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Spermatogonial stem cells, *in vivo* transdifferentiation and human regenerative medicine

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Abstract

Importance of the field—Embryonic stem (ES) cells have potential for use in regenerative medicine, but use of these cells is hindered by moral, legal and ethical issues. Induced pluripotent cells have promise in regenerative medicine. However, since generation of these cells involves genetic manipulation, it also faces significant hurdles before clinical use. This review discusses spermatogonial stem cells (SSCs) as a potential alternative source of pluripotent cells for use in human regenerative medicine.

Areas covered in the review—The potential of SSCs to give rise to a wide range of other cell types either directly, when recombined with instructive inducers, or indirectly, after being converted to ES-like cells. Current understanding of the differentiation potential of murine SSCs and recent progress in isolating and culturing human SSCs and demonstrating their properties is also discussed.

What the reader will gain—Insight into the plasticity of SSCs and the unique properties of these cells for regenerative applications, the limitations of SSCs for stem-cell-based therapy and the potential alternatives available.

Take home message—If methodologies for isolation and conversion of adult human SSCs directly into other cell types can be effectively developed, SSCs could represent an important alternate source of pluripotent cells that can be used in human tissue repair and/or regeneration.

Keywords

cell-based therapy; germ cells; pluripotency; transdifferentiation

1. Introduction

The potential use of stem cells in human regenerative medicine has captured the imagination of both the scientific and medical communities, as well as the general public, over the past few years. What would have seemed inconceivable only two decades ago, the use of stem cells to repair or replace damaged human organs and tissues, now seems almost within our grasp. One basic question that must be answered before progress in the stem cell field can be

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effectively translated into significant medical treatments is what type(s) of stem cells are most promising for human clinical use.

The development of human embryonic stem (ES) cells [1] ignited a boom in stem cell research that has continued and even accelerated since the initial development of these cells in 1998. The demonstrations that human ES cells could differentiate into tissues derived from all three germ layers and had developmental potential similar to that of mouse ES cells originally reported by Martin and Evans [2–4] suggested that these cells could have clinical applications in the treatment of diabetes, Parkinson's disease and many other serious human illnesses. However, human ES cells proved emblematic of the discipline in that they came to represent both the promise and problems of turning stem cells into viable medical treatments. Despite constant scientific progress in propagating and differentiating these cells [1,5–11], the potential of these cells for human regenerative medicine always appeared unclear due to the necessity of obtaining these cells from human embryos that were destroyed in the process and the anticipated need to make a clone of a patient during the process of generating allogenic stem cells for human clinical use. These concerns are exacerbated by the possible tumorigenic potential of ES cells [12–14].

The concerns regarding the ultimate clinical potential of human ES cells stimulated intense and ongoing investigations into alternate sources of pluripotent cells that may have applications in human regenerative medicine. In 2007, groups in Japan and the USA [15–17] demonstrated that fetal and adult mouse and human fibroblasts and other somatic cells [18–21] could be induced to become pluripotent following the introduction of key transcription factors using retroviruses. The emergence of techniques for making these induced pluripotent stem (iPS) cells provided a potentially powerful way to harness the promise of stem cells without the moral, legal and ethical problems of human ES cells. These iPS cells were initially generated by introduction of transcription factors including c-Myc into cells by the use of retroviral vectors, and this resulted in some of the induced cells becoming neoplastic. This problem was quickly circumvented by use of alternate cocktails of genes that did not contain c-Myc [22,23]. Despite the potential of these iPS cells, and their similarities to ES cells, underlined by the recent demonstration that iPS cells, like ES cells, can be used to produce viable offspring [24,25], significant obstacles to clinical utilization of these cells remain. In addition to the use of an oncogene in the initial transcription factor cocktail used to induce pluripotency, another major concern was the use of retroviral vectors [21,22,26,27] to introduce genes that conferred pluripotency on these cells. Although rapid progress has been made in the use of alternate adenoviral vectors (that do not incorporate into the genome) [21], and most recently through the use of non-viral plasmids [28] and recombinant proteins [29] to introduce the genes needed for pluripotency, concerns that the vectors or introduced genes themselves may produce complications in a human clinical setting remain and will have to be conclusively addressed before human clinical therapy becomes possible with this methodology.

