REVIEW ARTICLE

Stem cells and clinical practice: new advances and challenges at the time of emerging problems with induced pluripotent stem cell therapies

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ABSTRACT

Humans, like other species that reproduce sexually, originate from a fertilized oocyte (zygote), which is a totipotent stem cell giving rise to an adult organism. During the process of embryogenesis, stem cells at different levels of the developmental hierarchy establish all 3 germ layers and give rise to tissue-committed stem cells, which are responsible for rejuvenation of a given tissue or organ. The robustness of the stem cell compartment is one of the major factors that directly impact life quality as well as lifespan. Stem cells continuously replace cells and tissues that are used up during life; however, this replacement occurs at a different pace in various organs. The rapidly developing field of regenerative medicine is taking advantage of these physiological properties of stem cells and is attempting to employ them in clinical settings to regenerate damaged organs (eg, the heart, liver or bone). For this purpose, the stem cells most successfully employed so far are adult tissue-derived stem cells isolated mainly from bone marrow, mobilized peripheral blood, umbilical cord blood, fat tissue, and even myocardial biopsies. At the same time, attempts to employ embryonic stem cells and induced pluripotent stem cells in the clinic have failed due to their genomic instability and the risk of tumor formation. In this review, we will discuss the various potential sources of stem cells that are currently employed in regenerative medicine and the mechanisms that explain their beneficial effects. We will also highlight the preliminary results of clinical trials as well as the emerging problems relating to stem cell therapies in cardiology.

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Introduction Stem cell therapies began 50 years ago with the first transplantation of hematopoietic stem cells to replace damaged hematopoietic systems. The successful application of these cells in the hematological setting encouraged attempts to employ them in treating several other clinical problems encountered in cardiology, orthopedics, neurology, diabetology, opthalmology, dermatology, and gastroenterology.[1-5] Accordingly, stem cell-based therapeutic strategies have been proposed for treating a multitude of clinical problems, including damaged myocardium after heart infarction, damage to the brain after stroke, damaged spinal cord after mechanical injury, age-related macular degeneration of the retina, damaged liver, extensive skin burns, diabetes, and Parkinson disease. Unfortunately, beyond hematological applications, the results for other clinical applications of stem cells have been disappointing, and several encouraging results reported in laboratory animals have not been reproduced in humans.[6] Overall, the promise of clinical applications of stem cells and their success have often been exaggerated by the news media.

On the other hand, it is well known that stem cells residing in adult tissues are responsible for organ rejuvenation. However, this process occurs at a different pace in various tissues. While hematopoietic cells, intestinal epithelium, and epidermis are continuously replaced by new cells, this

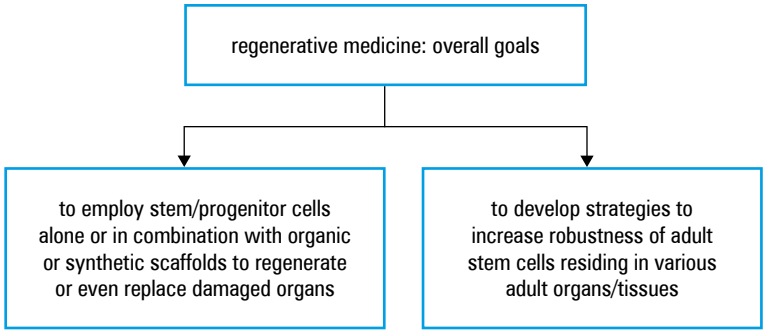

FIGURE 1 Two major goals of regenerative medicine

process is very slow in other organs (eg, heart or skeletal muscles) or its existence is still somewhat questionable (eg, for the brain and spinal cord).[7]

As depicted in FIGURE 1, the unique properties of stem cells make them candidates for 2 important clinical applications. First, they could be employed in certain clinical settings, provided that appropriate and efficient methods are available, to regenerate damaged tissues and improve the function of affected organs. These applications would require their systemic or local delivery. There is even hope that, in the future, transplantation of entire organs will be replaced by transplantation of a suspension of stem cells, alone or in combination with organic or synthetic scaffolds. However, this possibility would require that the stem cells are able to recapitulate organogenesis and give rise to cells from the different germ layers that usually comprise a given tissue and then establish a 3-dimensional functional organ or at least a significant functional fragment. This means that tissue derived by such means must be properly innervated and vascularized by blood and lymphatic vessels and responsive to external and internal stimuli. In other words, implanted stem cells should recapitulate mechanisms that regulate embryonic development in a given organ. Unfortunately, this requirement is still far from feasible technically.

