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Spermatogonial Stem Cells: An Alternate Source of Pluripotent Stem Cells for Regenerative Medicine

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1. Introduction

There is immense scientific and medical interest in stem cells as a potential source material for regenerative medicine to replace or restore lost, damaged, or aging cells, tissues or organs (Mason and Dunnill 2008). The ideal stem cell candidate for regenerative medicine is a pluripotent stem cell that is easily obtainable, has a stable developmental potential even after prolonged culture, forms derivatives of all three embryonic germ layers from the progeny of a single cell and generates teratomas after injection into immunosuppressed mice (Mason and Dunnill 2008). Stem cell-based therapy has the potential to offer important new treatment options for insulin-dependent diabetes, Parkinson's disease, cardiovascular, renal, musculoskeletal and retinal diseases and spinal cord diseases and trauma, among others. A critical question in this field is to establish which stem cells could be efficiently used clinically.

In 1981, Martin and Evans achieved a milestone in stem cell biology with the derivation of mouse embryonic stem cells (ESCs; Evans and Kaufman 1981). The subsequent derivation of pluripotent human ESCs in 1998 by James Thomson (Thomson et al. 1998) and pluripotent stem cells from human primordial germ cells (Shamblott et al. 1998) ushered in a revolution in the field of regenerative medicine and tissue engineering. However, the destruction of human embryos to obtain ESCs and the need for therapeutic cloning to use them optimally made their clinical application highly controversial. In addition to ethical, legal and moral issues, ESCs have inherent limitations that must be overcome before their clinical use. One prime concern is the potential tumorigenicity of these cells in vivo. Although efforts to eliminate this possibility are underway, it remains a serious issue (Blum and Benvenisty 2008; Fujikawa et al. 2005; Strulovici et al. 2007; Wu, Boyd, and Wood 2007). Other concerns are immune rejection (Drukker et al. 2002; Wobus and Boheler 2005), genetic instability and incomplete epigenetic reprogramming (Wobus and Boheler 2005). These concerns have

sparked interest within the scientific community in finding alternate sources of pluripotent/multipotent cells that have similar can as ESCs, but potentially circumvent the problems associated with these cells.

The derivation of induced pluripotent stem cells (iPSCs) through the introduction of a small combination of transcription factors into terminally differentiated cells has raised the possibility of producing an alternate pluripotent stem cell source for use in regenerative medicine. Researchers at Kyoto University, Japan, were first to identify conditions that allowed mouse skin fibroblasts to be induced into pluripotency (Takahashi and Yamanaka 2006) and a year later human skin fibroblasts were also induced to pluripotency using this same approach (Takahashi, Okita et al. 2007; Takahashi, Tanabe et al. 2007; Yu et al. 2007). The iPSC technology has immense potential for clinical therapy as these cells avoid some ethical and moral issues associated with ESCs. The technology involves producing critical levels of proteins needed for pluripotency through introducing the genes into the target cells exogenously using viral vectors (Takahashi, Tanabe et al. 2007; Takahashi and Yamanaka 2006; Yu et al. 2007), adenoviral vectors (Stadtfeld et al. 2008; Okita et al. 2008) or non-viral plasmids (Okita et al. 2008) or introducing the actual recombinant proteins themselves into cells (Zhou et al. 2009). Although initially there was concern over use of viral vectors integrating into the human genome and potentially inducing neoplastic changes, the use of alternate methods to deliver the pluripotency genes appears to circumvent that problem. Nonetheless, because there is genetic manipulation involved (Stadtfeld et al. 2010; Stadtfeld and Hochedlinger 2010) and an increased risk of tumorigenicity (Nakagawa et al. 2008; Ben-David and Benvenisty 2011; Kooreman and Wu 2010) extensive research has to be conducted before iPSCs are used clinically. Since iPSCs can be derived from patient-specific cells, one potential benefit of these cells was a low or no risk of immune rejection. However, a recent finding suggests that this might not be true: In contrast to derivatives of ESCs, abnormal gene expression in iPSCs introduced into syngeneic mice in vivo induced T-cell-dependent immune responses, leading to immune infiltration and ultimately rejection of these cells (Zhao et al. 2011). This raises concerns for use of these cells in regenerative medicine. As with any new technology, there are a number of other hurdles to overcome before using iPSCs clinically, such as refractoriness of many adult cells to reprogramming, transient epigenetic memory of donor cells (Kim et al. 2010; Polo et al. 2010) and possible non-recurrent mutations. Despite these concerns, the tremendous progress and obvious potential of iPSCs over the past few years has overshadowed work on other potentially pluripotent stem cells, although some of these cells may ultimately offer therapeutic potential equal to that of iPSCs.