The recent rapid progress with iPS cells has to some degree overshadowed parallel work on adult stem cells from various organs or tissues. However, if an adult source of allogenic stem cells with developmental potential equivalent to ES and iPS cells could be identified, this would probably be the most promising clinical approach for use in human regenerative

medicine. Similarly, as discussed above, the necessity of introducing several transcription factors into cells to induce pluripotency is an ongoing concern, and if endogenous stem cells could be identified that were capable of being induced to form iPS cells by a less extensive or severe genetic manipulation, this would also offer potential advantages for human applications.

Over the past few years, research at a number of laboratories has demonstrated that enriched populations of murine spermatogonial stem cells can give rise to ES-like cells that are pluripotent [30–34] and may have some advantages in terms of potential clinical applications [35–37]. The focus of this review is recent developments showing that both human and mouse spermatogonial stem cells can differentiate into a variety of tissues in different types of experimental paradigms. This review considers the potential these cells may have as a source of stem cells for human clinical applications, and discusses some of the major obstacles that must be overcome to conclusively prove or disprove the idea that these cells have clinical potential.

2. Spermatogonial stem cells

Spermatogonial stem cells (SSCs) are a subpopulation of unspecialized stem cells that lie along the basement membrane of the seminiferous tubules of the testes and give rise to the germline lineage in males. Testicular SSCs are typically unipotent and are only capable of giving rise to the germ cell lineage and ultimately spermatozoa. This ability of SSCs, the only adult stem cell population that transmits genetic information to the next generation [38–40], to generate spermatozoa is critically dependent on the microenvironment or niche that surrounds these cells (reviewed in [41–45]). When removed from their normal stem cell niche and cultured *in vitro*, SSCs display a broader developmental potential than they normally manifest *in vivo*. This has led to interest in their potential suitability for human regenerative medicine.

SSCs arise from primordial germ cells whose precursors are formed in the epiblast before gastrulation occurs in the embryo. Primordial germ cell precursors migrate into the extra-embryonic mesoderm and due to this initial location outside the embryo proper they are not classified as belonging to a particular germ layer. This location also allows germline stem cells to avoid some differentiation processes that other putative adult stem cell populations might undergo (Figure 1) [46].

Teratomas, tumors that contain cell types derived from all three germ layers, occur almost exclusively in gonads and are of germ cell origin [47]. This demonstrates that at least under pathological conditions, germ cells can give rise to a broad array of tissues. It has also been demonstrated that primordial germ cells from both mouse and human [48,49] can give rise to pluripotent embryonic stem cells when cultured *in vitro*. James Thomson, whose laboratory first derived human ES cells, has suggested that expression of key gene markers in ES cells is similar to that of early germ cells, even though recent results have identified other genes whose expression differs between ES cells, primordial germ cells and SSCs in mice (Table 1). Hence it was suggested that the closest *in vivo* equivalent of ES cells may be the early germ cell [50]. This suggests that due to their similarities to ES cells, germ-line

stem cells could be more plastic than other adult stem cell populations. If this is the case, SSCs may have greater potential for differentiation into cell types of the different germ cell layers upon exposure to the appropriate inductive signals or growth factors than other stem cells found in the adult, and thus may have significant clinical potential in human regenerative medicine.

