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What Are Stem Cells?-Review Paper

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Abstract: Modern medicine has moved by leaps and bounds throughout recent years to literally make science fiction part of our day-to-day reality. Undoubtedly one of the biggest breakthroughs was the discovery of stem cells, which was followed up in a breathtaking fashion by report after report of their promise, so much so that an almost miraculous scenario seems to be infolding in front of the world's eyes. Stem cells are classified mainly by their source into three basic types: Embryonic, umbilical and adult stem cells. Each differ in their plasticity and could thus potentially be used for different medical purposes. However, for this to be possible, lab techniques for collecting, culturing and perhaps even transdifferenciating different stem cells need to be studied and outlined, ideally while having a good background of the mechanisms underlying the processes of differentiation and transdifferentaition, with all the various combinations of growth factor cocktails needed for the cell culture to be viable commercially and the individual and additive effect of each ingredient on the resultant culture.

Key words: Stem cells, classification, differentiation, embryonic stem cells, umbilical cord blood

INTRODUCTION

Stem cell research was launched into the limelight recently by James Thomson, a scientist at the University of Wisconsin in Madison, who successfully removed cells from spare embryos at fertility clinics and grew them in the laboratory, establishing the world's first human Embryonic Stem cell (ES cell) line. In the same year, Dr. Gearhart isolated cells from 2-4-month-old foetuses (obtained from elective abortions with infromed consent from donors who had independently decided to terminate their preganacy). Gearhart's team established a different line of cell cultures, by collecting cells from the gonadal ridge from 9-week-old foetuses. Embryonic Germ (EG) cells from each foetus were used to establish a separate culture line.

These discoveries can be considered to be the staring point of what eventually became a plethora of evidence that has emerged since then, suggesting that these ES cells are capable of becoming almost any of the specialised cells in the body and therefore have the potential to generate replacement cells for a broad array of tissues and organs such as the heart, liver, pancreas and nervous system. Furthermore, other kinds of stem cells have been identified and it was found that the variety of cells that can be produced from each kind of stem cells differs under different laboratory conditions.

When taking in account all available evidence, the possibilities for stem research seem truly endless and yet unpredictable. The emerging field of stem cell research crosses many disciplinary boundaries, including reproductive biology, embryology, cell biology, molecular biology, endocrinology, immunology, foetal medicine, transplantation medicine and surgery.

MATERIALS AND METHODS

Classification of stem cells in terms of their plasticity. Methods of culturing each type of stem cells in the lab: Stem cells are defined as clonogenic, self-renewing progenitor cells that have the ability to divide for an indefinite period and can give rise to one or more differentiated cell types (Vejjajiva, 2002), which ability is known as developmental plasticity.

Stem cells are classified into different types and the different types exhibit a different degree of plasticity. A fertilized egg (zygote) is able to give rise to all cells of the body and has the highest degree of developmental plasticity. It is thus said to be totipotent. Blastomeres are initially totipotent but their level of plasticity decreases quickly. In fact, blastomeres from a five-day human embryo (consisting of about 200 cells) are said to be pluripotent because they can only give rise to a limited range of cell types. As the embryo continues to develop,

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Table 1: A comparison of the different types of stem cells in terms of their plasticity

Level of plasticity	Type of cells	Cell types obtainable
Totipotent	Zygote	All cell types-develops into embryo
Pluripotent	a)Embryonic Stem cells (ES cells)	All cell types-does not develop into an embryo
Pluripotent	b)Umbilical Stem cells	Same as ES cells
Pluripotent to multipotent	Adult Stem cells	
	a)Haematopoeitic stem cells	Red, white blood cells, immune cells, others? (refer in this study)
	b)Mesenchymal stem cells	lipocytes, cartilage, bone, tendon and ligaments, myocytes, skin cells, neurons
	c)Others (methods of collection still not very well-characterized)	Neurons, hepatocytes, pneumocytes…
	d)Transdifferentiation (occurs under special conditions)	Crossing of germ layers

individual cells become multipotent- they are able to give rise to only a few cell types. Hence it is now known that mesenchymal stem cells, which were the first non-haemotopietic progenitors to be isolated from the bone marrow and are now extensively characterised, can differentiate into osteocytes, tenocytes, adiposities and smooth muscle cells, in addition to their ability to support haematopoiesis (Koc and Lazarus, 2001). Finally, cells become specialized. This means that by definition, they either give rise only to cells of their own kind, or else lose the ability to reproduce completely (as in a neuron, for example).