There are extensive ongoing attempts to harness stem cells isolated from adult tissues in regenerative medicine. However, stem cells isolated from postnatal tissues have a very limited ability to differentiate and can contribute only to lineage-restricted progeny for which they are committed (eg, epidermal stem cells give rise to epidermis and mesenchymal stem cells give rise to cells of connective tissues).[1,8,9] On the other hand, it is premature to expect clinical applications for induced pluripotent stem cells (iPSCs) generated by genetic manipulation of adult immortalized cells.[10] While these cells may differentiate across germ layers in vitro, the first clinical trials with iPSCs have been suspended because of safety issues.[11]

The second, perhaps an even more important aspect of stem cell therapies, is related to the role stem cells play under steady-state conditions in tissue rejuvenation (FIGURE 1). Their robustness and regenerative potential can be manipulated directly in vivo in adult organisms by therapeutic means, including caloric restriction, regular physical activity, and by pharmacological interventions (in the future). This aspect of regenerative medicine, in contrast to stem cell applications as therapeutics in emergency situations, is still somewhat underappreciated but has important prophylactic and therapeutic significance. In particular, this area awaits development of more specific drugs that would increase stem cell robustness, and this provides a challenge to the development of stem cell-tailored pharmacology. Most likely, these strategies will have to target pluripotent or multipotent stem cells residing in adult tissues and protect them from premature depletion, for example, due to somatotropic and insulin-like growth factor signaling, which may negatively affect the most primitive stem cells residing in adult tissues. In support of this notion, there is strong evidence from animal models that a decrease in insulin/insulin-like growth factor signaling leads to prolonged lifespan, improvement in life quality, and a reduced risk of cancer, and all of these beneficial effects can be explained at the stem cell level.[12-14] At present, this is achieved, as mentioned above, by caloric restriction,[15] physical activity,[16] and administration of drugs interfering with insulin/insulin-like growth factor signaling, such as metformin or rapamycin.[17] However, this is a new area for clinical interventions and stem cell-tailored pharmacology.

Based on the foregoing, we look forward to technologies leading to optimization of the clinical use of stem cells and increasing their robustness in tissues, which no doubt will become a key to improving life quality and increasing human lifespan in the future.

The search for pluripotent or multipotent stem cells for therapeutic application in regenerative medicine

The differentiation potential of stem cells is required for their ability to contribute to a wide spectrum of tissues. Therefore, the ideal stem cells for application in regenerative medicine would be pluripotent stem cells (PSCs) or multipotent stem cells (MultiSCs).[18] While PSCs, according to their definition, may give rise to cells from all 3 germ layers (meso-, ecto-, and endoderm), the differentiation potential of MultiSCs is limited to only 2 germ layers. Below, we will discuss the currently available sources of such stem cells (FIGURE 2).

Embryonic stem cell lines isolated from early-stage embryos Under certain culture conditions, it is possible to establish embryonic stem cell (ESC) lines from early embryos at the blastula stage (FIGURE 2).[19] These cells have the ability to differentiate into stem cells for all of the different tissues. Nevertheless, the generation of immortalized ESC lines requires destruction or manipulation of embryos, which has been questioned from an ethical point of view. On the other hand, these cells are difficult to control, as they may grow teratomas, and it is still a problem to obtain from them fully functional differentiated somatic