2.1 Murine spermatogonial stem cells are pluripotent

Until recently it was believed that SSCs were unipotent and could only give rise to the spermatogenic lineage. However, landmark work in the early 21st century by Kanatsu-Shinohara *et al.* [32] challenged this concept when they showed that neonatal murine germ cells could give rise to ES-like cells when these cells are removed from the testis and grown for extended periods *in vitro*. They demonstrated that testicular cells isolated from neonatal mice and grown for a period of 4 – 7 weeks produced a low frequency of colonies that looked similar to ES cells. When these ES-like cells were subcultured, they formed colonies that could give rise to cell types derived from all three germ layers. Furthermore, these ES-like cells were capable of giving rise to teratomas when injected subcutaneously into nude mice and contributed to embryonic development when injected into blastocysts. Subsequently, enriched adult murine SSC preparations were also shown to be capable of giving rise to multipotent germline stem cells [30,31,33,34]. These ES-like cells apparently derived from SSCs appeared to have developmental potential at least comparable to ES cells. For example, two separate groups demonstrated that multipotent germline stem cells derived from enriched neonatal and adult mouse SSC populations could differentiate into mature cardiac and endothelial cells and that these cardiac cells were contractile and had electric potentials and ion channels [51,52]. Multipotent germ-line stem cells derived from adult mouse SSCs could also be differentiated into functional neurons and glia, again showing the plasticity of these stem cells [53,54]. More recently, Ko *et al.* [55] were able to derive pluripotent stem cells from adult mouse germline stem cells 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. SSCs isolated from neonatal or adult mice and humans are at best enriched in actual SSCs, and the cell populations of SSCs that we and others have isolated are by no means pure populations. However, for simplicity, ‘SSCs’ in this review is used to refer to the enriched population of SSCs or putative germline stem cells.

2.2 Human spermatogonial stem cells are multipotent/pluripotent

The studies showing that neonatal and adult murine SSCs were multipotent or pluripotent suggested a potential application for these cells in human regenerative medicine. The key question that remained was whether human SSCs, which had not previously been successfully isolated and cultured, would have similar potential. A series of recent papers have not only reported methodologies for the isolation and extended culture of human SSCs, but have also shown that enriched SSC cell populations derived from human testis are multipotent/pluripotent [56–59]. Some of these studies [56,58] used testicular biopsies, illustrating the feasibility of obtaining and propagating patient-specific SSCs which could be converted to desirable tissues types for therapy. In addition, the pluripotency genes *UTF-1* and *Rex-1* are expressed during human testis development and *UTF-1* is suggested to play a

role in spermatogonial stem cell renewal. Moreover, *UTF-1* and *Rex-1* are also expressed in high abundance in testicular germ cell tumors [60]. This also suggests that human spermatogonial cells have a marker profile similar to that of pluripotent cells, indicating that these cells are more undifferentiated than other adult stem cells.

3. Direct differentiation of spermatogonial stem cells to other cell types

Despite the demonstrated potential of human and mouse SSCs to give rise to ES-like cells that can differentiate into a variety of tissues, there are some obvious impediments to any possible clinical applications of this phenomenon. The extended culture time necessary for SSCs to give rise to ES-like cells is an obvious concern. Similarly, the low frequency of occurrence of these ES-like cells in the SSC cultures, and the extended time period necessary for subculturing and multiplication of these cells up to quantities that would be therapeutically useful, would all mitigate against use of SSCs in a clinical setting. Based on previous suggestions that SSCs were potentially more similar to ES cells than stem cells in other somatic tissues, we reasoned that SSCs might be able to directly differentiate into other cell types given the appropriate inductive environment, without the need to go through an ES-like stage. To test this hypothesis, we developed a new experimental system in which enriched populations of SSCs are obtained from the testis and recombined with fetal or neonatal mesenchyme from various organs that functions as an instructive inducer. Our results suggest that neonatal mouse stem cell spermatogonia can directly differentiate into a variety of other cell types in this experimental model [61].

3.1 Mesenchymal–epithelial interactions

Development of many organs is dependent on interactions between the mesenchyme, the undifferentiated connective tissue that is the precursor of the stroma in more mature organs, and the epithelium. These interactions are necessary for epithelial morphogenesis and cytodifferentiation in a wide variety of organs, and the mesenchyme regulates epithelial branching morphogenesis in organs such as lung, prostate, salivary glands, liver, pancreas and mammary glands [62]. The interactions between the mesenchyme and epithelium are reciprocal, although the specific factors involved in these interactions, and especially the epithelial factors that signal to the mesenchyme, are only now being identified [63–65].