2. Progress in regenerative medicine

In 2010, two ESC-based human clinical trials were approved by the U. S. Food and Drug Administration, one initiated by Geron Corporation and the other by Advanced Cell Technology. Though the clinical trials are underway, there is concern that introducing stem cells that have not transformed into specialized cells into patients may pose the risk of teratoma formation. However, remarkable progress has been made in cell-based regenerative medicine over the past decade. The cell-therapy based industry now has an annual revenue of over a billion dollars and it is projected to rise to $5.1 billion by 2014

(Mason and Manzotti 2010). The initial research and monetary investment to develop stem-cell based therapy is high. However, since the treatment involves transplantation of cells or tissues that can function normally for extended periods of time or even potentially for the remainder of a patient's life, there is negligible need for ongoing treatment, rendering this modality of treatment potentially cost-effective (Mason and Dunnill 2008).

The limitations and problems associated with iPSCs suggest that they may not offer the optimal solution for all aspects of regenerative medicine, raising the question of whether other stem cells may be preferable in some or potentially all regenerative medicine applications. Other stem cells that have received widespread attention in this regard include hematopoietic (HSC), mesenchymal and fetal cord blood stem cells. An adult allogenic stem cell source with developmental potential similar to ESCs and iPSCs would potentially be the most promising clinical approach if an optimal starting cell was found.

Another stem cell that has been studied extensively for years and has recently been shown to have potential in regenerative medicine is the spermatogonial stem cell (SSC). This chapter focuses on recent developments in this field and the advantages and limitations of SSC use in regenerative medicine. We also briefly discuss other multipotent stem cells that may have significant clinical use.

3. Spermatogonial Stem Cells

In mammalian testes, SSCs are found along the basement membrane of seminiferous tubules. They produce the spermatogenic lineage, ensuring lifelong fertility of the individual. Like other stem cells, SSCs are undifferentiated and capable of self-renewal. While in the testicular microenvironment, they differentiate only into one specialized cell lineage, spermatozoa. However, when SSCs are isolated from the testis and placed into a different environment, they acquire or manifest pluripotency and differentiate into tissues belonging to all three embryonic germ layers (Kanatsu-Shinohara et al. 2008; Golestaneh et al. 2009; Simon et al. 2009; Ning et al. 2010). This property makes SSCs a powerful potential source of cells for regenerative therapy.

3.1 Development and differentiation potential of SSCs

In the embryo, the germ line arises from primordial germ cells (PGCs). PGCs are initially identifiable as a small cluster of cells in the proximal epiblast near the extra-embryonic ectoderm. This lineage differentiates under the influence of bone morphogenetic proteins (BMPs) and diverges from the somatic lineage in the late embryonic and early fetal stage (Lawson et al. 1999; Ying et al. 2000). Due to their extra-embryonal origin, they are not classified as belonging to a particular embryonic germ layer. Therefore, they are not subjected to many of the differentiation signals other stem cells receive (Lawson and Hage 1994; Simon, Hess, and Cooke 2010); this may allow them to remain more undifferentiated than other stem cells. Following their initial development, PGCs subsequently migrate into the mesoderm, the endoderm (hindgut) and across the dorsal mesentery to reach the developing gonads at about 4-5 weeks of gestation in humans and 11-13 day post-coitum in rodents (Culty 2009). In the testis, PGCs then become mitotically quiescent until birth, and are called gonocytes. Shortly after birth, gonocytes resume mitosis and migrate to the basement membrane of seminiferous tubules, where they form SSCs and remain

