Stem Cells: what, how, and why?

Diane Carlisle
Department of Neurological Surgery, University of Pittsburgh, Pittsburgh, PA

Abstract

The use of stem cells clinically has a solid history and also broad undeveloped potential. As we learn more about these cells and their properties, our ability to harness their potential for future benefit will grow. However, there is also significant confusion surrounding discussions of stem cells, due to vague definitions regarding the types of stem cells and controversies that are inherent in the uses of some types of stem cells. This review discusses the types of stem cells, their advantages and disadvantages, and how they can be used clinically and for research. Specifically, the differences between various/common types of pluripotent stem cells is discussed. The utility of pluripotent stem cells compared to lineage restricted stem cells is also considered.

Keywords: Pluripotent stem cells, embryonic stem cells, tissue-specific stem cells, directed differentiation

Introduction

Although there are several kinds of stem cells, in scientific and lay conversations, often the term 'stem cells' is used without specificity, which can lead to confusion and misunderstanding. This review focuses on the major types of stem cells, including the types of pluripotent stem cells, as well as lineage-restricted stem cells, their advantages and disadvantages, and their uses in the clinic and in the laboratory.

Types of stem cells

Before discussion of the characteristics of stem cells, their advantages and disadvantages, and their clinical and research uses, it is important to clarify the types of stem cells. In general, there are 3 major types of stem cells: pluripotent stem cells, lineage restricted or somatic stem cells, and totipotent stem cells.

Pluripotent stem cells are the most versatile of stem cells. These cells are named as such because they have the potential to become any cell type that develops from an embryo. Indeed, the classic type of pluripotent stem cell is the embryonic stem cell. Other sources of pluripotent stem cells are somatic cell nuclear transfer stem cells and induced pluripotent stem cells. Beyond the ability to differentiate into any cell type in an embryo, pluripotent stem cell lines are immortal, with unlimited proliferation potential.

Embryonic stem cells

Embryonic stem cells are derived by dissecting and growing the cells that would become the embryo from a blastocyst stage embryo, before any differentiation into germ layers begins.[1-3] Human embryonic stem cells cells drew significant public attention upon publication in 1998,[3] because they are generated from human embryos after *in vitro* fertilization. However, embryonic stem cells generated from other animals were in existence for more than a decade before the generation of human embryonic stem cells by the Thomson group. Mouse embryonic stem cells were first published in 1981,[1] and other animal species followed.[3-6]

Somatic cell nuclear transfer stem cells

Somatic cell nuclear transfer can also be used to generate embryos for the production of pluripotent stem cells. These cells are generated by removing the haploid nucleus from an oocyte, transferring in the diploid nucleus from a somatic cell, followed by chemical activation of the oocyte.[7-11] Like embryonic stem cells, human somatic cell nuclear transfer cells are highly controversial. First, they are controversial because they can also be used for reproductive cloning, as first publicized by Dolly the sheep.[12] Second, much like human embryonic stem cells, the method involves the production of an early blastocyst from which the pluripotent stem cells are obtained.[13] Although human reproductive cloning has

not been achieved for ethical reasons, cloned nonhuman primates have been made[14] and thus it is theoretically possible, although inefficient.

Induced pluripotent stem cells

Induced pluripotent stem cells are somatic cells that are induced, through the re-expression of developmental genes, to become pluripotent. As with other types of pluripotent stem cells, this was first achieved in rodent cells,[15] followed by other animals including humans.[15] Because they do not require the use of an embryo or oocyte during their production, their generation and use is less controversial than other types of pluripotent stem cells.

Uses of pluripotent stem cells

Pluripotent stem cells are used for many purposes, some of which were mentioned above. The most well-known uses are the generation of genetically manipulated animals, the production of somatic cells for transplantation, and the production of somatic cells for research.

