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Introduction to Stem Cells and Regenerative Medicine

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Tissue-resident stem cells · Induced pluripotent stem cells · Mesenchymal stem cells

Abstract
Stem cells are a population of undifferentiated cells characterized by the ability to extensively proliferate (self-renewal), usually arise from a single cell (clonal), and differentiate into different types of cells and tissue (potent). There are several sources of stem cells with varying potencies. Pluripotent cells are embryonic stem cells derived from the inner cell mass of the embryo and induced pluripotent cells are formed following reprogramming of somatic cells. Pluripotent cells can differentiate into tissue from all 3 germ layers (endoderm, mesoderm, and ectoderm). Multipotent stem cells may differentiate into tissue derived from a single germ layer such as mesenchymal stem cells which form adipose tissue, bone, and cartilage. Tissue-resident stem cells are oligopotent since they can form terminally differentiated cells of a specific tissue. Stem cells can be used in cellular therapy to replace damaged cells or to regenerate organs. In addition, stem cells have expanded our understanding of development as well as the pathogenesis of disease. Disease-specific cell lines can also be propagated and used in drug development. Despite the significant advances in stem cell biology, issues such as ethical controversies with embryonic stem cells, tumor formation, and rejection limit their utility. However, many of these limitations are being bypassed and this could lead to major advances in the management of disease. This review is an introduction to the world of stem cells and discusses their definition, origin, and classification, as well as applications of these cells in regenerative medicine.

Introduction

Stem cells are undifferentiated cells that are present in the embryonic, fetal, and adult stages of life and give rise to differentiated cells that are building blocks of tissue and organs. In the post-natal and adult stages of life, tissue-specific stem cells are found in differentiated organs and are instrumental in repair following injury to the organ. The major characteristics of stem cells are: (a) self-renewal (the ability to extensively proliferate), (b) clonality (usually arising from a single cell), and (c) potency (the ability to differentiate into different cell types). These properties may differ between various stem cells. For example, embryonic stem cells (ESCs)

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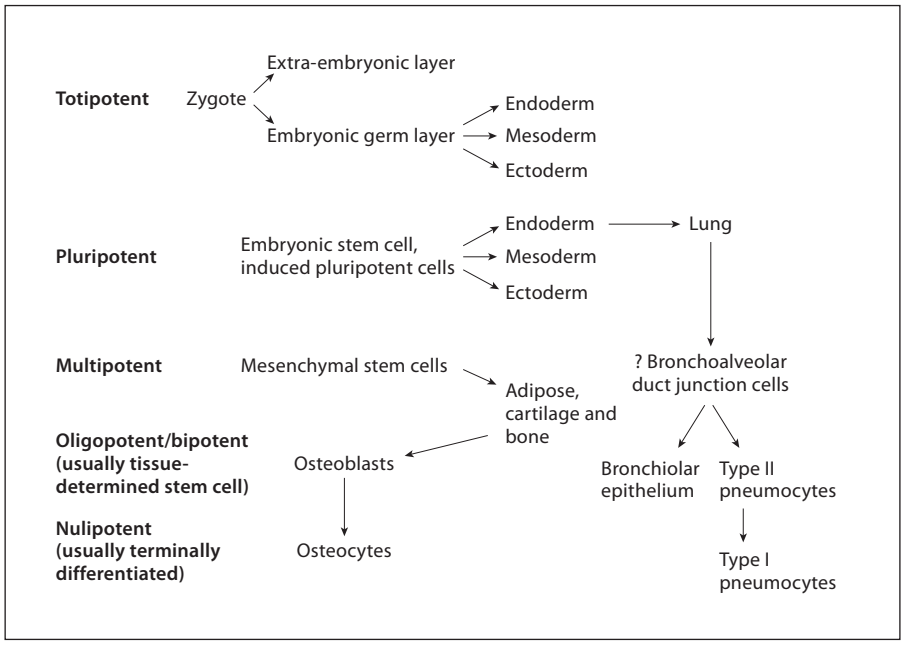

Fig. 1. The hierarchy of stem cells. Totipotent cells form embryonic and extra-embryonic tissue. Pluripotent cells form all 3 germ layers while multipotent cells generate cells limited to 1 germ layer. Bronchoalveolar duct junction cells in the lung may be multipotent while type II pneumocytes are oligopotent and differentiate into type I pneumocytes of the alveoli.

derived from the blastocyst have a greater ability for self-renewal and potency while stem cells found in adult tissue have limited self-renewal since they would not proliferate extensively and can only differentiate into tissue-specific cells.

