconstruction using bone marrow stromal cells (Özdal-Kurt et al., 2015); a similar approach was reported for the regeneration of dental pulp (Ravindran and George, 2015). The use of stem cells in neural differentiation/neuroregeneration was also reported, such as for induction of motor neuron differentiation applied with complex matrices that included collagen grafted nanofibers (Bagher et al., 2015), or peripheral nerve repair with single-walled carbon nanotubes/poly-lactic acid scaffolds (Kabiri et al., 2015).

Combination of multiple cell types, like hepatocytes and MSCs, with acellular matrixes for organ reconstruction is another field with promising results (Kadota et al., 2014).

7. Conclusions

Induced pluripotent stem cells together with embryonic stem cells and adult stem cells represent important candidates for regenerative medicine due to their extensive self-renewal and pluripotent proprieties. Stem cells have been tested for use in several diseases such as spinal cord injury, heart disease, stroke, and Parkinson disease, and also in various forms of hematological disorders. Nevertheless, the therapeutic use of stem cells is currently limited by several issues such as ethical considerations, teratoma development, and the long-term possibility of carcinogenesis, somatic mutations, and epigenetic defects induced by reprogramming. Further preclinical and clinical studies are needed in order to determine whether stem cell-based therapies can be useful in treating disorders for which available current treatments only succeed in slowing down the progression of the disease.

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