To generate genetically manipulated animals, pluripotent stem cells are isolated from embryos of the animal of interest, e.g. a mouse. Those cells are genetically manipulated, typically using CRISPR/Cas9 technology. The manipulated cells can then be injected into a blastocyst, which results in the generation of a chimeric animal. If any of the genetically altered cells contribute to the germline, then breeding the animal results in offspring that are genetically altered.[16] This is commonly used to generate research mice to model diseases in the laboratory.[16]

One of the most powerful uses of pluripotent stem cells is the ability to direct differentiation into any cell type in the body. In this context, pluripotent stem cells can be expanded into large batches and differentiated into the cell type of interest, which is then used for research purposes. This strategy is particularly important for cells that are postmitotic in adults, such as neurons. Proliferative cells can be obtained from a patient, grown in culture, and investigated; however, if the cell type of interest in the disease is postmitotic, obtaining a sufficient quantity of cells for experimental use is impossible. Examples include neurodegenerative diseases such as Alzheimer's Disease or amyotrophic lateral sclerosis (ALS), where the cell type of interest is the neuron. Live human neurons generally cannot be studied, except through the generation of neurons from pluripotent stem cells. Research in human neuroscience has accelerated exponentially since human pluripotent stem cells were discovered.

The same directed differentiation procedure can be used to generate somatic cells for transplantation, with the caveat that the pluripotent stem cell generation, growth, and subsequent differentiation are done under general manufacturing procedure conditions, which are the practices required to make clinical grade products. However, a significant caveat to the translation of this technology to the clinic is that it is extremely difficult to ensure that differentiation efficiency is 100% before transplantation. Less than perfect efficiency could result in the transfer of undifferentiated cells that result in teratoma formation in the patient.

Pluripotent stem cell characteristics

Pluripotent stem cells have incredible potential, but they are not easy to work with, for several reasons. They require a full knowledge of the starting cell source and ongoing quality control to be sure that they are and remain pluripotent.

Until this point, all the information above has applied to pluripotent stem cells from any source. This implies that embryonic stem cells, somatic cell nuclear transfer cells, and induced pluripotent stem cells are equivalent. However, there are data demonstrating that this is not true. When embryonic stem cells, somatic cell transfer stem cells, and induced pluripotent stem cells are examined side-by-side, there are phenotypic differences among them. Although the exact consequences of these differences are unclear, we cannot assume that all sources of pluripotent cells will perform equivalently in the generation of somatic cells, regardless of whether the final use is research or transplantation.

A key point that needs to be satisfied before using any pluripotent stem cell is to ensure that it is truly pluripotent. There are several levels of stringency that need to be met to prove pluripotency.[18] The

easiest is to look at molecular criteria. For example, cells can be analyzed for the expression of pluripotent genes and the absence of expression of somatic genes.[19] Similar protein profiles can also be examined.[19] However, molecular markers are not perfect and analysis of pluripotent functionality is always preferred. More rigorous is to look at the ability to differentiate into all 3 germ layers using *in vitro* differentiation protocols.[18] The most rigorous method is to truly test pluripotentiality by producing chimeric animals and demonstrating that the pluripotent stem cell contributed to each germ layer plus germ cells.[2]

Immortality is a key characteristic of pluripotent stem cells; however, this does not imply stability in culture. In fact, karyotypic instability was recognized as a concern early in the use of the cells.[20] Improved culture methods have decreased the occurrence of large chromosomal aberrations, but careful analysis demonstrates that with passage, pluripotent stem cell lines change over time, with numerous chromosomal differences, most less than 100 kilobases of DNA in size.[21] Furthermore, these changes can alter the ability to differentiate into certain somatic cell types.[21] Thus, careful and constant quality control of pluripotent stem cells is required.

Advantages and disadvantages

In summary, pluripotent stem cells are a powerful tool for medicine and research. Their potency can be harnessed to generate somatic cells for research and for the clinic, as well as to advance our understanding of developmental biology. However, they have some key disadvantages. Most importantly, anything less than completely efficient differentiation could result in development of subsequent cancer in a patient after transplantation.[22] Other disadvantages for clinical use include that differentiated pluripotent stem cells for transplantation are not immune matched to recipients and would be rejected without immune system suppression in the transplant recipient.

Lineage restricted stem cells

The other major stem cell type is the lineage restricted stem cell, also referred to as somatic stem cell, tissue stem cell, or multipotent stem cell. These cells are obtained from mature tissues and are the natural source of cell regeneration in tissues during healing or other physiological cell expansions. These cells are typically restricted to differentiation into cells within their own germ layer, or even within their own tissue.