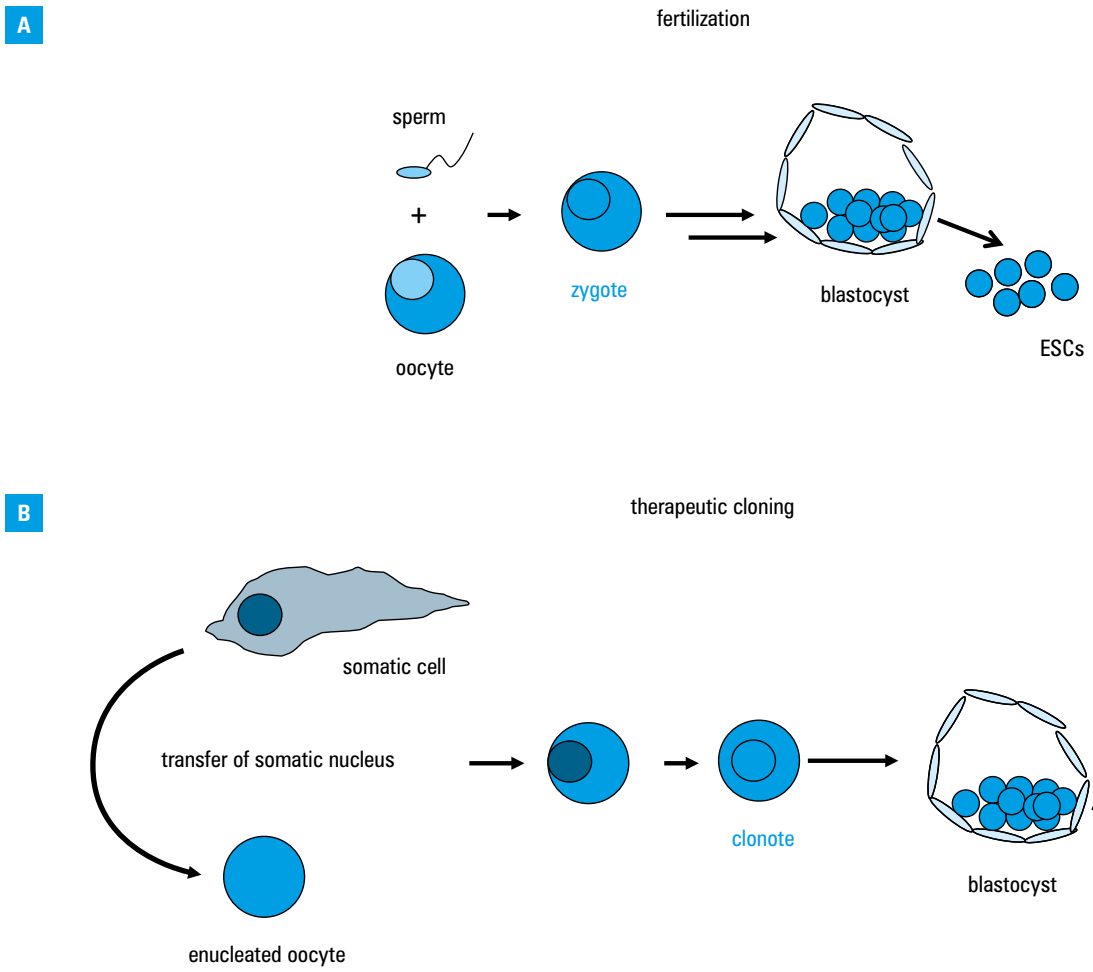

FIGURE 2 Embryonic stem cells (ESCs) obtained from embryos by physiological fertilization of the oocyte by sperm or after nuclear transfer of a somatic nucleus into an enucleated oocyte; **A** – ESCs isolated from blastocysts derived from an oocyte fertilized by sperm (zygote); **B** – ESCs can also be obtained by means of therapeutic cloning as the result of transfer of the nucleus from an adult somatic cell (eg, the nucleus of a fibroblast) into an enucleated oocyte. A totipotent stem cell generated by this strategy is called a clonote, which, like a zygote, gives rise to a blastocyst. Clonote-derived blastocysts are then a source of ESCs.

cells.[20] ESC lines can be generated from embryos derived by the physiological process of fertilization (FIGURE 2A) or derived by employing a nuclear transfer strategy (FIGURE 2B).

ESCs derived from zygotes generated by the physiological process of fertilization (FIGURE 2A) can be isolated from early embryos that have not been implanted into a uterus and are stored in liquid nitrogen at in-vitro fertilization clinics. Besides ethical considerations, the problem with such ESCs is that they give rise to differentiated cells that have a unique combination of histocompatibility genes inherited from the parents and would be rejected by a histoincompatible recipient.[21]

Another strategy is to create immortalized ESC lines, known in the literature as therapeutic cloning, which is based on the insertion of a donor-derived nucleus from a somatic cell into an enucleated oocyte derived from an ovulating female (FIGURE 2B). The basis of this method is that the nucleus of a differentiated somatic cell, when inserted into an enucleated oocyte, is reprogrammed by the enzymes, proteins, mRNA, and miRNA present in the oocyte cytoplasm to a state mimicking the nucleus in an embryonic cell.[22,23] Such artificially created totipotent cells (called, in contrast to physiologically fertilized oocyte zygotes, clonotes) have the potential for development and can be employed after initiation of embryogenesis to isolate ESCs from the inner cell mass of the blastocyst. Using this strategy, it is possible to create ESCs that are histocompatible with the donor of the somatic nucleus. This strategy, developed in animal models (eg, Dolly the sheep), has been recently demonstrated to work also in humans.[24] Nevertheless, there are similar serious ethical concerns and technical problems with ESCs obtained by this method, similar to those raised for ESCs isolated from embryos obtained in the process of physiological fertilization. As a result of these concerns and technical obstacles, no progress has so far been made with the application of such cells in humans, and this potential source of PSCs has at this point reached an ethical and technological dead end.[25]

Induced pluripotent stem cells generated from adult somatic cells In order to avoid using a controversial source of PSCs, such as cells isolated from early embryos, a strategy has been developed to