Stem cells are currently classified according to their origin, which may be from embryo, umbilical cords or adult tissue. Cord and ES cells are generally said to be equivalent to pluripotent blastomeres, while adult stem cells (which are also found in children as well as in adults) possess a wider range of plasticity ranging from pluripotent to multipotent (Table 1).

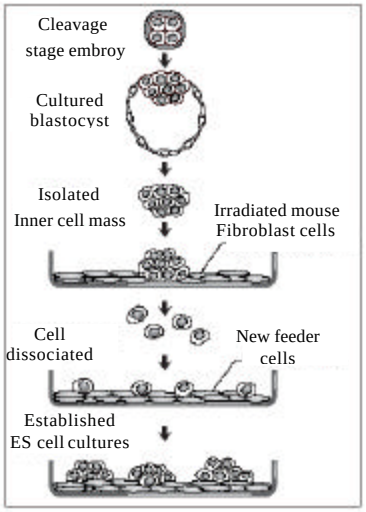

Fig. 1: Derivation of hES cells (Thomson *et al*., 1998)

Embryonic Stem cells (ES cells): ES cells are derived from the Inner Cell Mass (ICM) of the blastocyst and are capable of generating all differentiated cells types in the body, as represented the three germ layers. However, it is worth noting that since the association between the ICM and the trophoblast is disrupted (when ES cells are placed in culture), the ES cells can't develop into an embryo. Hence they are said to be pluripotent as opposed to totipotent. In culture, ES cells are immortal, proliferating indefinitely while retaining an embryonic phenotype.

Methods of collection of ES cells are continually being discovered. Two protocols are described here: One was used by Thomson (1998), the other by Klimanskaya *et al*. (2006) as a method by which hES cells were derived from single blastomeres without damaging their embryonic source (Klimanskaya *et al*., 2006).

The protocols utilised by Thomson *et al*. (1998), involved the isolation of stem cells from the ICM of human blastocysts, obtained from an IVF clinic. After culturing to the blastocyst stage, the ICMs were removed and cultured on irradiated mouse embryonic fibroblast feeder layers. After about two weeks in culture, the hES cells were dissociated and replated on fresh feeder plates in order to induce the formation of embryoid bodies (aggregations of cells into small clumps). The hES cells were after maintained for months in the undifferentiated state by serially sub-culturing cells from the edge of the embryoid bodies onto fresh, irradiated mouse embryonic fibroblast feeder layers (Fig. 1).

Thomson's team began with 35 embryos, of which 14 reached the blastocyst stage. The ICM was isolated from these embryos and used to establish 5 human embryonic cell lines: H7, H9, H1, H13 and H14, the former 2 being female and the latter three being male. The ES cell lines were continuously cultured for several months and expressed high levels of telomerase activity, characteristic of cells with high reproductive live span and expressed cell surface markers characteristic of ES cells, including Stage-Specific Embryonic Antigen m (SSEA)-3, SSEA-4, T-Cell Receptor Alpha (TRA)-1-1-60, TRA-1-81 and alkaline phosphatase.

The 5 original cell lines continue to divide without differentiating for 6 months and were able to form teratomas in mice when grown into Severe Combined

Immunodeficient (SCID) mice. Histology of the tumours revealed differentiated cells derived from all three embryonic germ layers including gut epithelium (endoderm), cartilage, bone, smooth and striated muscle (mesoderm) and neural epithelium, embryonic ganglia and stratified squamous epithelium (ectoderm) a result consistent with pluripotency. Cell line H9 went on to proliferate for more than two years and is now being used by research groups around the world. All cell lines continued to maintain a normal karyotype. This observation was crucial because if the cells grew well in culture but suffered chromosomal damage (broken chromosomes or daughter cells that received and incorrect number of chromosomes), they would have been useless for medical therapy.