The human body develops from the zygote and blastocyst from which ESCs are derived into the germ layers endoderm, mesoderm, and ectoderm. Specific organs arise from the germ layers. Some of the progenitor cells that have contributed to organ formation do not terminally differentiate but are retained as tissue stem cells and can be found in bone marrow, bone, blood, muscle, liver, brain, adipose tissue, skin, and the gastrointestinal tract [1, 2]. The tissue stem cells may be called progenitor cells since they give rise to terminally differentiated and specialized cells of the tissue or organ. These cells may be dormant within tissue but would proliferate under circumstances of injury and repair [3–5]. The dynamics of tissue stem cells or progenitor cells varies from tissue to tissue; for example, in bone marrow, liver, lung, and gut, stem cells regularly proliferate to supplement cells during normal turnover or injury [6–9], while in the pancreas, the heart, or the nervous system they proliferate to replace damaged cells following injury [10–14].

The idea of wound repair and organ regeneration is as old as humanity and is reflected in the ancient Greek myth of Prometheus, the Greek titan punished by Zeus for his disobedience in introducing fire and knowledge to human beings. In this myth, Prometheus is tied to a rock and everyday an eagle eats part of his liver which then regenerates. In modern-day medicine, work involving stem cells and organ regeneration commenced with the first attempts at bone marrow transplantation in animal models during the 1950s. These pioneering studies paved the way for human bone marrow transplantation [15], a therapy now widely used in various blood disorders [16]. This new therapeutic strategy revealed the existence of stem cells that regenerated adult tissue [17]. Presently, regenerative medicine is a major focus of research not only to find therapies but also to understand basic biology and the pathogenesis of disease [18–20]. Although a number of ethical issues have arisen in stem cell research [21], recent advances in stem cell isolation and development have helped scientists to identify and culture specific cell types for regeneration of tissue in various disorders such as Parkinson's [22], Alzheimer's [23], or diseases of the heart [24], muscles [25], lung [26, 27], liver [28], and other organs [14].

Stem Cell Classification Based on Differentiation Potential

The ability to differentiate, one of the two main characteristics of stem cells, varies between stem cells depending on their origin and their derivation (fig. 1). All

Table 1. Stem cell classification according to their differentiation potential and origin

Differentiation potential	Origin
Totipotent or omnipotent	
Pluripotent	ESCs, iPSCs
Multipotent	Fetal stem cells
Oligopotent	Adult or somatic stem cells
Unipotent	

stem cells can be categorized according to their differentiation potential into 5 groups: totipotent or omnipotent, pluripotent, multipotent, oligopotent, and unipotent (table 1) [29].

Totipotent Cells

Totipotent or omnipotent cells are the most undifferentiated cells and are found in early development. A fertilized oocyte and the cells of the first two divisions are totipotent cells, as they differentiate into both embryonic and extraembryonic tissues, thereby forming the embryo and the placenta [30].

Pluripotent Cells

Pluripotent stem cells are able to differentiate into cells that arise from the 3 germ layers – ectoderm, endoderm, and mesoderm – from which all tissues and organs develop [31]. Pluripotent stem cells called ESCs were first derived from the inner cell mass of the blastocyst [32]. Recently, Takahashi and Yamanaka [33] generated pluripotent cells by reprogramming somatic cells. These cells are called induced pluripotent stem cells (iPSCs) and share similar characteristics with ESCs. Notably, there has been no pluripotent cell population isolated from the lung.