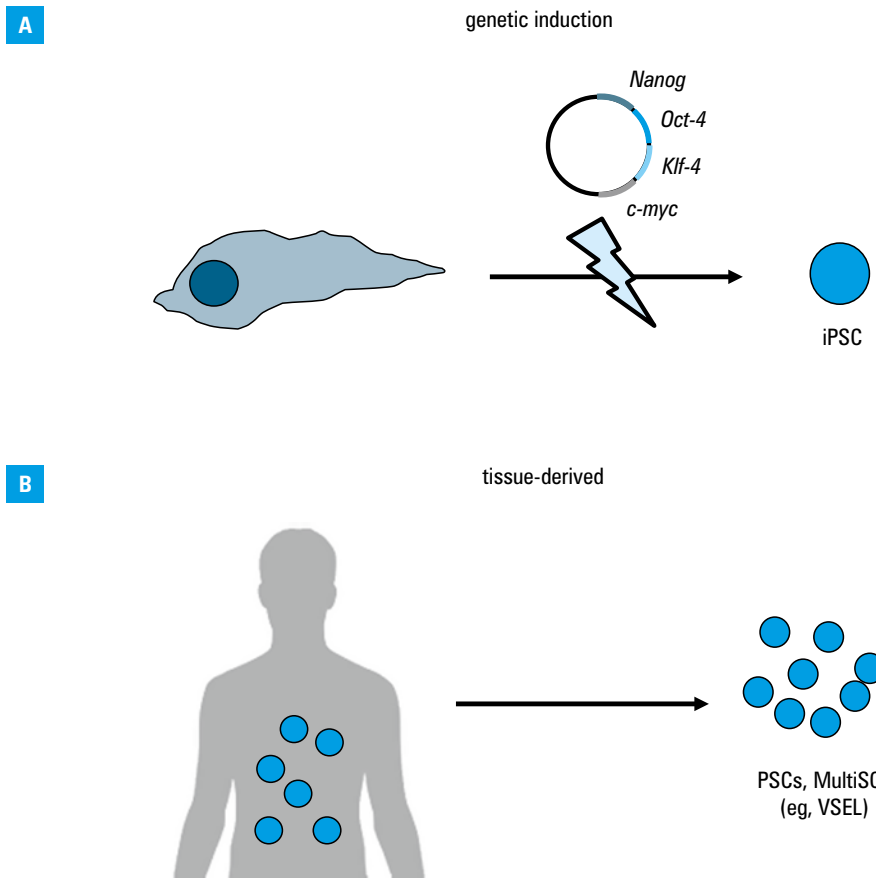

FIGURE 3 Pluripotent stem cells (PSCs) obtained from postnatal tissues; **A** – PSCs can also be obtained by transforming somatic cells (eg, fibroblasts) using genes that encode embryonic transcription factors (eg, Oct-4, Nanog, Klf-4, and c-myc). As mentioned in the text, there are also alternative strategies that replace DNA by mRNA, proteins, or regulatory miRNA and even by employing small-molecule modifiers of DNA; **B** – PSCs or multipotent stem cells can also be obtained from the tissues of mature individuals (eg, very small embryonic-like stem cells, also known as VSELs). With advances in the expansion strategy, these cells could become game changers in regenerative medicine.

obtain cells with multi-germ layer differentiation potential by inducing (transforming) adult somatic cells into the ESC state (FIGURE 3A). Since the target population of cells used for reprogramming is isolated from adult tissues (eg, skin fibroblasts), this source of PSCs, in contrast to cells isolated from embryos, is ethically acceptable.[26] Stem cells generated by this strategy differentiate into a wide spectrum of tissues, and these cells have been named "induced pluripotent stem cells". This technology allows us to obtain PSCs that are histocompatible with the donor of the cell used for reprogramming. Unfortunately, as with ESCs, several limitations have been identified for these cells, including the risk of teratoma and cancer formation as well as genomic instability.[27] These limitations explain why the first clinical trials using these "promising" cells were suspended, as mentioned in the introduction.[11]

The initial strategy for obtaining iPSCs, which earned Dr. Yamanaka a Nobel Prize in Medicine, was based on the transduction of somatic fibroblasts with a set of 4 genes that encode transcription factors (*Oct3/4*, *Sox2*, *c-Myc*, *Klf4*) governing pluripotency and the resulting proliferation of embryonic cells.[28] However, these genes integrate randomly into the chromosomes of manipulated cells, which raises concerns that randomly inserted genes may be incorporated into chromosome "hot spots" and, as a consequence, trigger activation of oncogenes or inactivate antioncogenes by insertional mutagenesis.[29] In response to this possibility, several alternative strategies have been proposed, such as employing nonintegrating DNA plasmids[30]; replacing DNA with mRNA or miRNA[31,32]; employing protein products in the form of cell-penetrating Oct3/4, Sox2, c-Myc, and Klf4 proteins (eg, poly-arginine-modified) instead of the genes themselves[33,34]; and even employing small molecules that modify the DNA structure of target cells and induce the pluripotent state.[35]

Despite the hope that these cells will be employed in the clinic, several problems have emerged, as mentioned above.[27,36] Besides the risks of teratoma formation, cancerogenesis, and insertional mutagenesis, the most important problems are summarized in TABLE 1. First, it was found relatively early that iPSCs may trigger an immune response in animal models. This problem is still under debate, but convincing results demonstrate that this complication occurs even in autologous iPSCs. The second problem is

TABLE 1 Molecular obstacles to clinical translation of induced pluripotent stem cells (iPSCs)

Obstacle	Reference
risk of teratoma and other tumor formation	97
risk of insertional mutagenesis	29
immune response to autologous iPSCs	98
genomic instability	99
transcriptional and epigenetic instability	100
variability in differentiation capacity	101
variability among iPSC clones derived from the same donor cells	102

TABLE 2 Strategies to improve the safety of induced pluripotent stem cell therapies (iPSCs)

Strategy	Reference
using suicide genes to eliminate any remaining undifferentiated iPSCs after therapy	103
proper selection of cells as the source of iPSCs	104
employing cells from younger donors (umbilical cord blood?)	105
better delivery methods for reprogramming genes (eg, using nonintegrating vectors, Sendai virus, episomal plasmid vectors)	38,39
for reprogramming, replacing the delivery of DNA with proteins, mRNA, or regulatory miRNAs	31,32,40
reprogramming with small DNA-modifying molecules	35
depletion of reprogramming-inhibiting genes (eg, *Mbd3*)	106
using low-passage iPSCs	107

related to the genomic, transcriptional, and epigenetic instability of iPSCs. Third, it has been demonstrated that there is variability in the differentiation capacity of iPSCs and even variability among iPSC clones derived from cells from the same donor. Moreover, it is technically difficult to obtain fully functional cells and, as of today, fully functional hematopoietic stem cells that can establish long-term hematopoiesis after transplantation in animal models have not been derived from iPSCs.[37]