The concept that tissue development was regulated by signals from the surrounding microenvironment was first postulated by Pander in the 1800s (cited in [66]) and was experimentally established by the elegant experiments of Spemann in the early 20th century that were conducted with amphibian embryos [67]. Spemann demonstrated that mesenchyme instructively induces epithelial fate and he termed this as the ‘organizer-effect’. This concept is well illustrated by the classical experiments of Saunders [68,69] who used tissue recombination experiments involving mesenchyme from various skin regions recombined with epidermis from different regions of the chick to demonstrate that the mesenchyme dictates the type of epithelial differentiation as well as various epithelial modifications (scales, presence and type of feathers, etc.).

These tissue recombination approaches have also yielded important insights into tissue interactions in the male and female reproductive tract. The urogenital sinus is an initially

ambisexual fetal organ that gives rise to the prostate in males and a portion of the vagina in females. Cunha and colleagues [70–72] were able to demonstrate that not only did the urogenital sinus mesenchyme (UGM) regulate development of the urogenital sinus epithelium (UGE), but it could also function as an instructive inducer. For example, bladder epithelium can be instructively induced to form prostatic epithelium when recombined with UGM and grown in the androgenic environment of a male host *in vivo*. Similarly, neonatal uterine mesenchyme instructively induces neonatal vaginal epithelium to form uterine epithelium in tissue recombinants *in vivo* [73].

3.2 Human ES cells can differentiate into various epithelia when recombined with instructive inducers

Melding the tissue recombination methodology with the known pluripotential nature of human ES cells, Taylor *et al.* [74] produced chimeric prostatic tissue in which the ES cells had differentiated into prostatic epithelium by recombining mouse UGM and human ES cells and growing these tissue recombinations *in vivo*. They showed that the prostatic epithelium formed from the ES cells expressed human prostate-specific antigen, a unique prostatic epithelial secretory protein. Using a similar methodology [75,76], others were able to directly differentiate human ES cells or bone marrow-derived mesenchymal stem cells into bladder epithelium by exposing these cells to the inductive influence of bladder mesenchyme in a tissue recombinant. These results powerfully demonstrate that the mesenchyme can instructively direct the differentiation of ES or other stem cells into a specific cell fate.

3.3 Spermatogonial stem cells directly transdifferentiate into other cell types

Based on previous studies showing the pluripotential nature of SSCs and the instructive potential of various mesenchymes, we were able to show that neonatal mouse SSCs could directly differentiate into prostatic, uterine and skin epithelium [61] when recombined with the appropriate mesenchyme and grafted *in vivo*. Critically, the original SSCs expressed molecular and functional markers that were entirely characteristic of the induced cell type following tissue recombination and grafting *in vivo* and totally lost all characteristics of the germ cells from which they differentiated. For example, the epithelium in tissue recombinations of UGM and SSCs expressed NKX3.1, a critical prostatic epithelial transcription factor. These tissue recombinants also expressed high levels of androgen receptor; neither NKX3.1 nor androgen receptor are expressed by the original SSCs (Figure 2). Thus, this methodology provides a way to directly derive various epithelial cell types from SSCs, and shows that these cells can give rise to a variety of other cell types without the need to form an ES-like cell as an intermediate step.

Boulanger and colleagues [77] were able to direct the differentiation of testicular stem cells into mammary epithelia when the enriched SSCs were mixed with mammary epithelial cells and grafted into the mammary fat pad *in vivo*. These findings, like our recent study, demonstrated that the microenvironment or niche has a more important role to play in deciding the fate of the stem cell than the intrinsic properties of the cell itself. However, in that study an enriched SSC population alone was unable to form mammary epithelium when these cells alone were implanted into the cleared mammary fat pad, suggesting that SSCs

were unable to form mammary epithelium *de novo* unless a preexisting mammary epithelial population was present. This contrasts with our results, which show that the inductive environment provided by the mesenchyme in the tissue recombination is alone sufficient to induce SSCs to undergo extensive cytodifferentiative events required to form an epithelial cell.