Multipotent Cells

Multipotent stem cells are found in most tissues and differentiate into cells from a single germ layer [34]. Mesenchymal stem cells (MSCs) are the most recognized multipotent cell. They can be derived from a variety of tissue including bone marrow, adipose tissue, bone, Wharton's jelly, umbilical cord blood, and peripheral blood [35]. MSCs are adherent to cell culture dishes and are characterized by specific surface cell markers. These cells can differentiate into mesoderm-derived tissue such as adipose tissue, bone, cartilage, and muscle [35–38]. Recently, MSCs were differentiated into neuronal tissue which is derived from the ectoderm. This is an example of transdifferentiation, i.e. when a cell from one germ layer (mesoderm) differentiates into neuronal tissue (ectoderm) [39]. Tissue-resident MSCs have been isolated from the lung; however, no other multipotent cell has been isolated to date [40].

Oligopotent Cells

Oligopotent stem cells are able to self-renew and form 2 or more lineages within a specific tissue; for example, the ocular surface of the pig, including the cornea, has been reported to contain oligopotent stem cells that generate individual colonies of corneal and conjunctival cells [41]. Hematopoietic stem cells are a typical example of oligopotent stem cells, as they can differentiate into both myeloid and lymphoid lineages [42]. In the lung, studies suggest that bronchoalveolar duct junction cells may give rise to bronchiolar epithelium and alveolar epithelium [43].

Unipotent Cells

Unipotent stem cells can self-renew and differentiate into only one specific cell type and form a single lineage such as muscle stem cells, giving rise to mature muscle cells and not any other cells [44–47]. In the lung, type II pneumocytes of the alveoli give rise to type I pneumocytes.

Stem Cell Classification Based on Origin

Stem cells can be grouped into 4 broad categories based on their origin: ESCs, fetal and adult stem cells, and iPSCs (table 1) [48, 49]. In general, ESCs and iPSCs are pluripotent, whereas adult stem cells are oligopotent or unipotent.

Embryonic Stem Cells

ESCs are pluripotent, derived from the inner cell mass of the blastocyst, a stage of the pre-implantation embryo, 5–6 days post-fertilization [32]. These cells can differentiate into tissue of the 3 primary germ layers but can also be maintained in an undifferentiated state for a prolonged period in culture [50]. The blastocyst has 2 layers of cells, i.e. the inner cell mass, which will form the embryo, and the outer cell mass, called trophoblasts, that will form the placenta. Cells from the inner cell layer are separated from trophoblasts and transferred to a culture dish under very specific conditions to develop ESC lines [51]. ESCs are identified by the presence of transcription factors such as Nanog and Oct4 [52, 53]. These factors maintain the stem

cells in an undifferentiated state, capable of self-renewal [53, 54]. ESCs that have been cultured in an undifferentiated state with no genetic abnormalities are propagated as an ESC line. These cells could be frozen and thawed for further cultures and experimentation [55]. Culture conditions are critical in maintaining ESCs in an undifferentiated state. A feeder layer of embryonic fibroblast cells (MEFCs) or medium that contains the anti-differentiation cytokine leukemia inhibitory factor (LIF) are used. Withdrawal of LIF from the medium or removal of the ESCs from the feeder layer results in the formation of 'embryoid bodies', in which all 3 germ layers (endoderm, mesoderm, and ectoderm) are present [56–60].

Adult Stem Cells

Adult stem cells are derived from adult tissue. Examples include MSCs as well as stem cells derived from placental tissue such as human amnion epithelial cells. These cells have been shown to be anti-inflammatory and augment repair of animal models of injury. They have limited differentiation capacity although these cells have been differentiated into tissue from different germ cell layers in vitro [61, 62].

Adult stem cells are of advantage since autologous cells do not raise issues of rejection or ethical controversies [21, 63]. Adult stem cells could be obtained from all tissues of the 3 germ layers as well as placenta. Several studies have demonstrated that transplantation of adult stem cells restores damaged organs in vivo, such as bone tissue repair and revascularization of the ischemic cardiac tissue via stem cell differentiation and generation of new specialized cells [64–66]. Other studies have shown that cultured adult stem cells secrete various molecular mediators with anti-apoptotic, immunomodulatory, angiogenic, and chemoattractant properties that promote repair [67, 68].

Tissue-Resident Stem Cells

The ability of some tissues and organs in the adult to renew and repair following injury is critically dependent on tissue-resident stem cells that generate tissue-specific, terminally differentiated cells [69]. Studies suggest that these cells originate during ontogenesis and remain in a quiescent state till local stimuli activate their proliferation, differentiation or migration [70, 71].