In response to these obstacles, some attempts to mitigate the risk of therapies using iPSCs and increase their differentiation into fully functional stem cells are listed in TABLE 2. These strategies include the use of suicide genes to eliminate any remaining undifferentiated and highly proliferative iPSCs from the recipient's body after therapy. Another important issue is the selection of a proper source of cells that will be immortalized by transduction. For example, fibroblasts from the skin are commonly used for obtaining iPSCs and may already carry mutations that would be propagated in the iPSCs created. It is also known that cells from younger donors are more free of genomic changes than cells from older tissues. Therefore, umbilical cord blood cells that are obtained from a patient and stored for later use could be used for the generation of iPSCs. In parallel, better gene delivery methods for reprogramming have been proposed, such as using nonintegrating vectors, Sendai virus, or episomal plasmid vectors or replacing DNA with protein, mRNA, miRNA, or small-molecule DNA modifiers for iPSC induction. Reprogramming may also be facilitated by the removal of genes that hamper this process, such as *Mbd3*. Finally, since mutations may accumulate in cells during subsequent passages, a lower passage number for iPSCs would be more suitable for clinical applications.[32,38-41]

Nevertheless, several problems that have been identified so far make the potential application of these cells in the clinic highly questionable and suggest that expectations of a therapeutic payoff are premature. Until significant progress is made in increasing the safety of these cells, iPSCs can serve only as experimental models to study cell differentiation processes or as tools to identify the genes responsible for the development of certain disorders. However, even in this setting, some doubts have been raised about the utility of these cells because of their genomic instability and variability.[42]

Stem cells isolated from adult tissues So far, adult stem cells are the only cells to be employed safely in regenerative medicine. However, their clinical efficacy in other than hemotological applications has not been tested yet. These cells are isolated from postnatal tissue sources, such as bone marrow (BM), umbilical cord blood, umbilical cord, mobilized peripheral blood (PB), adipose tissue, skin epithelium, myocardium, and skeletal muscle biopsies, and are employed in the clinic as safe sources of stem cells for treating patients.[43] Nevertheless, despite several clinical trials, there is no solid and reproducible evidence in humans that these cells (except hematopoietic transplants) contribute to generating functional cells in damaged organs. The beneficial effects of stem cell therapies in cardiology, hepatology, neurology, and orthopedics is mostly related to their paracrine effects. It is well known that adult stem cells are a rich source of growth factors, cytokines, chemokines, and bioactive lipids, which have trophic, antiapoptotic, and proangiopoietic effects.[44,45] Moreover, in addition to soluble factors, stem cells also secrete membrane-derived extracellular microvesicles (ExMVs), which may deliver mRNA, miRNA, and certain proteins to target cells, thereby promoting cell survival and proliferation. The evidence suggests that these paracrine effects, mediated by soluble factors or by ExMVs, are major factors responsible for some of the positive results observed in patients after cell therapies.[46]

The cells most commonly employed for regeneration of damaged organs are mesenchymal stroma cells (MSCs). In the literature, these cells are usually (and wrongly) called mesenchymal stem cells, as only a very low percentage has the properties required for clonal growth and are real progenitors of connective tissue.[47] The bulk of these cells derived from expansion protocols are merely fibroblasts.

MSCs are isolated from BM, adipose tissues, and recently even from dental pulp. These cells can

proliferate and be passaged several times; however, this process is limited due to the shortening of telomeres that stabilize the ends of chromosomes. While MSCs are safe for clinical applications, when passaged many times ex vivo they can also acquire mutations. Nevertheless, because of the easy procedure to isolate and grow these cells, they are the most common source of cells currently employed in regenerative medicine.[48-51] This is so even though it is now well known that their beneficial effects are transient and mainly due to the release of soluble paracrine factors and ExMVs.[52]

However, evidence has accumulated that adult tissues harbor a population of very rare stem cells with PSC and MultiSC characteristics that express early-development embryonic markers (FIGURE 3B). Specifically, several types of putative PSCs and MultiSC have been described and isolated, primarily from BM, which are able to give rise to cells from more than one germ layer. These very rare cells were purified from adult cell suspensions by employing various strategies, mostly by the ex-vivo expansion of cells partially purified by immunomagnetic means or sorted by fluorescence activated cell sorter. Therefore, depending on the strategy by which they were isolated or cultured ex vivo, such cells have been given different names. The list of these cells is quite long and includes multipotent adult stem cells,[26] multilineage-differentiating stress-enduring cells,[53] multipotent adult progenitor cells,[54] unrestricted somatic stem cells,[55] marrow-isolated adult multilineage-inducible cells,[56] multipotent progenitor cells,[57] and spore-like stem cells.[58] However, these rare PSCs or MultiSCs that are able to change germ layer commitment were never well characterized at the single-cell level,[57,59] and their PSC/MultiSC-like properties were identified post factum, after phenotyping clones of already differentiated, in vitro-expanded cells.[26,53,56,57] Since it is unlikely that so many PSC and MultiSC types exist in adult tissues, all these early-development stem cells endowed with broader differentiation potential and residing in adult tissues are most likely closely related and represent overlapping populations of cells.