3.4 Mechanism of transdifferentiation

The mechanism by which SSCs lose their germ cell characteristics and begin expressing all the phenotypic and functional characteristics of a totally unrelated tissue, such as prostatic epithelium, under the inductive influence of mesenchymal cells is poorly understood. There may be a distinct subpopulation of SSCs that are undifferentiated and thus capable of giving rise to pluripotent cells when they are removed from their microenvironment in the testis or in response to a novel inductive environment, such as that provided following tissue recombination with mesenchyme from other organs. However, Kanatsu-Shinohara and coworkers [33] 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 argument was also supported by a recent study [55] showing that pluripotent stem cells can be derived from unipotent germline stem cells. Conversely, Izadyar *et al.* [31] 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⁺ and gives rise to the spermatogenic lineage. Further work is required to establish exactly how SSCs can give rise to other cell types. Identifying the exact cells in the SSC population responding to the inductive signal from the mesenchyme and giving rise to a new epithelial cell type may also greatly facilitate the clinical application of these cells.

4. Expert opinion

A critical question is whether human SSCs have the same differentiation potential as mouse SSCs and if these adult cells are equally as plastic as neonatal SSCs. Recent work showing that there is marked conservation in gene expression between human spermatogonia and mouse gonocytes [78] and the observation that adult human SSCs can give rise to ES-like cells that can further differentiate into a variety of cell types [56–59] suggest that it is likely that adult human SSCs may be similar in developmental plasticity to neonatal mouse SSCs and could differentiate into prostatic, uterine or other types of epithelium in our tissue recombination methodology, although this remains to be directly established.

There is great clinical interest in the regeneration of bladder and skin [75,79]. One approach to obtain these tissues would be to differentiate SSCs in the presence of an appropriate inductive stroma. Bladder mesenchyme [75] and skin dermis [61] in the fetal stages are instructive inducers. However, a significant challenge for translation of this methodology into a viable human clinical therapy would be to determine whether stromal cells from an adult could be obtained that would provide the appropriate microenvironment to instructively induce SSCs to form a desired tissue. For example, it is presently unknown

whether adult bladder stroma and skin fibroblasts are able to instructively induce SSCs to form bladder or skin epithelium, respectively.

As discussed above, progress toward establishing the mechanistic basis of stromal–epithelial interactions has been modest. However, the relevance of our study is that stroma or mesenchyme can instructively induce SSCs to express a new fate. Even if it is not possible to obtain adult fibroblastic stroma from organs such as bladder or skin that are capable of instructively inducing SSCs, or if these adult tissues do not have the inductive capabilities of the younger mesenchyme, this does not preclude use of SSCs for various clinical applications involving tissue regeneration. If we can determine the stromal factors responsible for inducing an uncommitted or previously differentiated cell type, then it may be possible to induce SSCs to form tissues such as bladder and skin epithelium (Figure 3). This could be done not by recombining them with an inductive stroma, but merely by exposing them to the inductive signals normally produced by these tissues, perhaps using some sort of artificial scaffold to stimulate the correct three-dimensional development of these tissues, as is presently being done in skin biology. Based on the pluripotential nature of SSCs revealed in recent studies, it is possible that in the right microenvironment SSCs will differentiate into specific non-epithelial cell types, in addition to forming various epithelial cells under the inductive influence of mesenchyme. However, this system is not applicable to tissues whose development does not involve mesenchymal–epithelial interactions (e.g., cartilage, bone, neurons, blood cells). As depicted in Figure 3, for neurons, pancreatic beta cells and a number of other cell types, another inductive approach would have to be used to convert SSCs (or ES or iPS cells) into the desired cell types, but the demonstrated plasticity of SSCs documented in the literature suggests this may be feasible. Another area worth exploring is whether SSCs could home and differentiate into a specific tissue type when injected directly to a site of injury. For example, hematopoietic stem cells injected directly into injured cardiac tissue generate functional myocardial tissue [80].

SSCs, as well as other adult-derived stem cells, may be safer to use therapeutically than ES or iPS cells. ES and iPS cells are totally undifferentiated and capable of forming all tissue types (Figure 1); therefore, it is not surprising that one of the greatest concerns in using ES cells in medicine is their potential to induce the formation of teratomas or other tumors in a patient [12–14]. SSCs are at least one step more differentiated than ES or iPS cells (Figure 1) and thus less likely to induce teratomas [58].