throughout life. Thus, the embryological origin of SSCs is unique. This may facilitate their potential for differentiation into cell types of different germ cell layers and underlines their clinical potential for regenerative medicine. Furthermore, teratomas occur exclusively in gonads (Stevens 1964) and are of germ cell origin, and gene expression in early germ cells is very similar to ESCs (Zwaka and Thomson 2005; Simon, Hess, and Cooke 2010) , which emphasizes the broad developmental potential of SSCs. SSCs express genes such as POU domain class 5, transcription factor 1 (Pou5f1; Huang et al. 2009; Bhartiya et al.), Lin 28 (Zheng et al. 2009), undifferentiated embryonic cell transcription factor 1 (UTF-1) and Zinc finger protein 42 (Rex-1; Kristensen et al. 2008), which impart pluripotency. However, expression of another pluripotency gene, Nanog, is repressed in the testis by transformation related protein 53 (TRP53) and phosphatase and tensin homolog (PTEN). Both proteins belong to a critical signaling pathway preventing SSCs from being pluripotent while in the testis (Kuijk et al. 2009). Overall, this gene expression pattern suggests that SSCs have a gene profile similar to ESCs and thus are more undifferentiated than other adult stem cells.

3.2 Current methods of isolation and propagation of SSCs with emphasis on human SSC isolation

Spermatogonial stem cell constitute only 0.03% of the total germ cell population in rodent and human testis (Tegelenbosch and de Rooij 1993). Thus, their small numbers and the lack of specific markers are the main hurdles to their characterization. Nonetheless, significant progress has recently been made in the isolation and propagation of cells with SSCs properties from rodent (Dym et al. 2009; Guan et al. 2009; Kanatsu-Shinohara, Takehashi, and Shinohara 2008; Kubota, Avarbock, and Brinster 2004; Oatley and Brinster 2006, 2008; Ogawa et al. 2003; Hofmann et al. 2005; Kanatsu-Shinohara et al. 2010) and human testes (Conrad et al. 2008; Glaser et al. 2008; Golestaneh et al. 2009; Kossack et al. 2009; Zovoilis et al. 2008; Izadyar et al. 2011; He et al. 2009). Human testicular tissue is currently obtained from testicular biopsies (Izadyar et al. 2011; Kossack et al. 2009), orchiectomies (Izadyar et al. 2011) and organ donors (Golestaneh et al. 2009). Testicular biopsies of approximately one gram can yield sufficient number of human SSCs for most clinical applications. Although our knowledge of mouse and human SSCs phenotype is still limited, studies suggest that human SSCs express proteins such as cluster of differentiation antigens 49f, 90 and 133 (CD49f, CD90, and CD133, respectively), glial cell line-derived neurotrophic factor family receptor alpha 1 (GFRA1), G protein-coupled receptor 125 (GPR125), melanoma antigen family A 4 (MAGE4), promyelocytic leukemia zinc finger (PLZF) and stage-specific embryonic antigen-4 (SSEA-4; Costoya et al. 2004; Conrad et al. 2008; He et al. 2009; Izadyar et al. 2011). Using these markers in magnetic- or fluorescent-activated cell sorting, SSCs can be isolated with a high degree of purity (Gassei et al. 2009; Izadyar et al. 2011; Kokkinaki et al. 2009; Simon et al. 2010). Although there have been no definitive culture conditions for propagation of either mouse or human SSCs, culture systems established by different groups seem to be conducive for their propagation. At least in rodents, glial cell line-derived neurotrophic factor (GDNF) was found to be essential to maintain SSCs in an undifferentiated state in vivo and in vitro (Tyagi et al. 2009; Hofmann 2008; Sariola and Immonen 2008; Oatley, Avarbock, and Brinster 2007; Oatley et al. 2006; Naughton et al. 2006; Kubota, Avarbock, and Brinster 2004; Meng et al. 2000).