At the single-cell level, the best-characterized population of PSCs or MultiSCs residing in adult tissues are very small embryonic-like stem cells (VSELs).[60,61] The presence of these developmentally primitive stem cells in adult tissues can be explained by the possibility that early during embryogenesis not all of the stem cells differentiate into monopotent tissue-committed stem cells, but some survive in developing organs as a dormant backup population of primitive stem cells.[62] VSELs, which express a primitive embryonic phenotype, are detected in increasing numbers in PB during tissue or organ injuries (eg, heart infarct, stroke, intestinal inflammation, or skin burns).[63-65] The existence of these cells has recently been confirmed by several independent groups of investigators, and they have been postulated to play a role in physiological organ and tissue rejuvenation and regeneration after injury. Moreover, the number of these cells circulating in PB may be of prognostic value. The quiescence of VSELs, due to changes in expression of certain parentally imprinted genes, was until recently a major obstacle to their application in the clinic. However, this problem, has recently been overcome by employing small-molecule DNA modifiers that allow efficient ex vivo expansion of these cells. We envision that, since VSELs do not grow teratomas in experimental animals and can currently be expanded ex vivo, they will in the near future become "game changers" in the field of regenerative medicine.[66-68]

Emerging technologies to improve stem cell delivery and organ regeneration As mentioned above, the most crucial need in regenerative medicine is to identify a reliable source of stem cells that has the potential for cross-germline differentiation and would be able to establish the 3-dimensional, functional structure of damaged organs. It is obvious that, even if we have in hand PSCs that can differentiate into all types of cells of a given organ, the next major step will be to harness these cells to recover the 3-dimensional tissue structure. In other words, stem cells employed as therapeutics will have to repeat the process of organogenesis that occurs during embryonic development.

A temporary solution is to employ 3-dimensional scaffolds, which are prepared using organic or synthetic fibers or derived from "de-cellularized" normal organs.[69,70] Such organ-derived scaffolds, which contain connective tissue fibers, could be reseeded with stem cells in order to establish tissue. This procedure has been successfully employed in recovering functional murine kidney fragments. It is hoped that scaffold technology, in combination with 3-dimensional printing, will also enable significant progress in regenerating human organs.[71,72] However, this still seems to be a remote possibility, and, unfortunately, premature clinical trials using normal tissue-derived scaffolds to reconstitute trachea have failed.[73]

Other important aspects of stem cell therapeutics are cell delivery and cell dosage. These could be delivered directly to the damaged organs, infused into the arteries supplying damaged tissues, or infused systemically. For example, for treatment of damaged myocardium, cells could be injected directly into damaged heart tissue by introducing a catheter or infused into coronary arteries.[2,74]

Another possibility for improvement of current cell therapies is to increase the therapeutic power of stem cell paracrine effects.[75,76] As mentioned above, stem cells are a rich source of growth factors, cytokines, chemokines, and bioactive lipids, which may promote the proliferation of residual stem cells, inhibit apoptosis and promote neovascularization in the damaged tissues, and activate local tissue-committed stem cells. Stem cells can be conditioned for more efficient secretion of

soluble paracrine factors by exposure to hypoxia or by manipulation to overexpress genes that encode antiapoptotic or proangiopoietic factors (eg, kit ligand, vascular endothelial growth factor, or fibroblast growth factor 2) before infusion into patients.[77-79]

Moreover, as mentioned above, the effect of cell therapeutics may depend not only on the trophic effects of soluble factors but also on the similar effects of cell-derived ExMVs. These small spherical structures derived from cell membrane encapsulate and deliver to the cells in damaged organs fragments of cell cytoplasm enriched for mRNA, miRNA, proteins, and bioactive lipids. As recently demonstrated, the therapeutic effect of MSC-derived ExMVs when employed to improve the function of damaged kidney in mice was comparable to that of intact cells.

Based on the important finding that ExMVs have similar beneficial effects in regenerative therapy as the intact cells from which they are derived,[46] producing ExMVs on a large scale and even modifying their composition should be considered.[45] For this purpose, one might employ established immortalized ESCs or iPSCs as the cells producing ExMVs, which are enriched for several embryogenesis- and development-related paracrine factors. In order to be highly enriched for the desired molecules, such cell lines could be additionally manipulated to overexpress genes for growth factors or cytokines that would protect target cells in damaged organs from apoptosis and for factors that effectively induce angiogenesis. Similarly, ExMVs derived from cells cultured under hypoxic conditions would be enriched for factors that promote angiogenesis. At the same time, in order to improve delivery, ExMVs-producer cell lines could be manipulated to enrich ExMVs for molecules that would facilitate their tropism to the specific damaged organs and retention in damaged tissues after infusion into patients.

Clinical results with stem cell therapies for damaged organs related to cardiology Stem cells derived from adult tissues have been employed in several clinical settings to treat damaged organs (eg, heart, liver, spinal cord, retina, joints, and brain). However, as mentioned above, any improvements observed have most likely been due to the paracrine effects of the cells employed in the therapy. Below, we highlight the most important results of clinical trials using adult stem cells for tissue or organ regeneration related to cardiology.