There are concerted efforts underway to understand the natural regeneration that occurs in organisms like planarians and salamanders, and to determine if this could have relevance for human regenerative medicine [81]. Regenerative biologists believe that many genes and molecules that are responsible for the ‘regeneration recipe’ [81] are conserved between species and they could be extrapolated to higher mammals like humans. It is difficult to predict whether work in this area will lead to any viable strategies for human regenerative medicine, or even what direction the basic thrust of research in human regenerative medicine will be. However, the demonstrated plasticity of SSCs from men and other species emphasizes that these cells could have utility in treating some diseases and in human regenerative medicine in general.

One great advantage of using SSCs for human regenerative medicine is that they can be obtained through testicular biopsies and could be used for autologous transplants. However, pluripotent cells (multipotent germline stem cells, ES and iPS cells) could be targets of cytotoxic T lymphocytes and thus increase the risk of rejection after transplantation [82]. Moreover, these cells would seem to have limited potential in non-autologous transplants, due to the concerns related to transplant rejection and the consequent host immune suppression that is necessary in these cases. However, if medicine can overcome or minimize the present immunological hurdles that limit autologous or non-autologous transplants, then SSCs could be a promising source of pluripotent stem cells that could be used in clinical situations.

Other major challenges in the use of human SSCs are obtaining sufficient numbers of these cells to use for therapeutic purposes and the development of a standardized system of isolating and propagating these cells. A large testicular biopsy (1 g) might contain approximately 5 million germ cells, and obviously not all these are stem cells [57]. Given this relatively modest number of stem cells available, it will be of paramount importance to identify specific markers for human SSCs that will facilitate their efficient isolation and culture, as well as identify any subsets of SSC populations that may have greater plasticity and thus have greater clinical potential. In addition, initial descriptions of these cells indicated that they were problematical to maintain in long term culture [36]. However, He *et al.* [83] and Wu *et al.* [78] recently demonstrated that human SSCs share many characteristics with SSCs of other species and the work of He *et al.* [83] suggests that human SSCs can be cultured effectively *in vitro*. Clearly, optimization of culture conditions for human SSCs that will allow extended multiplication of these cells without differentiation is essential for any potential therapeutic application given the limited number of spermatogonial cells obtained from a testicular biopsy [83].

Apart from the role of SSCs in human regenerative medicine, advances in human SSC biology would also facilitate preservation/expansion of these cells for reproduction for men planning to undergo medical treatments such as radiation or chemotherapy that sometime render them infertile. Conceivably, a testicular biopsy could yield SSCs that, if needed, could be returned to the testes to restore fertility using the stem cell transplantation assay developed by Brinster (Figure 3) [84–86].

In spite of the promise of SSCs as a source of pluripotent cells, extensive research is required to more accurately identify, propagate and induce the differentiation of SSCs into desired cell types. Inherent similarities to ES cells may give the SSCs significant advantages as a starting material for human tissue regeneration. The precise future of stem cells in human medicine is unclear, but SSCs appear to be a powerful tool that biologists can utilize as they work toward regenerating human tissue in various clinical settings.

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Highlights

- Spermatogonial stem cells (SSCs) are a subpopulation of unspecialized stem cells in the testes and when removed from their normal stem cell niche, they display a broader developmental potential than they normally manifest *in vivo*.
- Murine SSCs can give rise to a wide range of other cell types either directly, when recombined with instructive inducers, or indirectly, after being converted to ES-like cells.
- Recent work shows that stem cells from human testis are multipotent/pluripotent and thus have promise in the field of regenerative medicine.

