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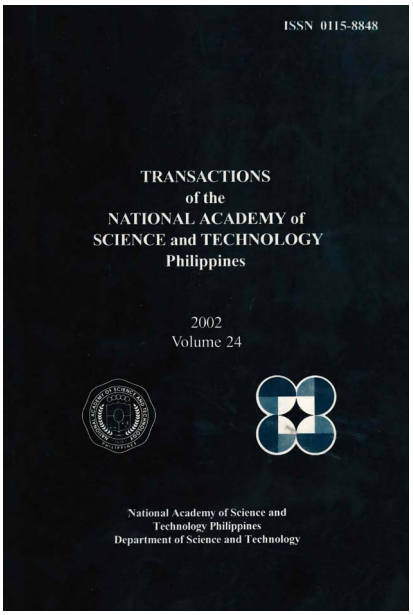

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THE PROMISE OF STEM CELL-BASED THERAPIES: AN UPDATE

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ABSTRACT

Stem cell-based therapies are based on the unique ability of stem cells to self-renew and to generate a number of specialized cell types. Sources of stem cells include the embryo, fetal tissues, umbilical cord blood and the adult. Stem cells from adult bone marrow are the most studied and most frequently used. Hematopoietic stem cells from adult bone marrow are used in the treatment of leukemias and lympohomas, in restoring blood and immune cells destroyed by chemotherapy and in the treatment of autoimmune diseases. On the other hand, embryonic stem cells are totipotent and have the potential to develop to more than 200 types of cells needed for all tissues and organs in the body. While significant progress has been made in the development of stem cell-based therapies, more studies are needed to determine their long term effects, the immunogenicity and safety of stem-cell derived transplants. The ethical issues involving their derivation and use of embryonic and fetal tissue also need to be resolved.

Keywords: stem cell; stem cell-based therapy; immunogenicity

SOURCES OF STEM CELLS

Stem cell-based therapies rely on the unique ability of stem cells to self-renew and to generate a number of specialized cell types. These characteristics offer the possibility of growing large numbers of cells in culture which can be used as replacement cells in damaged tissues or as a vehicle for delivering genes/ drugs to specific tissues in the body. Stem cell-based therapies, therefore, have the potential to cure diseases like diabetes, Parkinson's disease, myocardial infarction, and cancer (DHHS, 2001).

Stem cells have marked proliferative capacity and give rise to a variety of highly differentiated cells. A number of sources of stem cells have been identified. These include the embryo, fetal tissues, umbilical cord blood and the adult (Snyder and Vescovi, 2000). Adult stem cells are present in mature tissues throughout life and are used to regenerate existing tissues such as blood and skin. These cells are characterized as being multipotent, that is, they can give rise to all the different specialized cell types of the tissue from which it originated. Aside from the bone marrow and the skin, adult stem cells have also been isolated in the brain, muscle and intestines. Among these, stem cells from adult bone marrow are the most studied and most frequently used. Hematopoietic stem cells from adult bone marrow have been in clinical use for years in the treatment of leukemias, lymphomas and hereditary blood disorders; in restoring blood and immune cells destroyed by chemotherapy; in the treatment of some autoimmune diseases; and in the graft-versus-tumor treatment of cancer (DHHS, 2001; Snyder and Vescovi, 2000; McKay, 2000; Cassidy and Frisen, 2001).

EMBRYONIC STEM CELL

Another source of stem cells is the embryo. Embryonic stem cells are present only during early development. These cells, at the early stages of development are totipotent, that is, they have the potential to develop into the more than 200 different cell types needed for all tissues and organs in the body (Pera et al., 2000). Human embryonic stem cell lines (HESCs) can be derived from human embryos that have been created by in vitro fertilization. The embryonic stem cells are harvested from the inner cell mass of an embryo at the blastocyt stage of development. These cells are grown in culture and develop into HESCs after about 25 days (DHHS, 2001; Pera et al., 2000).

There are many different potential uses for HESCs (DHHS, 2001; McKay, 2000; Pera et al., 2000; Edwards et al., 2000; Edwards, 2001; Kaji and Leiden, 2001). These cells may be used to decipher the fundamental questions about life with the goal of improving health. HESCs could be used in studying early human development and in researches dealing with the elucidation of genetic factors involved in the regulation of gene expression and cell differentiation. These researches will result in the identification of genetic, molecular and/or cellular events that lead to congenital birth defects, and help in the development of methods for preventing them. Another use for HESCs would be in drug development and toxicity testing. The use of HESCs may be a safer and cheaper model since the source of the cells would be human and the screening tests would better mimic the in vivo response compared to drug screening using animal models (or cells derived from them) that are currently in use.