The majority of cardiovascular cell therapy studies used unmodified autologous nonselected BM-derived mononuclear cells (BMMC). The BMMC fraction is relatively easy to isolate, but also heterogeneous and consists mostly of mature cells with negligible regenerative potential. Also since there is no consistency across the studies in details of cell isolation, the protocol comparison between the studies, such as for example the use of heparin as an anticoagulant, is difficult. Data from large clinical trials showed that heparin might affect the viability, migratory potential, and regenerative capacity as well as the clinical outcomes.[80] The key limitation of cardiac application of cell therapy is the very low retention of injected cells (3%–5% after 24 hours).[81] Therefore, as discussed above, the most probable mechanism of cell-mediated effects are paracrine mechanisms (antiapoptotic, proangiogenic, antifibrotic, activation of resident cardiac stem cells), as well as proangiogenic actions.[77,78] Paracrine effects are mediated by secretion of cytokines, chemokines, growth factors, and ExMVs containing micro-RNAs.[46,52] Also the clinical presentation (acute versus chronic) and local environment (presence of scar) influence the clinical results. In acute myocardial infraction (MI), the most beneficial effects would be the immunomodulation and antiapoptotic effect, while in chronic heart failure and refractory angina, it would be the stimulation of angiogenesis.[45,82] The reparative capacity of autologous cells is limited by the fact that cells isolated from patients with multiple comorbidities (diabetes, renal failure) display impaired migratory properties, ability to differentiate and form colonies, as well as signs of cellular senescence (telomere attrition).[83] More recently, instead of patient-isolated autologous BMMC, allogenic cells, such as MSC, attracted a significant scientific interest. Cardiac cell therapy can be delivered either by intracoronary infusion or intramyocardial delivery (transendocardial or epicardial). In clinical trials in acute MI, the intracoronary cell delivery is used, and in the setting of refractory angina and heart failure, the intramyocardial cell delivery is preferred because it provides better cell retention.[84]

Acute myocardial infarction So far, studies in cardiovascular cell therapy focused on 3 different populations of patients: acute MI, refractory angina, and heart failure. In the majority of trials in acute MI, the autologous BMMC were delivered into the infarct-related coronary artery using the "stop-flow technique" between 24 hours and 7 days after the primary percutaneous coronary intervention. Numerous meta-analyses of the trials showed that the single intracoronary injection of cells led to an increase in left ventricular ejection fraction measured between 4 and 6 months after the infarction, a trend towards less left ventricular remodeling, and favorable safety profile. Importantly, the obtained results are not consistent across the studies with more recent and larger trials using magnetic resonance imaging for endpoint evaluation showing no significant benefit of cell therapy. Moreover, a recent patient-level meta-analysis provided neutral results.[85-87] However, so far no cell therapy study has been powered to evaluate the long-term clinical outcomes, especially in terms of so called hard endpoints. The results of phase III BAMI trial will provide a definitive answer on the role of autologous BMMC in the setting of ST-segment

elevation MI (unpublished data; http://euram.ltd.uk/BAMI).

Refractory angina The frequency of refractory angina is from 1% to 4% of the patients with coronary artery disease, and this population includes also patients with complex coronary atherosclerosis not eligible for revascularization.[88] Such patients have frequent hospital admissions and report significantly impaired quality of life. Several randomized clinical trials with placebo showed improved exercise tolerance and decreased angina frequency, and some also showed improved stress myocardial perfusion with autologous mononuclear cell therapy. It seems that such effects persisted up to 5 years.[89,90] The most practical way of cell application in therapy is to use their endomyocardial delivery with the locatable catheter connected to an endocardial mapping and navigating system (NOGA), which allows for injection of cells in the areas of viable dysfunctional myocardium (hibernated segments). Also, the most recent meta-analysis provided encouraging conclusions showing improvement of the quality of life supported by encouraging imaging endpoints.[91]

Heart failure Heart failure is clearly a field where the unmet needs for new therapies are most significant. This population of patients is the most heterogenous, so the assessment of the effects of cell therapy is most difficult. Also there are multiple cell therapy studies using different delivery routes (transendocardial, intracoronary) and types of cells such as MSC, mononuclear cells isolated from either BM or PB, or adipose tissue-derived cells. In general, there is a positive effect of the cell therapy in terms of surrogate endpoints (improved results of 6-minute walk test and levels of N-terminal pro-B-type natriuretic peptide) and, in some studies, even increased left ventricular ejection fraction. It seems that intramyocardial application of at least 50 million cells within the areas of viable myocardium may provide beneficial effects.[92] However, there are some differences in terms of outcomes in nonischemic versus ischemic cardiomyopathy, so further clinical studies are needed.[92,93]

In conclusion, the cell therapy has been investigated in the clinical trials in cardiology for 13 years, and, despite the excellent safety profile and some encouraging signals of its efficacy in terms of clinical status and surrogate endpoints, it still remains an experimental treatment option.

New concepts in cardiovascular cell therapies In order to explore new sources of stem cells for therapy, 2 recent clinical trials SCIPIO and CADUCEUS showed safety and feasibility of autologous culture-expanded cardiac stem cells or cardiosphere-derived cells in patients with ischemic cardiomyopathy. However, larger randomized trials are needed to prove the efficacy.[74,94]