3.3 Spermatogonial stem cells are pluripotent

In the testicular microenvironment, SSCs produce only the spermatogenic lineage, and the assumption was that this was their sole potential developmental fate. However, the iconoclastic finding of Kanatsu-Shinohara et al. (Kanatsu-Shinohara et al. 2004) demonstrated that neonatal murine SSCs produced ESC-like cells when isolated from the testis and grown for extended periods in ESC culture conditions. Although initial work was done with neonatal SSCs (Kanatsu-Shinohara et al. 2004; Kanatsu-Shinohara et al. 2008; Simon et al. 2009), subsequent work showed that adult murine SSCs (Glaser et al. 2008; Izadyar et al. 2008; Seandel et al. 2007; Guan et al. 2006) grown for 4-7 weeks in vitro also produced a low frequency of ESC-like colonies. These ESC-like cells can give rise to cell types derived from all three embryonic germ layers, and also produce teratomas when injected subcutaneously into nude mice. In addition, these ESC-like cells contribute to embryonic development when injected into blastocysts. ESC-like cells derived from SSCs have been termed multipotent germline stem cells (mGSCs) and these cells have been differentiated into many cell types. For example, two separate groups demonstrated that mGSCs could differentiate into mature cardiac and endothelial cells and that these cardiac cells were contractile and had electric potentials and ion channels (Baba et al. 2007; Guan et al. 2007). mGSCs derived from adult mouse SSCs could be differentiated into functional neurons and glia (Glaser et al. 2008; Streckfuss-Bomeke et al. 2009). Pluripotent stem cells were derived from adult mGSCs that not only could differentiate into a variety of cell types both in vivo and in vitro, but also showed germline transmission to the next generation when injected into blastocysts (Ko et al. 2009). Moreover, mGSCs have pluripotency characteristics similar to ESCs such as telomerase activity, telomere length and hypomethylation of pluripotency marker genes (Zechner et al. 2009)

In recent years, several research groups reported methodologies for isolation and culture of human SSCs and also demonstrated that these cells were pluripotent/multipotent (Conrad et al. 2008; Golestaneh et al. 2009; Kossack et al. 2009; Dym et al. 2009; Izadyar et al. 2011). SSCs isolated from human testicular tissues and cultured for a week or more spontaneously produced ESC-like small colonies, which were then transferred into ESC media and cultured for about 4 weeks to get sufficient numbers of ESC-like colonies and these ESC-like cells could then be differentiated into specific cell types. These results indicate that human SSCs have the potential to be used as an alternate source of pluripotent stem cells in regenerative cell therapy, without the ethical concerns of ESC and the concerns involving exogenous gene introduction in iPSCs.

4. Direct differentiation of spermatogonial stem cells

4.1 Epithelial-mesenchymal interactions

Epithelial-mesenchymal interactions are critical for organogenesis in many organs such as lung, prostate, mammary gland, liver, pancreas and salivary glands (Grobstein 1953). Mesenchyme, which is undifferentiated connective tissue, signals to the epithelium to induce epithelial morphogenesis and cytodifferentiation in a wide variety of organs. The central role of epithelial-mesenchymal interactions in organ development was postulated initially by Pander and later experimentally demonstrated by Spemann and Saunders (in Simon, Hess, and Cooke 2010).

Classical tissue recombination experiments conducted by Cunha and coworkers with reproductive tissue demonstrated that the mesenchyme dictates the fate of the epithelium in various reproductive tissues. The urogenital sinus is an ambisexual fetal organ that gives rise to the prostate in males and a portion of the vagina in females. Under the influence of androgen, urogenital sinus mesenchyme (UGM) induces urogenital sinus epithelium (UGE) to differentiate into prostatic epithelium and UGM also regulates epithelial ductal morphogenesis and cytodifferentiation in prostate. UGM also instructively induces prostatic morphogenesis in other epithelia (Cunha et al. 1983; Cunha, Lung, and Reese 1980; Cunha, Sekkingstad, and Meloy 1983). For example, UGM instructively induces adult bladder epithelium to form prostatic epithelium in tissue recombinants in vivo (Cunha et al. 1983). Similarly, neonatal uterine mesenchyme instructively induces neonatal vaginal epithelium to form uterine epithelium (Cunha 1976).

