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Differentiation Plasticity of Germline Cell-Derived Pluripotent Stem Cells and Their Potential Application in Regenerative Medicine

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Abstract

Pluripotent stem cells hold the key to replacing cells in several degenerative and intractable diseases as well as offer the possiblity of modeling human diseases and developing new drugs diseases. Stem cells can be differentiated into specialized cell types and can be a useful source of healthy cells in genetic disease, especially those which can be corrected by only a small amount of functional protein. In the last years impressive efforts have been made in understanding the potential application of pluripotent stem cells in the field of regenerative medicine.

In these review, we will discuss the differentiation plasticity of mouse Germline Cell-derived Pluripotent Stem Cells (GPSCs) into different cell types with the aim to translating these potentialities to human organ regeneration.

Keywords: Embryonic stem cells, Spermatogonial stem cells, iPS, differentiation, regenerative medicine.

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4.1 Introduction

Most of the evidence that pluripotent stem cells can be directed to differentiate into specific types of cells suitable for transplantation comes from experiments with mouse cells, and offers the cues for translational research. The application of stem cells in human regenerative medicine could be an alternative to organ transplantation, avoiding the problem of donor shortage and rejection [1]. One important question that has to be answered before stem cells can be effectively translated into significant medical treatments is what type of pluripotent stem cells are the most suitable for human clinical application.

Embryonic stem cells (ESC) are the most versatile cells among pluripotent stem cells. These cells, derived from the inner cells mass of blastocysts, are able to give rise to all type of adult differentiated cells. The development of human embryonic stem cells (hESC) gave an incredible acceleration to stem cell research [2, 3]. Human ESC, like murine ESC, can be differentiated into tissue derived from all three germ layers and have a limitless reproductive capacity. Despite their huge potentiality, the use of human ESC in cell therapy is impeded by moral and ethical concern of destroying human embryos for derivation of ESCs [4]. The recent discovery of the ability to reprogram adult cells into pluripotent embryonic-like stem cells (known as induced pluripotent stem cells; iPS) has profound implications for stem cell therapy [5–8]. The first generation of iPS was generated by introduction of transcription factors, including c-Myc, by retroviral vectors, this probably lead to the generation of neoplastic cells from some induced cells. This problem was solved by using alternative vectors that do not comprise c-Myc [9]. Rapid progress has been made in finding alternative ways to reprogram cells. Now virus-free iPS are available from adult somatic cells [10, 11], this could have important implication in terms of clinical application. iPS have shown remarkable promises in many ways, including the generation of patient-specific iPS [12]. However, the drawback of iPS-based therapy is the need of transducing cells with reprogramming factors to achieve an efficient generation of iPS. Moreover, the existence of inherent epigenetic differences between iPS and ESC can affect iPS functionality [13, 14]. Adult stem cells have been identified in several human tissues, such as liver [15], blood [16], skin [17] and testis [18]. Adult stem cells could be an autologous, free-from-ethical concern, source of pluripotent stem cells. A particular type of adult stem cells that have attracted the interest of the scientific community in the last years are spermatogonial stem cells. Spermatogonial stem cells (SSCs) reside in the basal membrane of testis, these cells are the only stem

cells that undergo self-renewal during all life and generate male gametes. Spermatogonial stem cells derive from primordial germ cells (PGCs) which migrate from proximal epiblast to the gonocytes. Around 7 days post-partum in mice, the gonocytes turn into SSCs that then provide the basis for the first and the following rounds of spermatogenesis [19, 20]. This rare population of cells (0.03%) can give rise to spermatozoa through subsequent division and it is able to restore spermatogenesis once injected in the testis of infertile mice [21]. The capacity to generate spermatozoa seems to be strictly related to the microenvironment, that probably inhibits the potentiality of SSCs and maintains their unipotent state [22, 23]. When transferred *in vitro*, SSCs get free from the inhibitory action of niche, show a broad developmental potential. SSCs are highly specialized cells but several lines of evidence highlight the multipotency of these cells. In fact, teratomas, that are particular types of benign tumors containing derivates of the three germ layers, occur almost exclusively in the gonads [24]. Moreover, PGCs have the unique feature to give rise to pluripotent ESCs once cultured *in vitro* [25, 26]. In the last ten years, the research demonstrated that SSCs can undergo a spontaneous process to re-acquire pluripotency when cultured *in vitro* for a long period of time [27–33]. In 2004, the first report about SSCs conversion of Kanatsu-Shinoara proved that murine neonatal SSCs could be converted to a ESC-like feature. Since 2004, a large number of groups demonstrate that not only murine neonatal SSCs can form ES-like colony but adult murine SSCs as well. The ES-like colonies derived from SSCs (called Germline Cell-derived Pluripotent Stem Cells, GPSCs) share many hallmarks with ES cells. GPSCs are able to self-renew and differentiate into derivatives of all three germ layers [32]. GPSCs also display a broad potential in that they can be differentiated *in vitro* into functional cardiomyocytes, neurons, glia and hepatocytes [34–36]. Furthermore, GPSCs are capable of forming teratomas once injected subcutaneously in immunocompromized mice and to generate chimeras when injected into blastocytes [27]. Taken together, these data show a huge potential of GPSCs in regenerative medicine. In 2008, Conrad et al. for the first time, claimed to be able to obtain ES-like cells starting from human SSCs [29]. Initially, the pluripotency of these ES-like cells has been demonstrated [30, 31, 33], but more recently, some researchers challenged the concept of pluripotency of human SSCs (hSSC). Despite the murine counterpart, hSSC seems to have less developmental potential in that they are not able to give rise to teratomas or to form all three germ layers after EBs induction [37]. The debate is currently open and more investigation is needed.