In a more recent development, Klimanskaya *et al*. (2006) published a series of 10 separate experiments demonstrating that hES cells can be derived from single blastomeres (as opposed to removing the whole ICM). The results consisted of nineteen ES-cell-like outgrowths and two stable hES cell lines. The latter hES cell lines maintained undifferentiated proliferation for more than 8 months and showed normal karyotype and expression of markers of pluripotency, including Oct-4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, nanog and alkaline phosphatase. It was noted that the cells retained the potential to form derivatives of all three embryonic germ layers both *in vitro* and in teratomas. These lines displayed undifferentiated proliferation for more than 8 months and appeared to be comparable in all aspects to lines derived with conventional techniques. Hence, it follows that the cells obtained in this experiment are comparable to those obtained by Thomson *et al*. (1998) as regards plasticity. Furthermore, numerous reports suggest that neither the survival rate nor the subsequent development and chances of implantation differ between intact human embryos at the blastocyst stage and those following blastomere biopsy for Preimplantation Genetic Diagnosis (PDG).

The ability to create new stem cell lines and therapies without destroying embryos would address the ethical concerns of many and allow the generation of matched tissue for children and siblings born from transferred PGD embryos.

Umbilical Cord Stem (UCS) cells: The collection of UCS cells involves the insertion of a needle into the umbilical cord, in order to allow the blood to drain into a specially designed collection bag. Once at the storage lab, the sample is processed to concentrate the stem cells and the cells are then frozen in liquid nitrogen. At this very low temperature the cells are completely stable and can be stored for many years in case they may be needed. In case of need, the stem cells are released to the physician treating the person at precisely the time they are needed. Hence there is no need to search for a donor. Furthermore, the collection procedure has no effect whatsoever on the baby or the mother.

UCS cells have been shown to have an excellent developmental plasticity, even equalling that of ES cells. Other advantages include a significantly reduced rate of Graft-Versus-Host Disease (GVHD) and greater immunological naivete compared to bone marrow (Laughlin *et al*., 2001); a higher acceptance rate for cord blood for HLA matched siblings (Rocha *et al*., 2000) as well as for HLA mismatched recipients (Long *et al*., 2003) and a significantly reduced chance of transplant patients receiving umbilical cord blood transplants from a relative, to develop GVHD (Gluckman *et al*., 1997) although other factors may have to be considered (Madrigal *et al*., 1997). Furthermore, the rapid availability of cord blood for transplantation is a particular advantage for those patients requiring urgent transplantation (Barker *et al*., 2002; Sanz and Sanz, 2002).

The first umbilical cord blood transplant was performed in 1970 on a 16-year-old boy with acute lymphoblastic leukaemia (Ende and Ende, 1972). The boy received cord blood units from 8 different unrelated donors, untested for any HLA compatibility, over 18 days. Only 1 unit engrafted, but the patient remained in complete remission with maintenance chemotherapy until his last follow-up appointment at 9 months.

Since then, umbilical cord blood storage and transplantation is now widely accepted by the medical and scientific profession as a valid and immensely useful procedure. After expansion in culture, cord blood cells are able to express the phenotype of multiple lineages, including for example adhesion molecules CD13+, CD29+ and CD34+. Cord blood may prove to be a new source of cells for cellular therapeutics for stromal, bone and potentially, neural repair (Goodwin *et al*., 2001). More than 3,000 patients with at least 45 different pathologies have now been treated using cord blood cells (Fasouliotis and Schenker, 2000; Barker and Wagner, 2002). Diseases which have been cured by UCS cells include Fanconi anemia (Croop *et al*., 2001), Chronic Myelogenous Leukemia (Wadhwa *et al*., 2002), breast cancer (Paquette *et al*., 2000) and many others. In 2004, the first case of autologous cord blood transplantation was reported, in relation to the treatment of severe aplastic anaemia secondary to liver transplantation (Fruchtman *et al*., 2004).

UCS cells, collected from millions of infants, could in time, become an extremely valuable tissue bank that could

be used to treat many diseases without prompting immune rejection. Additionally it does not raise ethical problems associated with ES cells.

Adult stem cells: Adult stem cells (also known as somatic stem cells, derived from Greek GTµ"J46óH, of the body) are defined as undifferentiated cells found throughout the body that divide to replenish dying cells and regenerate damaged tissues. The term adult stem cells are somewhat misleading as they can be found in both children and adults.