USES AND APPLICATIONS

The primary use for these HESCs would be as a potential unlimited source of replacement cells to repair organs and tissues. This application of HESCs hinges on the ability of HESCs to undergo directed differentiation in order to generate large numbers of cells that would be sufficient enough for use in therapeutic transplants. This sets the stage for cures against diseases like Parkinson's, Alzheimers, diabetes, spinal cord injuries, Duchenne muscular dystrophy, myocardial infarction and heart failure.

In 1998, James Thomson and his colleagues at the University of Wisconsin showed that HESCs grown in culture can develop into cell types found in the gut, brain, bone marrow, cartilage, muscle and kidney (Thomson et al., 1998). The differentiation into the various specialized cell types, however, occurred at random.

The development of stem cell-based therapy for a specific disease is premised on the idea that purified stem cells grown in culture can be directed to differentiate into a specific cell type prior to use. Research is currently underway to determine which combination of culture conditions and growth factors are needed. Different laboratories have shown that mouse embryonic stem cells can be directed to differentiate into mature nerve neurons, heart muscle cells or pancreatic islet cells (Rossant, 2001; Rathjen et al., 2002; Fraichard et al, 1995; Boheler et al, 2002; Vogel, 2001; Hori et al, 2002; Shiroi et al., 2002; Yamada et al, 2002). Researches using HESCs have likewise shown that these cells can be directed to differentiate into mature nerve cells, insulin-producing cells, muscle cells which are structurally and functionally similar to cardiomyocytes, endothelial cells and into multiple hematopoietic lineages (Schuldiner et al, 200; Rathjen et al., 2002; Assady et al, 2001; Kehat et al., 2002; Levenberg et al., 2002; Kaufman et al., 2001). Apart from directing the HESCs to differentiate into a specific cell type, the cells must be generated in large numbers enough to carry out a therapeutic transplant. Once transplanted into the recipient must be able to survive, make the appropriate connections with the surrounding cells, and restore the function of the damaged tissue. In a rat model of Parkinson's disease transplantation of stem cell-derived neurons into the brain relieved symptoms associated with the disease (McDonald et al., 1999). Parallel studies in humans with Parkinson's disease have been encouraging although with limited success (Harley et al., 2001). It appears that the stem cell source and the degree of differentiation of the developing neuron are important in determining the success of the stem cell transplantation. At this point in time, a number of researches are being undertaken which directly address the potential use of HESCs in the cure of specific diseases. These researches however, are still relatively limited because only few laboratories have access to HESCs. Thus, any therapy-based use of HESCs at present, although very promising remain highly experimental.

The use of HESCs in the treatment of diseases offer several advantages (Pera et al., 2000; Edwards et al., 2000; Kaji and Leiden, 2001). They are immortal,

flexible and available; immortal since these cells have both the ability to self-renew and to proliferate; flexible in the sense that these cells can be directed to differentiate into many specialized cell types; and available with the derivation and continued culture of well-characterized HESC lines. There are, of course, several possible disadvantages to using HESCs. These include the fact that these cells at present are hard to control and may be rejected by the immune system of the recipients. There is a need to study the immunological status of these cells. It may be possible to genetically engineer HESCs to express the major histocompatibility antigens of the transplant recipient. Another method to create HESCs that are genetically identical to that of the transplant recipient is through somatic cell nuclear transfer technology (SCNT) (DHHS, 2001; Pera et al., 2000; Edwards et al., 2000). SCNT is a technique by which mammals could be cloned from adult cells. This was the method employed by Ian Wilmut and his group at the Roslin Institute in Edinburg, Scotland in creating Dolly, the first sheep cloned from an adult mammary gland cell. In this technique, donor egg cell enucleated and fused with an adult cell from the recipient by applying an electrical current. The embryo that develops will thus have all of its the genetic information (nuclear DNA) identical to that of the prospective recipient. This is a customized way of creating HESCs. It is, however, labor extensive.

ETHICAL CONSIDERATIONS

Aside from this, another disadvantage of using HESCs is that it is ethically controversial. The ethical issues involved may be broken down into three: (1) Is it ethical to produce and/or use living human embryos for the preparation of HESCs ?; (2) Is it ethical to engage in therapeutic cloning?; and 3) Is it ethical to use HESCs and the differentiated cells obtained from them? (Kaji and Leiden, 2001; NABAC, 1998; CIN, 2000; Juengst and Fossel, 2000).