4.2 Hepatocytes Derived from GPSCs

Hepatic disorders affect hundreds of millions of people worldwide. The mild conditions, if not cured properly, may lead to progressive liver injury, liver fibrosis and ultimately cirrhosis, portal hypertension, liver failure, and, in some instances, cancer [38]. Orthotopic liver transplantation (OLT) has become the standard of care for the treatment of patients with end-stage liver disease resulting in elevated request for OLT. However, the ongoing organ shortage has impeded the treatment of all recurrent-incurable hepatic diseases. Importantly, adult hepatocytes are capable of replicating under particular conditions, for e.g. after a partial hepatectomy [39]. The human liver also contains resident stem cells and the bipotential oval cells that can differentiate into hepatocytes when necessary [15, 40]. However, in a severely compromised liver, the regenerative capacity of hepatocytes and liver stem cells is impaired and can no longer restore functionality. Thus, cell therapy may be the only way out. Hepatocytes transplant have been reported in several cases. For e.g., repeated hepatocyte transplantation in a patient with acute liver failure due to mushroom poisoning has been shown to improve patient's condition and liver functionality [41]. The reported viability of the thawed primary hepatocytes was 62%. This loss in cell viability upon thawing may be a problem, especially when billions of cells are needed for transplant in patients. Hence, alternative sources of hepatocytes are being looked for. Human bone marrow-derived stem cells have been shown to differentiate to hepatocytes *in vitro* and reverse liver failure *in vivo*, but these cells are present in minute fractions and thus are tedious to isolate and difficult to expand [42]. ESCs are considered a very promising source of hepatocytes for cell therapy due to their limitless capacity for self-renewal and proliferation, and their ability to differentiate into all major cell lineages [42]. However, the allogenic nature of these cells as well as the ethical burden, has impeded their use in the clinical setting. GPSCs are an interesting alternative. The differentiative potential of GPSCs are being extensively studied in the mouse with the aim of extending the results to human. EBs generated from mouse GPSCs express the early hepatic marker, alpha fetoprotein. We and others have reported on the directed differentiation of GPSCs into hepatocyte-like cells [36, 43]. Metabolically active hepatocytes, capable of albumin and haptoglobin secretion, urea synthesis, glycogen storage, and indocyanine green uptake can be derived from GPSCs *in vitro* [36]. Our large scale microarray analysis comparing GPSCs to ESCs during hepatocyte differentiation revealed that there was a marked similarity in gene expression profile between these two

cell lines. The GPSC-derived hepatocytes, at Day 28 of *in vitro* differentiation, were closer to fetal hepatocytes (embryonic day 16) than adult hepatocytes (post natal day 1) [36]. *In vivo* studies in mouse models of liver diseases will reveal if these GPSC-derived hepatocytes can home to liver and restore functionality.

GPSCs are thus a promising tool for the treatment of liver diseases. Hepatocytes derived from GPSCs may provide the minimal amount of functional protein that is required to correct certain metabolic diseases. These cells may also be useful in the period awaiting transplant as in the case of newborns with genetic defects [44]. As liver transplant requires mainly blood group compatibility, there is the possibility of transplanting GPSC-derived hepatocytes in both male and female patients [45].

4.3 Cardiac Cells Derived from GPSCs

Heart transplantation is one the most effective treatment for severe cardiomyopathies. However, the insufficient number of matching donor hearts has elicited search for other sources of cardiomyocytes including extracardiac ones. Transplantable cell sources identified hitherto comprise cardiac progenitor cells [46], mesenchymal stem cells [47], fetal cardiomyocytes [48], bone marrow cells [49], ES cells [50] and iPS cells [51]. Due to the advantages described above, GPSCs may also offer a substitute to ES cells or iPS cells in cardiomyocytes generation. EBs formed from mouse GPSCs form contracting areas which show cardiomyocyte phenotype characterized by sarcomeric striations when stained for α-sarcomeric actinin, sarcomeric MHC and cardiac troponin T, organized in bundles [32]. Functional cardiomyocytes could also be generated from these GPSC-derived EBs [34]. Through molecular, cellular, and physiological assays, Guan et al. demonstrated that GPSC-derived cardiomyocytes engrafted into the left ventricular free wall of mice one month post-injection. These cells proliferated in the normal heart without giving rise to teratoma. However, no spontaneous *in vivo* differentiation into cardiomyocytes were observed in the normal heart. The GPSCs differentiated *in loco* into vascular endothelial and smooth muscle cells probably related to the fact that these cells were not induced to differentiate into cardiomycoytes prior to transplantation [52]. Interestingly, Flk1+ cells from differentiating GPSCs could give rise to mature cardiomyocytes and endothelial cells as efficiently as ES cells [53]. Transplantation of these Flk1+ GPSCs directly into the heart of ischemic mice improved cardiac function [54]. Four weeks after treatment,

