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Stem Cells: Outstanding Potential and Outstanding Questions.

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Introduction

Stem Cells

Stem cells are unspecialized cells found in multi-cellular organisms that have the capacity to self-renew, proliferate for long periods in culture and which can be induced to become specialized cell types. Despite a legion of research publications in recent years, stem cell research has been ongoing for over half a century. As now, this research has often been dogged by controversy and has even been ignored by mainstream science. Initial reports, as far back as the 1960s, of adult neurogenesis in the brain[1,2] were largely ignored, as they contradicted the then existing dogma, stemming from Santiago Ramon y Cajal's (1852-1934) original hypothesis, that no new neurons were formed in the adult brain.

Pioneering work on mouse bone marrow transplantation,[3] however, did indicate the presence of putative stem cells in bone marrow, kick starting today's rapidly evolving field of regenerative medicine.

What are the Properties of Stem Cells?

Stem cells can be isolated from both the embryo and adult and display three general properties which distinguishes them from other cells in the body:

(i) They are unspecialized (i.e. they do not have an overt, specific developmental function).

(ii) Unlike other primary cell types, which senesce, stem cells are able to self renew and can do so for extensive periods of time while retaining an unspecialized phenotype.

(iii) They have the potential to give rise to specialized cell types with specific functions (e.g. neurones, insulin producing cells, liver cells). This capacity is termed 'potency' and can be sub-divided into four categories:

- Totipotency: capable of producing all embryonic and post embryonic cells, tissues and organs. (e.g. a fertilized egg).
- Pluripotency: capable of producing cells from any of the three germ layers (ectoderm, endoderm, mesoderm).
- Multipotency: capable of only producing cells of a closely related family of cells (e.g. hematopoietic stem cells).
- Unipotency: can self renew, but can only produce a single cell type upon differentiation, (e.g. muscle stem cells).

Embryonic Stem Cells

Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst of fertilized embryos. Unambiguous characterization of a cell as an ESC is complex and no defined standard test to characterize them exists. A number of features are used for more routine definition.

(i) Maintenance of undifferentiated growth in culture and the expression of markers called Oct 4, nanog and Sox-2, which are required for maintenance of self-renewal characteristics, in combination with a variety of cell surface markers.[4] Human ES cells are also routinely identified using the markers SSEA3, SSEA4, Tra-1-60 and Tra-1-81.[5]

(ii) Possession of a stable karyotype, despite extended growth in culture.

(iii) The ability to undergo multiple freeze/thaw cycling and retain viability.

(iv) The capacity to demonstrate pluritpotency in vitro and to form teratomas, following transplantation into an immunosuppressed mouse.

Adult Stem Cells

Adult Stem cells (ASCs) are undifferentiated cells found in a developed tissue or organ.

Their origin remains unproven and it has not been determined if they are residual ESCs. ASC numbers are thought to be limited and their degree of plasticity remains to be determined. They are, however, unspecialized, self renew and demonstrate multipotency; being able to produce the differentiated cells and tissues of the organ in which they reside, possibly as part of a tissue repair and maintenance system. It is unknown if ASCs are homogeneous in nature, or are distinct to their tissue and organ of isolation. The molecular signals controlling their biology also remains unclear. Typically, ASCs are described by their tissue origin (neuronal stem cell, mesenchymal stem cell).[6,7]

Despite all these uncertainties, their use in transplantation is widely recognized. ASCs from bone marrow have been used in transplants for 40 years. As ASCs can be obtained from the patient, they have significant therapeutic potential, as their use evades the side-effects of any immuno-suppression to prevent rejection, including a significantly enhanced risk of cancer. However, limited plasticity (termed ‘restricted lineage’) means that unlike ESCs, they might be able to make fewer desired cell types for transplantation. The term trans-differentiation has been applied to describe ASCs with the capacity to cross the lineage divide and produce differentiated cell types from more than one lineage, though not all ACS have this potential.[8] This situation is typically compounded by an inability to get sufficiently large numbers of cells required for therapeutic use.[9]

Limited potency/plasticity in ASCs, in contrast to ESCs, can also be considered an improved safety factor, as uncontrolled differentiation, often associated with ESC derived teratomas, would be significantly reduced and the risk of cancer post transplant thus decreased. This is pertinent to the cancer stem cell hypothesis, which has emerged in recent years. This espouses that ASCs, apart from providing a maintenance and repair system for adult tissues and organs, act as a reservoir for genetic and epigenetic changes that will predispose to the development of cancer at a later stage, as these cells will persist in tumors, forming a cellular pool that may give rise to relapse and metastasis.[10]

Stem Cell Therapies

Stem cell research holds immense promise to treat previously untreatable conditions, such as Parkinson’s disease, macular degeneration, ALS and spinal cord injuries, while it offers hope of novel therapies for treating diseases such as diabetes. Social and scientific uncertainty, however, still dogs the field. This either arises around moral or ethical issues associated with the use of human ESCs, or through technical difficulties. These difficulties include controlling the directed differentiation of ESCs, the prevention of teratoma formation post transplant, expanding adult stem cell populations to derive enough cells for therapeutic efficacy, and identifying cryptic damage pre-transplant, that may affect post transplant safety and efficacy.[11] All these issues remain to be solved.

A number of ASC based treatments do, however, already exist, including bone marrow transplantation used in leukemia treatment.[12] The use of autologous cell transplants remains a ‘holy grail’ for much research, despite a growing movement to explore stem cell biology purely for scientific understanding. As yet, no bona fide treatment for a major disease has emerged using stem cells, despite numerous claims not subject to peer review. Stem cell treatments are, however, in clinical trials in Japan, Korea, Australia, USA and the UK. Interestingly, these include such procedures as breast augmentation. This work remains unpublished, but has received much press attention (http://news.bbc.co.uk/2/hi/health/6354451.stm).