4.1.1 Potential signaling pathways involved in epithelial-mesenchymal interactions

Although mesenchymal paracrine signaling is essential for determining epithelial fate, the specific signaling pathway(s) involved in this phenomenon remain unclear. Some possible signaling molecules are Wnt7a (Miller and Sassoon 1998), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) family molecule(s) in the uterus (Chen, Spencer, and Bazer 2000, 2000), HGF and FGF family molecule(s) for lung organogenesis (Ohmichi et al. 1998) (for review see Kumar et al. 2005,) and FGF and Wnt and Hedgehog signaling in the prostate (for review see Thomson, Cunha, and Marker 2008; Cunha, Cooke, and Kurita 2004; Lin and Wang, 2010; Taylor et al. 2009).

4.1.2 Mesenchyme dictates the fate of stem cell differentiation

The demonstrations that epithelial-mesenchymal interactions are essential for organogenesis also triggered interest in using this approach in regenerative therapy. ESCs were differentiated into prostatic epithelium by recombining mouse UGM and human ESCs and growing these tissue recombinations in vivo (Taylor et al. 2006). Using a similar methodology (Oottamasathien et al. 2007; Anumanthan et al. 2008), human ESCs or bone marrow-derived mesenchymal stem cells have been differentiated into bladder epithelium by exposing these cells to the inductive influence of bladder mesenchyme in a tissue recombinant. More recently, Taylor et al. demonstrated that stroma could induce adult stem cells to express dual phenotypes (Taylor et al. 2009). Prostatic stroma induced putative mammary epithelial stem cells to generate glandular epithelia expressing both prostatic and mammary markers. These results demonstrate that the mesenchyme can instructively direct the differentiation of ESCs or other stem cells into a specific cell fate.

4.2 Spermatogonial stem cells differentiate into tissues of all three embryonic germ layers in response to instructive inducers

Based on demonstrations of the importance of the stem cell niche (Tyagi et al. 2009; de Rooij 2009; Hess et al. 2006; Simon et al. 2010; Oatley, Racicot, and Oatley 2010), the pluripotential nature of SSCs and the instructive potential of various mesenchymes, we postulated and subsequently demonstrated that neonatal mouse SSCs could directly differentiate into prostatic, uterine and skin epithelium (Simon et al. 2009) when recombined with the

appropriate mesenchyme and grafted in vivo (Fig. 1). To track cell lineages derived from SSCs in tissue recombinations and verify that these cells were undergoing differentiation, wt transgenic C57BL/6 mice expressing enhanced green fluorescent protein (GFP) ubiquitously were used. For example, UGM derived from wild-type mice (wt-UGM) were recombined with SSCs derived from mice expressing GFP (G-SSC) and grafted under the renal capsule of syngeneic male hosts. After 4 weeks of growth, the epithelium in these, wt-UGM + G-SSC, tissue recombinants expressed NKX3.1, a prostatic epithelial marker and androgen receptor but not germ cell nuclear antigen 1 (GCNA1), a germ cell marker. The tissue recombinants had an epithelium that stained intensely for GFP (Fig. 1A), indicating that it was of SSC origin, while stromal cells lacked GFP staining. Similarly uterine mesenchyme (UtM) from mice expressing GFP (G-UtM) recombined with SSCs derived from wt-mice (wt-SSC) differentiated into uterine epithelium that expressed cytokeratin 8 (CK8), estrogen receptor–alpha and progesterone receptor. In these G-UtM + wt-SSC tissue recombinants, stromal cells strongly expressed GFP, while epithelium did not, indicating that the epithelium was of SSC origin (Fig. 1B). This approach provides a method to directly differentiate SSCs into specific cell types from all three embryonic germ layers without the extended culture period needed in vitro to produce ESC-like cells that can subsequently differentiate into various derivatives.