Adult Stem cells were first cultured and transplanted in lab animals by Alexander Friedenstein, Maureen Owen and their coworkers. Transplantation was performed in either in closed systems (diffusion chambers), or open systems (under the renal capsule, or subcutaneously) to characterize cells that compose the physical stroma of bonemarrow (Friedenstein *et al*., 1966, 1970; Owen, 1988). Since there is very little extracellular matrix present in marrow, gentle mechanical disruption (usually by pipetting and passage through syringe needles of decreasing sizes) can readily dissociate stroma and haematopoietic cells into a single-cell suspension. These cells are then plated at low density, resulting in the rapid adhesion of Bone Marrow Stromal Cells (BMSCs), which can then be easily separated from the nonadherent haematopoietic cells by repeated washing. With appropriate culture conditions, distinct colonies are formed, each of which is derived from a single precursor cell, the CFU-F.

As regards plasticity, tissue-specific adult stem cells can generate a whole spectrum of cell types of other tissues, even crossing germ layers (Filip *et al*., 2004), under special conditions, which phenomenon is referred to as stem cell transdifferentiation. It was noted that transdifferentiation can be induced by modifying the growth medium when stem cells are cultured *in vitro* or by transplanting them to an organ of the body different from the one they were originally isolated from.

A novel use for adult stem cells has been reported recently by Voltarelli and his team, who transplanted haematopoietic stem cells in 15 newly diagnosed Type 1 diabetes mellitus patients. Of these, 14 became insulin-free (for a variable length of time), leading the scientists to infer that autologous non-myeloablative haematopoietic stem cell transplantation increased $ cell function in all but one patient. Furthermore, it was found that prolonged insulin independence resulted in the majority of patients (Voltarelli *et al*., 2007).

Despite their enormous potential, protocols for expanding the numbers of adult stem cells in cell culture have not yet been worked out, primarily because of an inability to reliably identify and isolate these rare cells from the heterogeneous populations in which they typically reside and perhaps more importantly, to grow populations of cells that retain stem cell properties over multiple cell division. This latter observation is crucial, as large numbers of cells are needed for stem cell replacement therapies. In fact, research today tries to analyse the optimum conditions for culturing of adult stem cells by altering culture microenvironment, although a lot needs to be done to determine the mechanism(s) which allows this to happen. Hence, the developmental potential of adult somatic stem cells are continually being reassessed and redefined, in this fast-moving science (Magli *et al*., 2000).

Two examples of adult stem cells which are well-characterised today are haematopoietic and mesenchymal stem cells:

- Haematopoietic stem cells were the first stem cells to be used successfully in therapies and have been used for decades to treat leukaemia and other blood disorders (Bush *et al*., 2000).
- More recently, their use in treatment of breast cancer (Damon *et al*., 2000) and coronary artery diseases (Strauer *et al*., 2001) has also been explored. While this and other results are encouraging, it is still not clear whether they could be used on a clinical scale to restore tissues and organs other than blood and the immune system. In fact haematopoietic stem cells' potential to produce cell types other than blood cells has become the subject of intense scientific controversy and will perhaps continue to dominate the scientific community for years to come.

Mesenchymal cells, also found in the bone marrow, can form a variety of cell in the laboratory, including lipocytes, cartilage, bone, tendon and ligaments, myocytes, skin cells and even neurons.

An advantage of using adult stem cells is that the patient's own cells could be expanded in culture and then reintroduced into the patient, meaning that the cells would not be rejected by the immune system. This represents a significant advantage as immune rejection is a difficult problem that can only be circumvented with immunosuppressive drugs.

CONCLUSION

The promise of stem cells lies in the fact that unlike skin, liver, bone marrow and other tissues in the body, some organs do not retain the ability to regenerate. Hence, scientists are now learning how to coax stem cells

into producing the latter kind of cells in the hope of being able to treat diseases which were hitherto only managable and not curable. Such disease include neurodegenerative diseases like Parkinson's Disease (PD) and Alzheimer's Disease (AD), tumours such as luekemia and brain tumours, anaemias, autoimmune disease such as Multiple Sclerosis and Systemic Lupus Erythomatosus (SLE) and cardiovascular diseases to mention just a few examples.

It is important to remember however, that this platonic scenario is only possible at the cost of doing away with some of the values which form the very basis of our society. Hence it is up to us, today's students and tomorrow's researchers, to contribute and shape our future by striking a balance between old and new, permanence and change, tradition and innovation. We can only achieve this by understanding the scientific, legal, social, ethical and economic aspect which influence this crucial area of research.

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