Figure 1. Origin and development of spermatogonial stem cells (SSCs), embryonic stem (ES) cells and induced pluripotent stem cells

During embryo development in mammals, the inner cell mass of the blastocyst develops into the embryo proper and trophoblasts develop into the placenta and extraembryonic membranes. Embryonic stem cells are derived by *in vitro* culture of the inner cell mass and are pluripotent. As the inner cell mass develops into the epiblast, primordial germ cell precursors are formed and migrate away from the embryo's body before gastrulation. Primordial germ cells and the spermatogonial stem cells that arise from these cells are pluripotent and can give rise to ES-like cells that are not germ-layer-restricted in terms of the tissues they can form. Other adult somatic stem cell populations that are multipotent arise after the three germ layers, ectoderm, mesoderm and endoderm, are formed during gastrulation and hence may be more differentiated and less plastic than SSCs. An alternate source of pluripotent cells, induced pluripotent stem cells, are derived from terminally

differentiated somatic cells by introducing exogenous genes that confer pluripotency to these cells.

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Figure 2. Direct differentiation of spermatogonial stem cells into prostatic epithelium

Spermatogonial stem cells (SSCs) isolated from green fluorescent protein (GFP)-expressing mice were recombined with wild-type (non-green) urogenital sinus mesenchyme and grafted under the renal capsule of a syngeneic male host. Immunofluorescence for GFP showed that the epithelium (E) expresses GFP confirming that GFP⁺ SSCs have differentiated into prostatic epithelium. Stroma (S) does not express GFP. The epithelium also expresses androgen receptor (red nuclei), which is typical of prostatic epithelium but is not seen in SSCs.

Figure 3. Potential therapeutic applications of spermatogonial stem cells

Spermatogonial stem cells (SSCs) are isolated from testicular biopsies or orchiectomies. SSCs can be cryopreserved and transplanted back into the testis of patients following radiation/chemotherapy treatments that might render them infertile. In addition to this, SSCs are a potential alternative source of pluripotent stem cells. Some of the possible methods of differentiating SSCs into specific cell types for use in regenerative medicine are (1) Recombination of SSCs with mesenchymes that are instructive inducers to differentiate SSCs into specific epithelial cell types; (2) Artificial induction of SSCs using inductive stromal signals that induce these cells to differentiate as a specific cell type together with the use of artificial scaffolds for the three-dimensional development of these tissues; (3) Embryonic stem (ES)-cell-like induction by long-term culture of SSCs and selection for cells that morphologically resemble embryonic stem cells and subsequent culture and differentiation of these pluripotent cells into specific cell types; (4) Induced pluripotent stem (iPS) cell induction by introducing genes that confer pluripotency to cells. Since SSCs are more plastic than terminally differentiated or adult stem cells and express Oct4, it might be possible to induce SSCs to form iPS cells through a less extensive manipulation than that is now used on other somatic cells to induce them to form iPS cells and (5) Direct injection at the site of an injury or infarction. Since SSCs are unspecialized, they could home and respond to signals in the new microenvironment and generate normal tissue at the site of injury.

Table 1

Genes expressed by inner cell mass (ICM), embryonic stem cells (ES), primordial germ cells (PGC) and spermatogonial stem cells (SSC) in mouse.

Gene	ICM	ES	PGC	SSC
<i>Akp2</i>	+ [87]	+ [87]	+ [88]	– [32]
<i>Blimp1</i>	– [89]	– [89]	+ [90]	+ [91]
<i>C-myc</i>	ND	+ [92]	– [93]	+ [94]
<i>Dazl</i>	– [95]	+ [95]	+ [95]	+ [96]
<i>Ddx4(Mvh)</i>	– [97]	– [97]	+ [97,98]	+ [98]
<i>Dppa3(Stella)</i>	+ [99]	+ [99]	+ [99]	+ [100] – [101]
<i>Flt1m3(Fragilis)</i>	+ [90]	+ [90]	+ [90]	+ [100]
<i>Kit</i>	– [102]	+ [102]	+ [102]	– [103]
<i>Klf4</i>	ND	+ [104]	– [93]	+ [94]
<i>Nanog</i>	+ [105]	+ [105]	+ [105]	– [106]
<i>Pou5f1(Oct4)</i>	+ [107]	+ [107]	+ [107]	+ [108]
<i>Sox2</i>	+ [109]	+ [109]	+ [110]	+ [94]

Modified from [50].

ND: Not determined.