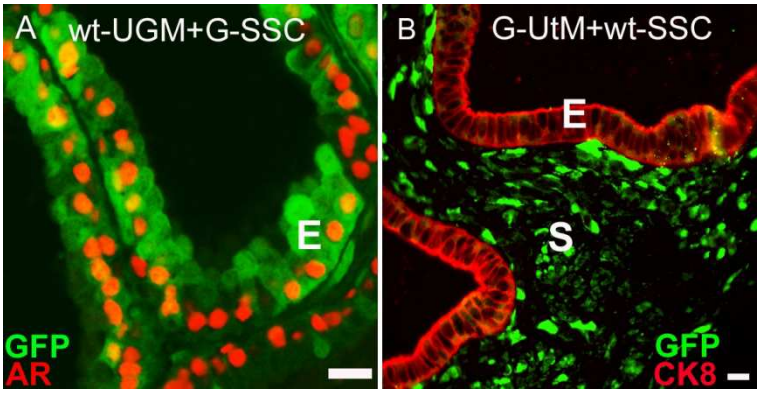

Fig. 1. Differentiation of SSCs into prostatic and uterine epithelium. A) Urogenital sinus mesenchyme (UGM) from wild-type (wt) mice was recombined with SSCs derived from mice that expressed enhanced green fluorescent protein (G) ubiquitously and was grafted under the renal capsule of syngeneic male hosts. After 4 weeks of in vivo growth, the wt-UGM + G-SSC tissue recombinants formed prostatic epithelium (E) that was of SSC origin expressing GFP and androgen receptor (AR, red nuclei). B) Uterine mesenchyme (UtM) from GFP mice was recombined with SSCs derived from wt mice and grafted under the renal capsule of syngeneic female hosts. After 4 weeks of growth, the G-UtM + wt-SSC tissue recombinants formed uterine epithelium (E) that was of SSC origin expressing cytokeratin 8 (CK8). Stromal cells (S) express GFP but E does not. SSCs do not express AR or CK8.

When SSCs were mixed with mammary epithelial cells and grafted into the mammary fat pad in vivo, SSCs differentiated into mammary epithelial cells, but the stem cells alone could not be differentiated into mammary cells (Boulanger et al. 2007). This suggests that the

inductive potential of the adult mammary fat pad might not be sufficient for directing the differentiation of SSCs into mammary epithelial cells. This is a potential hurdle for the use of this methodology, as the inductive mesenchyme for most of the organs is present during fetal life, and as they transition to form adult stromal cells they might lose this ability to instructively induce other epithelia. However, a recent study demonstrated direct differentiation of SSCs into hematopoietic cells (Ning et al., 2010). When SSCs were injected into the bone marrow of adult female mice, these mice had Y chromosome positive-cells that had phenotypical and functional characteristics of hematopoietic cells both in vivo and in vitro, emphasizing both the pluripotential nature of SSCs and that the microenvironment even in adult organs plays a decisive role in directing the differentiation of SSCs.

As discussed earlier, our understanding of the mechanistic basis of epithelial-mesenchymal interactions is fragmentary. Fetal mesenchymes are potent instructive inducers, and one of the challenges for using this methodology will be to determine whether or not adult stroma is capable of similar instructive inductions. Once we are able to dissect out the molecular mechanisms used by mesenchyme to instructively induce epithelial morphogenesis, it will be feasible to expose SSCs to the inductive signals produced by these tissues in vitro, on artificial scaffolds or some other arrangement. Another major limitation of this methodology is that it is inapplicable to tissues that do not involve epithelial-mesenchymal interactions during development. However, the direct differentiation of SSCs into hematopoietic cells (Ning et al., 2010) discussed above suggests that SSCs will differentiate into a specific tissue type when exposed to an appropriate microenvironment, even in the absence of epithelial-mesenchymal interactions. Thus further research is necessary to determine both the full developmental potential of SSCs and the most appropriate methodology for inducing specific cell types, but SSCs appear to have great potential in this regard.