Are ASCs Needed for Organ Repair?

A number of recent developments indicate that potential stem cell therapies may need modulating and that the biology of tissue repair is less well understood than previously thought.

A seminal paper from Dor et al[13] has indicated that the only source of beta cell regeneration, even after injury, is the existing beta cells, and that there is no murine stem cell involvement in the generation of the mouse pancreas following damage. This work has been extended by Nir et al[14] with lineage tracing experiments in transgenic animals, demonstrating that endogenous beta cells are involved in pancreas regeneration following injury. Significantly, treatment with immuno-suppressants, such as Sirolimus and Tacrolimus routinely used in the Edmonton protocol for human islet transplantation, inhibited beta cell regeneration and prevented the normalization of glucose homeostasis in diabetic animals. These observations have important implications for any regenerative medicine approach using stem cells to treat type 1 diabetes. They suggest that any therapeutic regime used to treat auto immunity as part of such an intervention, must be compatible with islet regeneration.

Other Cell Types for Cellular Therapies

A novel cell population has been isolated from both adult rat and human pancreatic ductal tissue termed Pathfinder cells[15] on the basis that they appear to navigate a path towards sites of damage in vivo. Direct intravenous injection of rat or human Pathfinder cells into streptozotocin induced diabetic mice normalizes blood glucose levels, primarily by stimulating host tissue to regenerate. Crucially, the insulin produced by these treated animals is principally mouse in origin and is of both type I (embryonic) and II (adult). These observations demonstrate the feasibility of regenerating damaged tissue and enhance our understanding of the mechanisms relating to tissue repair. Such observations are also in keeping with those of Dor et al[13] and Nir et al[14] and suggest a means for novel therapeutic intervention in diabetes without making differentiated cells for transplantation ex vivo. This cell population has also been used to derive bi-potential endodermal progenitor cells, capable of making functional islets and hepatocytes in vitro,[16] which holds further promise for clinical transplantation.

A number of other strategies that evade the use of a pre-existing stem cell population have emerged. These involve genetically manipulating adult cells to express a number of transcription factors normally required for maintaining stem cells in an undifferentiated state. Meissner et al have demonstrated the in vitro reprogramming of somatic cells into a pluripotent embryonic stem cell-like state, using expression of Oct4, Sox2, c-myc and Klf4 in combination.[17] These 'induced pluripotent stem' (iPS) cells were isolated through stringent selection for drug resistance to a neomycin reporter inserted into the endogenous Oct4 or Nanog loci. This achievement mirrors that of Okita et al[18] who derived pluripotent stem cells from mouse fibroblasts using induced expression of Oct3/4, Sox2, c-Myc and Klf4, with subsequent selection for Nanog expression. These derived cells show many similarities to native ESCs in morphology, proliferation and teratoma formation. The observation of tumour formation on chimaeras derived using these cells, however, currently precludes their use in transplantation, despite the avenue they open for evading the constraints around human ESC derivation.

The Future

The use of cellular therapies to mitigate previously intractable conditions is exciting. Translation of any of these in the near future is still hazardous. Our basic understanding of tissue repair is being challenged, while the underlying biology of ESCs and ASCs is incompletely understood.

With the exception of bone marrow transplantation, obtaining enough of a given cell type free from the risk of cancer formation post transplant, is still a problem. As yet, getting a clinical translation of any cellular therapy is accompanied by a legion of unanswered questions.

How can one differentiate a given SC into the desired cell type(s) with sufficient purity and in sufficient numbers? How long will these cells survive in the recipient after transplant? Will they integrate into the surrounding tissue after transplant and function appropriately for the remainder of the recipient's life? Will simply replacing tissue address any underlying pathology? What immune issues need to be dealt with? The latter is pertinent, given the findings on pancreas regeneration.[14]

Despite this, stem cell research offers great potential and hope for treating disease and understanding basic biological processes involved in development and in tissue homeostasis.

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References

1. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats, J Comp Neurol 1965; 124: 319–335.

2. Altman J, Das GD. Postnatal neurogenesis in the guinea-pig, Nature 1967; 214: 1098–1101.

3. Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 1963; 197: 452-4.

4. Boyer LA, Lee TI, Cole MF, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 2005; 122(6): 947-56.

5. Adewumi O, Aflatoonian B, Ahrlund-Richter L, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol 2007; 25(7): 803-16.

6 Barrilleaux B, Phinney DG, Prockop DJ, O'Connor KC. Review: ex vivo engineering of living tissues with adult stem cells. Tissue Eng 2006; 12(11): 3007-19.

7 Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ. Res. 2007; 100(9): 1249-60

8. Filip S, English D and Mokry. Issues in stem cell plasticity. J Cell Mol Med 2004; 8(4): 572-577.

9. Ratajczak MZ, Machalinski B, Wojakowski W, et al. A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues. Leukemia 2007 21 (5): 860-7.

10. Bonnet D and Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997 3: 730-7.

11. Shiels PG and Jardine AG. Dolly, no longer the exception: telomeres and implications for transplantation. Cloning Stem Cells. 2003; 5(2): 157-60.

12 Gahrton G, Björkstrand B. Progress in haematopoietic stem cell transplantation for multiple myeloma. J Intern Med 2000; 248(3): 185-201.

13 Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004; 429(6987): 41-6.

14 Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. J Clin Invest 2007; 117(9): 2553-61.

15 Shiels PG. The use of adult stem cells to mitigate the effects of diabetes Horizons in Medicine 2005; 17: 241-249.

16 Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. Nat Biotechnol 2007; 10: 1177-81.

17 Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448(7151): 313-7.