Tissue-resident stem cells reside in a 'stem cell niche' [72]. The stem cell niche is a microenvironment that controls the self-renewal and differentiation of these cells [73]. There is a growing body of evidence that stem cell function is critically influenced by extrinsic signals from the microenvironment; therefore, the niche plays a crucial role in stem cell homeostasis and tissue repair [74–76]. The majority of tissue-resident stem cells are dormant but are activated by specific signals during injury and repair [73]. This dormancy of tissue-resident stem cells is not well understood but is likely influenced by the niche environment. This property is critical to maintaining a population of cells that do not perform other functions apart from generating tissue-specific cells during repair [77]. The niche environment consists of various signals from extracellular matrix and soluble mediators that mediate cell signaling and gene expression [78–81], thereby regulating stem cell proliferation, migration, differentiation, or apoptosis [82, 83]. We still need to elucidate what the triggers are for stem cells to move from a self-renewal state and proliferation to differentiation and whether these signals are tissue specific.

Furthermore, the types of cell division that a stem cell undergoes determine the cells the type of cells generates. Symmetrical cell division by a stem cell results in identical daughter cells, which provides new cells to restitute damaged cells following injury [84, 85] (fig. 2). It is important to note that an uncontrolled increase in stem cell proliferation could lead to stem cell hyperplasia and/or carcinogenesis while a reduction in stem cells would impair organ repair; thus, balance in stem cell homeostasis is very important [86].

Asymmetric division occurs when a stem cell generates an identical daughter cell and a second differentiated daughter cell. This process allows for organ repair and regeneration while maintaining a population of stem cells [87–89].

Induced Pluripotent Stem Cells

iPSCs are produced from adult somatic cells that are genetically reprogrammed to an 'ESC-like state' [90]. Mouse iPSCs were reported for the first time by Takahashi and Yamanaka [33] in 2006 by transducing mouse fibroblasts with 4 genes encoding the following transcription factors: octamer-binding transcription factor 3/4 (OCT3/4), SRY-related high-mobility group box protein-2 (SOX2), the oncoprotein c-MYC, and Kruppel-like factor 4 (KLF4). A year later, in 2007, Yamanaka and collegues [91] described the generation of human iPSCs from adult human dermal fibroblasts with the same 4 factors: Oct3/4, Sox2, Klf4, and c-Myc. They demonstrated that these cells were similar to human ESCs in terms of morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity, and they could differentiate into cell types of the 3 germ layers in vitro [91]. iPSCs are cur-

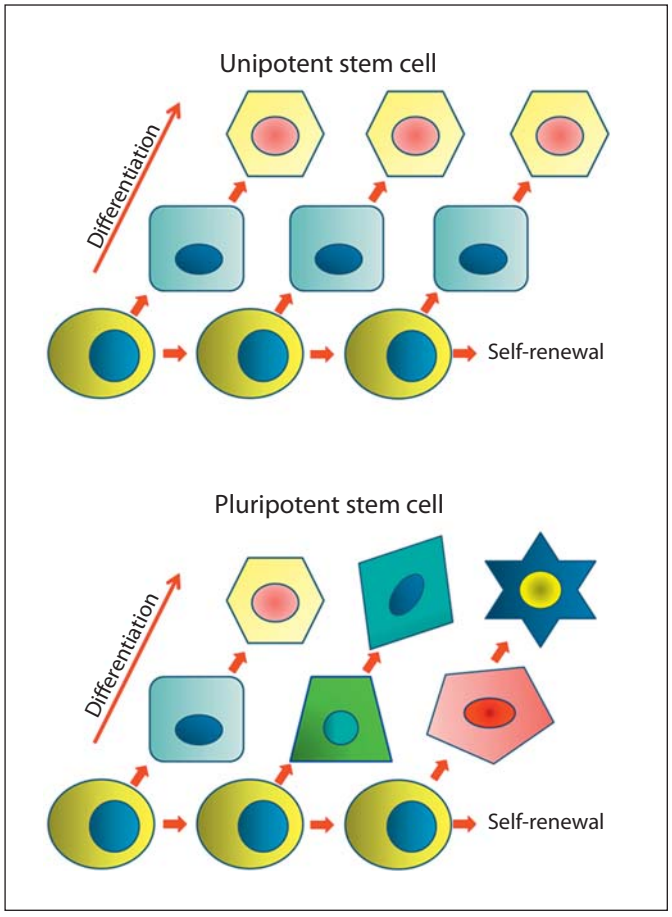

Fig. 2. Stem cells are characterized by their ability to self-renew and differentiate. Each individual daughter cell could go further in a symmetric division giving more stem cells or differentiate into one or more specialized cells, depending in the pluripotency of the stem cell, maintaining the population of tissue cells.