there was a small number of cardiomyocytes derived from Flk1+ GPSCs in these mice. Enhanced angiogenesis and decreased senescence around the ischemic area was noted the early phase of ischemia [54]. These results are quite promising and indicate GPSCs as potential cardiomyocyte-generating cells for cardiac repair.

4.4 Neuronal Cells Derived from GPSCs

Stem cells that have the propensity to form neural precursors, mature neurons and glia upon induction *in vitro* are good candidates for cell therapy of neurodegenerative diseases. These include fetal neuroprogenitor cells, bone marrow stem cells [55, 56], ES cells [57] and iPS cells [58]. Recently, the direct conversion of adult human bone marrow stromal cells or skin fibroblasts to neurons have been reported [56, 59, 60]. GPSCs are also capable to differentiating into different neural cell types when given the right cues *in vitro* [35]. Electrophysiological analyses showed maturation of these progenitors into functional neurons (GABAergic, glutamatergic, serotonergic, and dopaminergic neurons) and glial cells (astrocytes and oligodendrocytes) was achieved in the mouse. It was also shown that GPSC-derived neurons can give rise to functional networks which use both GABAergic synaptic transmission and engage in synchronised oscillatory activity [52, 61]. Another important point is that GPSC-derived oligodendrocytes are capable of undergoing complete maturation and ensheathing host axons in myelin-deficient tissue in organotypic slice cultures of the myelin-deficient rat cerebellum [61]. Neural cells can thus be derived from mouse GPSCs and assuming translation to the human system, these cells represent a great potential for the treatment of neurodegenerative diseases.

4.5 Hematopoietic Cells from GPSCs

GPSCs are also promising candidate for the generation of hematopoietic stem cells for transplantation in patients suffering from hematological malignancies and inherited disorders. GPSCs have been shown to generate CD45+ hematopoietic cells, including Gr-1+Mac1+ myeloid cells and Ter119+ erythroid cells [27]. In a recent report, Yoshimoto *et al.* reported the generation of multipotent hematopoietic progenitor cells with myeloid and lymphoid potential emerging from Flk1+ differentiating GPSCs and described the localisation of GPSC-derived hematopoietic cells in the bone marrow cavity

after intra-bone marrow injection in immunodeficient mice [52, 62]. These GPSC-derived hematopoietic cells, however, failed to expand and showed stem cell repopulating ability *in vivo*. Improved *in vitro* differentiation conditions may be needed to generate a more efficient multipotent hematopoietic progenitor that is capable of proliferating *in vivo*. But there may be also no need to leave the SSCs to acquire pluripotency for so long *in vitro* prior to the transplantation experiments. Ning et al. have shown that GFP^+ $Sca\text{-}1^+$ $H\text{-}2K^{b+}$ cells isolated from mouse testis and transplanted directly into the bone marrow of GFP^- male busulfan-treated recipient mice acquired the functional properties of hematopoietic stem cells. Donor derived-GFP+ cells were present in the bone marrow, peripheral blood and spleen 12 weeks after transplantation**.** These observations indicate the transdifferentiation potential of mouse SSCs to hematopoietic stem cells *in vivo* [63]. This concords with a previous observation, in another system, that mouse SSCs, when transplanted into the renal parenchyma of whole body irradiated adult mice, could directly differentiate into mature renal parenchyma cells [64].

4.6 Vascular Cells Derived from GPSCs

Most recently, Im et al. demonstrated that GPSCs can be induced to differentiate into into vascular endothelial cells and smooth muscle cells *in vitro* [65]. The vascular differentiation process of GPSCs displayed a developmentally appropriate sequence of transcription factor expression, similar to ES cells, indicating again that GPSCs and ES cells have similar differentiation properties [36]. The GPSC-induced vascular endothelial cells expressed VE-cadherin or CD31 proteins at their cell-cell junctions as observed in primary endothelial cells and these VE-cadherin- or CD31-positive cells formed sprouted branch-like structures. The efficiency of conversion of GPSCs to vascular endothelial cells was 7% compared to 18% of ES cells. The authors also demonstrated that GPSCs could be differentiated into vascular smooth muscle cells. Some differentiated cells from GPSCs were co-stained with anti-SM22-α and anti-α-SMA IgGs, typical markers of adult vascular smooth muscle cells, and exhibited the intracellular fibril structure seen in the control vascular smooth muscle cells. These findings indicate that GPSCs may be considered as a source of vascular smooth muscle cells and endothelial cells for potential therapeutic applications like for the treatment of ischemic vascular diseases.

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