4.3 Mechanism of differentiation of spermatogonial stem cells into other cell types

The mechanism of differentiation of SSCs into other cell types under the influence of instructive inducers is poorly understood. One possibility is that in response to an inductive mesenchyme, the SSCs de-differentiate into ESC-like cells (as has been reported in vitro), and then subsequently differentiate into a new epithelia. However, our preliminary studies indicate that SSCs may not undergo a de-differentiation step in the presence of an inductive mesenchyme, but instead may differentiate directly from SSCs to another epithelial type without going through an intermediate ESC-like cell stage. Shinohara and coworkers (Kanatsu-Shinohara et al. 2008) demonstrated that a single spermatogonial stem cell could produce an embryonic stem-like line that was multipotent and germline stem cells that were committed to spermatogenesis, indicating that all SSCs may be capable of becoming pluripotent. This is supported by other studies (Ko et al. 2009). Conversely, Izadyar et al. suggested that there are two distinct populations of SSCs, one that is $OCT4^+$ and c-KIT^- that gives rise to multipotent cells and another that is $OCT4^+$ and c-KIT^+ that gives rise to the spermatogenic lineage (Izadyar et al. 2008). A definitive elucidation of how SSCs differentiate into other tissue types, as well as definitively establishing whether all SSCs or a specific subpopulation can be converted into other tissues, will be an essential prerequisite for successful use of this approach in a clinical setting. Nonetheless, SSCs can be differentiated into specific cell types using different approaches and be a potential source for pluripotent cells for stem cell-based therapy (Fig. 2).

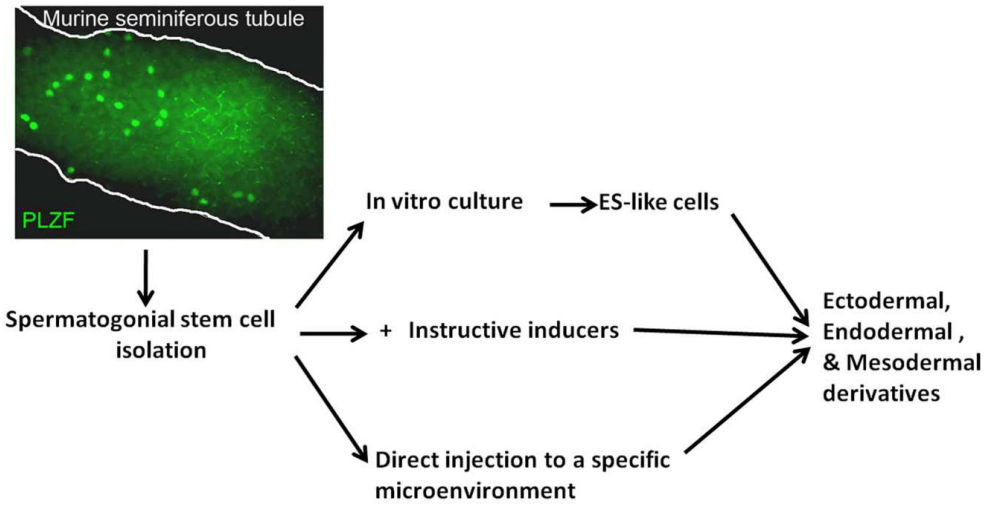

Fig. 2. Potential use of spermatogonial stem cells (SSCs) in regenerative medicine. Spermatogonia in the murine seminiferous tubule expressing PLZF, a SSC marker. SSCs are isolated from the testis using magnetic- or fluorescent-activated cell sorting. Some of the possible methods of differentiating SSCs into specific cell types are 1) Long-term culture of SSCs, selection for cells that form embryonic stem cell-like colonies and subsequent culture and differentiation of these pluripotent cells into specific cell types. 2) Recombination of SSCs with instructive inducers to directly differentiate SSCs into specific epithelial cell types; 3) Direct injection to a specific site of injury or specific microenvironment. Since SSCs are unspecialized, they could home and respond to signals in the new microenvironment and differentiate into cells of all three embryonic germ layers.

5. Advantages and limitations of SSCs over other pluripotent stem cell sources

SSCs, as well as other adult derived stem cells, may be safer to use therapeutically than ESCs or iPSCs. Since SSCs are more differentiated than ESCs, they are less likely to induce teratomas (Kossack et al. 2009). However, the risk of malignant transformation cannot be totally rejected since SSCs are relatively more undifferentiated than other adult stem cells. This type of problem is illustrated by the formation of brain tumors from donor-derived cells in patients who received fetal stem cells for treatment of ataxia-telangiectasia (Amariglio et al. 2009), emphasizing that tumorigenicity is the biggest impediment for the use of pluripotent stem cells in cell therapy. Another obstacle is the immunogenicity of SSCs and potential risk of rejection of the cells (Dressel et al. 2009). But immune rejection of autologous or allogenic stem cell transplants can be minimized by routine immunosuppression treatments as is used for organ transplantation. Another possible alternative is genetic manipulation of stem cells and elimination of genes responsible for immune rejection (Wobus and Boheler 2005).