rently useful tools for drug development, modeling of diseases, and regenerative medicine, but although these cells express identical characteristics of pluripotent stem cells [92] it is not yet known if iPSCs and ESCs would significantly differ in clinical practice.

Retroviral vectors, used to introduce the reprogramming factors into adult cells, and oncogenes like c-Myc limit the use of iPSCs in a clinical study since the vectors used to introduce transcription factors to adult cells can cause cancers [93]. Researchers are currently investigating new methods to generate safe iPSCs without genomic manipulation [94]. New techniques have been described, using several types of mouse and human adult somatic cells. To avoid the use of oncoproteins c-MYC and KLF4 they have used one factor (OCT3/4 or KLF4) or they have substituted them with combinations of other factors [95, 96], including the use of non-retroviral vector approaches, such as chemical compounds, plasmids, adenovirus, and transposons [97–100].

Despite the safety issues, this innovative discovery has created a powerful tool to reprogram somatic adult cells 'sending them back' to earlier undifferentiated stages and generating iPSCs, thereby creating an identical match to the cell donor and thus avoiding issues of rejection.

Stem Cells in Clinical Practice and Regenerative Medicine

The contribution of stem cells in modern medicine is of paramount importance, both for their broad use in basic research and for the opportunities they give us to develop new therapeutic strategies in clinical practice [101]. Their characteristics make them valuable in a wide range of applications in biological and medical sciences [18]. For example, ESCs are excellent tools to understand human development and organogenesis. Stem cells such as iPSCs will be critical in the investigation of new and safe therapies.

In addition, stem cells may be able to replace damaged tissue or even regenerate organs [14]. iPSCs provide the opportunity to set up human models of diseases that would improve the understanding of the pathogenetic mechanisms of human diseases and would enable improvements in cell-based therapy for degenerative disorders [18].

Cell therapy has been investigated in almost every degenerative disorder. Promising results from preclinical studies and clinical trials have already been described in several diseases, such as diabetes mellitus [102, 103], chronic myeloid leukemia [104, 105], cirrhosis [106, 107], pulmonary fibrosis [108, 109], Crohn's disease [110, 111], heart failure [66, 112], and disorders of the nervous system [113–117], and the immunomodulatory effects of stem cells have found their utility in several conditions characterized by predominant inflammation [118, 119].

There are issues to consider in cell therapy and regenerative medicine. Immunorejection is still a consideration although MSCs and placental tissue as well as iPSCs circumvent the problem. The genetic stability of stem cells, especially iPSCs, remains to be elucidated. Genetic instability can give rise to tumor formation. Indeed the plasticity and self-renewal that characterize stem cells could lead to carcinogenesis in the host tissue [120], while spontaneously occurring teratomas and related tumors could develop from the use of ESCs or iPSCs in therapeu-

tic cell transplantation [121]. Finally, a number of ethical concerns have been raised mainly in the use of ESCs [122]. These include the ethical controversies of destroying an embryo in generating ESCs. This can now be potentially bypassed by iPSCs.

Conclusions

Stem cells are an important tool for understanding both the organogenesis and the continuous regenerative capacity of the body. They could be a model for the study of pathogenetic mechanisms and could assist researchers in understanding the pathophysiology of various diseases. They also offer the possibility of developing biological models for the study of new pharmacological agents. However, the most important potential of these cells is to replace damaged tissue and even regenerate organs. To date, a large number of research protocols, preclinical studies, and clinical trials have been published. Although, several clinical studies have already reported encouraging results for the development of new therapeutic strategies in cell-based medicine, there are a number of risks and obstacles. Despite this, there is ongoing research and development that gives us great optimism about regenerative medicine.

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