Because the autologous BM cells have low engraftment potential and no transdifferentiation capacities, an interesting concept of cardiopoiesis-guided cells was introduced. It was based on predifferentiation of autologous BMMC, using multiple growth factors. It was evaluated in the C-Cure trial with promising safety and feasibility; however, the recently presented CHART-1 trial showed no substantial effects on the primary endpoint (*Eurpean Heart Journal*, in press). In addition, recently allogenic MSC isolated from healthy donors and expanded in bioreactor emerged as a promising option for treatment. Such a population of cells can be standardized and used in an "off-the-shelf" approach.[74,92,93] A study by Kastrup et al[95] showed promising results and an excellent safety profile,[95] and a multicenter clinical trial funded by the Horizon 2020 scheme (Stem Cell Therapy in IschEmic Non-treatable Cardiac Disease SCIENCE, NCT02673164) evaluating endomyocardial delivery of 100 million of allogenic adipose-issue MSCs in patients with heart failure will validate this concept. Another potential source of regenerative cells is the Wharton's jelly, which provides a rich source of MSCs for cardiac repair.[96] A cardiovascular cell therapy program using Wharton's jelly-derived MSCs (CIRCULATE) is currently starting under the STRATEGMED II funding scheme and will investigate their applications in acute MI, heart failure, and peripheral artery disease. Finally, the most recent data with successful ex-vivo expansion of VSELs in chemically defined media will certainly encourage clinicians to employ these cells in clinical trials.

Another important possibility for stem cell therapies is a recent postulate by Dr. Roberto Bolli from the University of Louisville, Louisville, United States, to employ repeated infusions of stem cells, as all clinical trials reported so far employed single doses of cells (personal communication).

Conclusions Stem cells and their potential applications in regenerative medicine have generated immense interest in society at large, and this topic has been extensively covered both by nonprofessional and professional news media. Unfortunately, news stories predicting that clinical applications for a variety of medical problems will soon be available fosters unrealistic expectations in the public. Other concerns include the negative consequences for stem cell therapies and stem cell research from patent issues and the financial involvement of biotechnology companies, which frequently drive competition to the exclusion of cooperation. However, one of the most disturbing problems is stem cell tourism, in which patients seek stem cell therapies abroad in poorly qualified clinics that falsely promise therapeutic benefits that are still not approved for use in established medical centers.

Therefore, one intention of this review was to present the current state of regenerative medicine in an unbiased way. On the one hand, we have addressed the ethical and technical problems with the application of stem cells isolated from embryos generated by fertilization or by

nuclear transfer (therapeutic cloning) and, on the other hand, we tried to cool down the overheated expectations for the clinical application of iPSCs derived by ex-vivo dedifferentiation and immortalization of somatic cells.[11,25,42] We also addressed the current state of adult stem cell therapies that, except in the hematological field, are mainly based on paracrine and trophic effects that increase survival and regeneration of damaged organs.[45] These effects explain why adult stem cell therapies may have a positive effect on damaged tissues, even if a significant level of donor-derived chimerism is not detected.

Despite all the problems and limitations discussed above, a new era of regenerative medicine is approaching, and the coming years will bring further exciting discoveries. The identification of developmentally primitive stem cells residing in adult tissues and promising evidence that these cells can be isolated and expanded ex vivo opens the door to a new chapter in regenerative medicine. Therefore, we are at a critical point for stem cell therapies, even if the road to more efficient clinical applications is bumpy and sometimes difficult—as it is for all major breakthroughs in science.

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ARTYKUŁ POGLĄDOWY

Komórki macierzyste w praktyce klinicznej – postępy i wyzwania wobec problemów z klinicznym wykorzystaniem indukowanych pluripotencjalnych komórek macierzystych

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SŁOWA KLUCZOWE

efekty parakrynne, indukowane komórki pluripotencjalne, komórki macierzyste embrionalne, komórki macierzyste izolowane z dojrzałych tkanek, mikrofragmenty błonowe

STRESZCZENIE

Człowiek – podobnie jak inne gatunki, które rozmnażają się płciowo – powstaje z najwcześniejszej totipotencjalnej komórki macierzystej, którą jest zapłodniona komórka jajowa (zygota). Podczas embriogenezy powstają komórki macierzyste, które utworzą trzy listki zarodkowe oraz dadzą początek komórkom ukierunkowanym do różnych tkanek i narządów. Potencjał proliferacyjny i zdolność samoodnowy tych komórek to jeden z najważniejszych czynników warunkujących jakość i długość życia. Komórki macierzyste są źródłem komórek w dorosłych tkankach podczas życia osobniczego, aczkolwiek odnowa komórek w poszczególnych narządach następuje w różnym tempie. Celem dynamicznie rozwijającej się medycyny regeneracyjnej jest wykorzystanie tych właściwości komórek macierzystych oraz zastosowanie ich w praktyce klinicznej do regeneracji uszkodzonych narządów (np. serca, wątroby czy kości). W tym celu z największym powodzeniem wykorzystywano dotychczas komórki macierzyste izolowane ze szpiku kostnego, mobilizowanej krwi obwodowej, krwi pępowinowej, tkanki tłuszczowej, a nawet bioptatów mięśnia sercowego. Jednocześnie próby wykorzystania w medycynie regeneracyjnej komórek embrionalnych czy też tzw. indukowanych komórek pluripotencjalnych zakończyły się niepowodzeniami ze względu na niestabilność genetyczną tych komórek oraz ryzyko powstawania potworniaków. W niniejszym artykule omówimy różne potencjalne źródła komórek macierzystych wykorzystywane w medycynie regeneracyjnej oraz mechanizmy warunkujące ich korzystne działanie. Przedstawimy także pokrótce wstępne wyniki badań klinicznych oraz rodzące się wyzwania związane z wykorzystaniem terapii komórkowych w kardiologii.

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