Advantages and disadvantages of lineage restricted stem cells:

The major disadvantage of lineage restricted stem cells is that they are not pluripotent. They can become some cell types in the body, but not all. However, for clinical purposes, they have some significant advantages. First, since they are not pluripotent, they will not form teratomas upon transplantation. Second, they have a long history of clinical use, demonstrating safety and efficacy. Some examples include hematopoietic stem cells, mesenchymal stem cells, and tissue-specific stem cells.

Hematopoietic stem cells have been in use in the clinical setting for decades, particularly in the treatment of cancer. For this purpose, a person's natural hematopoietic stem cells are destroyed and replaced using donated bone marrow, a major source of hematopoietic stem cells.[23] Mesenchymal stem cells are highly versatile, although their differentiation capabilities are controversial. Initial studies demonstrated increased proliferation of mature cell types in many organs, including from other germ layers, after the injection of mesenchymal stem cells, leading to speculation that these cells could be pluripotent.[24] Other studies that traced the source of increased cell proliferation after mesenchymal stem cell transplantation demonstrated that the new cells generated do not come from the transplant, but from the tissue, suggesting that mesenchymal stem cells are powerful endocrine modulators that activate endogenous tissue stem cells without directly contributing to the tissue.[25]

Other tissue specific stem cells are known to exist through their properties: the ability to proliferate to maintain the tissue stem cell reservoir while at the same time providing cells for differentiation into mature cell types. An example is spermatogonial stem cells, which have been

characterized by their properties in the testes, although they are difficult to specifically isolate for study.[26,27]

Totipotent stem cells

Totipotent stem cells are the least understood and also the least studied, but they are known to exist by their function. These cells distinguish themselves from pluripotent stem cells because they can become any cell type in the body (as pluripotent cells can) as well as the placenta (pluripotent cells cannot).[2] These cells exist in the very earliest stages of development before the blastocyst forms; however, because they have not been isolated or grown in culture, relatively little is known about their biology.[28]

Cell fate specification

Given that the uses of stem cells that generate the most interest are for regeneration of somatic cells for clinical and research uses, understanding the tenets of cell fate specification are key to utility of stem cells. Two approaches to cell fate specification are direct reprogramming of somatic cells and use of developmental biology signaling programs for specification.

In direct reprogramming, somatic cells of 1 type are treated with cocktails (genetic, protein, or small molecules) to directly turn 1 cell type into another. This approach skips the intermediate step of creating a stem cell, which makes it relatively fast. The biological methods for this are still being refined, but it has been accomplished for some applications, such as turning fibroblasts into neurons.[29] However, the methods are experimental, and it has yet to be done under GMP conditions. Additionally, because it eliminates the intermediate step of creating an immortal stem cell that allows for rapid expansion, it is not ideal when starting material is limited.

The more established approach is to use the known signaling pathways of developmental biology to specify cell fate. This technique can be used starting from pluripotent stem cells, by initiating primitive streak specification, or it can be used on lineage restricted stem cells by initiating maturation of tissue specific progenitors into mature cell types. Pluripotent stem cells can be guided through specification to endoderm, mesoderm, or ectoderm then to mature cells within each germ layer using developmental growth factors or with small molecule analogues that evoke the equivalent cellular signaling response.[30] Bioengineered matrices can also contribute to cellular signaling to direct differentiation.

Confirmatory assays

Similar to the confirmation of pluripotency, there are multiple tests of varying rigor for verifying the effectiveness of directed differentiation. The least rigorous is analysis of the gene expression profile, i.e. mRNA from differentiated cells can be analyzed to determine if the expression profile matches that of a somatic cell of the same type that is obtained directly from the tissue of interest. Similarly, but more rigorous is the analysis of the protein profile, demonstrating that the cell not only generated appropriate mRNA, but translated that mRNA to the appropriate protein profile. However, as previously mentioned, functional testing is the gold standard for confirmation of cell specification, demonstrating that the cell functions as and, ultimately, can integrate into the desired tissue.[30]

Conclusion

Stem cells are fascinating with a solid history of providing clinical benefit and broad applicability for new uses as our understanding of how to harness their potential grows. The applications continue to expand as we learn how to tap into the potential of all that stem cells have to offer.

Conflict of interest

There are no conflicts of interest to declare.

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