6. Alternate sources of stem cells for use in regenerative medicine

The use of stem cells in regenerative medicine began when bone marrow cells were transplanted to treat acute leukemia (Thomas et al. 1959), and presently HSC therapy is the only stem cell therapy widely used clinically (Helmy et al. 2010). There is growing evidence that HSCs are plastic—and can give rise to tissues other than those of the blood system, e.g., liver cells (Lagasse et al. 2000). Another readily available source is mesenchymal stem cells that can be isolated from adult tissue, fetal tissue and umbilical cord blood. These cells can differentiate into osteoblasts, chondrocytes and adipocytes (Friedenstein et al. 1974; Pittenger et al. 1999) neurons (Cho et al. 2005), astrocytes (Kopen, Prockop, and Phinney 1999) and hepatocyte-like cells (Petersen et al. 1999). The use of adipose stem cells as a source for cell therapy is increasing rapidly as methods of isolation and culture are standardized; subcutaneous depots are easily accessible, replenishable and are often abundant. Adult stem cells derived from adipose tissues can differentiate in vitro into many cell types including adipocyte, chondrocyte, endothelial, epithelial, hematopoietic support, hepatocyte, neuronal, myogenic, and osteoblast lineages (Gimble and Guilak 2003; Halvorsen et al. 2001; Safford et al. 2002; Zuk et al. 2001). Fetal stem cells are self-renewing cells located in various types of fetal tissue, including umbilical cord blood, umbilical cord matrix, fetal blood and the amniotic membrane (Reinisch and Strunk 2009; Jager et al. 2009; Zeddou et al., 2010). Umbilical cord blood contain multiple populations of stem cells that can be effective in treating many diseases such as hematological malignancies, hemoglobinopathies, metabolic disorders and the greatest advantage of these cells is decreased immune rejection (Liao et al., 2011).

Stem Cells	Advantages	Major limitations
Embryonic stem cells	Pluripotent Indefinite self-renewal potential	Ethical concerns Tumorigenicity Therapeutic cloning involved
Induced pluripotent cells	Pluripotent Initial source of cells are easy to obtain No ethical or moral concerns Indefinite self-renewal potential	Introduction of exogenous genes Tumorigenicity Genetic instability Use of viral vectors to introduce genes
Spermatogonial stem cells	Pluripotent No ethical or moral concerns Relatively easy to obtain Less tumorigenic potential	Relatively small numbers Difficult to maintain in cultures
Fetal stem cells (Fetal cord blood, umbilical cord tissue)	Relatively easy to obtain Minimal immunorejection during autologous transplantation	Restricted differentiation and self-renewal potential
Adult stem cells (hematopoietic, mesenchymal, adipose)	Easy to obtain Reduced risk of tumorigenicity	Restricted differentiation and self-renewal potential

Table 1. Potential advantages and limitations of stem cells in regenerative medicine

7. Conclusions

Among pluripotent/multipotent stem cells (Table 1), spermatogonial stem cells have great potential and some unique advantages. Despite their promise, numerous hurdles must be overcome before clinical use of SSCs. The small population of SSCs in the testis and the difficulty in propagating and maintaining them in culture is one major hurdle. The methodology proposed here is promising but extensive work is needed before its application in regenerative medicine.

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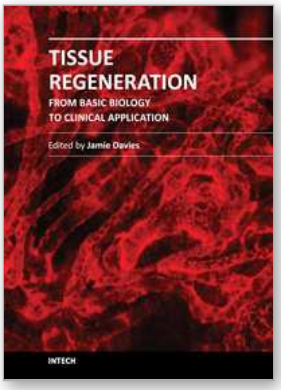

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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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