The first issue is concerned with the moral status of the embryo. Should a fertilized egg have the same moral status as a baby and what sorts of protection, if any, should it have? In what ways, if any, is it ethical to use human embryos? The answer to these questions depend on one's concept of when does life begin. In the Catholic tradition, life begins at conception, when the egg meets the sperm (CIN, 2000). If this is the case, then the inner cell mass that is harvested from the embryos at the blastocyst stage is not just a mere cluster of cells with no moral status, but a human being with a right to life. There are some theologians who believe that the moral status varies according to the stage of development of the embryo. Some Catholic moral theologians do not consider the human embryo in its earliest stages as constituting an individual human entity. Other religious traditions (Protestant, Jewish, Islam) support a view of fetal development that does not assign full moral status to the early embryo (NBAC, 1998).

The scientific community is unanimous that it is unethical to create human embryos by in vitro fertilization primarily for research purposes. The main source

of human embryos for the derivation of HESCs has been human embryos created by IVF that are no longer needed. In these cases, the potential donors are informed on how to dispose of the excess human embryos. They may opt to keep the excess embryos in storage, donate it to other couples or to discard them. If they opt to discard the embryos, then, they have a choice of whether they would like to donate the embryos to research (NBAC, 1998).

Christopher Reeve, in his Senate testimony (NBAC, 1998) said, "Is it more ethical for a woman to donate unused embryos for research or to let them be tossed away as garbage when they could help save thousands of lives?" The dilemma is between the commitment to cure disease against the commitment to protect human life. The Declaration on the Production and the Scientific and Therapeutic Use of Human Embryonic Stem Cells (CIN, 2000) maintains that "a good end does not make right an action which in itself is wrong."

With regard to whether it is ethical to engage in therapeutic cloning, which is the use of somatic cell nuclear transfer to generate embryonic stem cells, this basically implies producing customized human embryos and destroying them to obtain HESCs.

The third issue, regarding the use of HESCs and the differentiated cells obtained from them tries to distinguish between derivation and use of HESCs. Are these two activities ethically distinct and/or ethically justifiable? Is participation a sign of cooperation? Knowing that derivation of HESCs involves destruction of the human embryo. There are countries which distinguish derivation from use of HESCs. In particular, in Germany, it is illegal to derive HESCs, however, it is legal to import and use HESCs (Breithaupt, 2001; Breithaupt and Owens, 2002). In the United Kingdom, derivation and use of HESCs are allowed, so long as the HESCs are derived from embryos at its early stages (within the first 2 weeks/ 14 days) (DHUK, 2001; Dickson, 2000; Mayor, 2002).

In the United States, Pres. George Bush declared in August 2001 that he will allow federal funding for research on existing ES cell lines all created using embryos remaining after fertility treatment (Martin, 2001). Sixty-eight such cell lines have been identified. There are stem cell researchers who feel that the existing ES cell lines may not be enough to develop therapies effectively, especially because out of these 68 cell lines, less than half have been developed in the United States, and although a repository for these existing cell lines has been established at the National Institutes of Health in Bethesda, Maryland, there is always the question of who will have access to these cell lines and how quickly can the cell lines be distributed (Bonetta, 2001) (35). Of course, this pronouncement does not limit the derivation of new HESCs from frozen embryos by researchers who rely on private funding.

In the Philippines, the guidelines on assisted reproductive technology research put forth for biomedical and behavioral research by the Philippine Council for Health Research and Development (PCHRD) specifically prohibits the "intentional creation of human zygotes, embryos or fetuses for study, research and

experimentation or for commercial and industrial purposes". It also maintains that the "embryos formed by in vitro fertilization shall be given respect commensurate to their status" (PCHRD, 2000).

ALTERNATIVES

Given these limitations and ethical considerations, what are the alternatives? Would it be possible to use adult stem cells to attain the same goals? Mouse stem cell lines have been created using adult stem cells. Recent studies on stem cells have also indicated that they may be more plastic than previously believed (Andersson et al, 2001; Jackson et al, 1999; Terskikh et al, 2001; Verfaillie et al, 2002). That is, hematopoietic stem cells have been shown to generate not only the different mature blood cell types but may also be coaxed under highly specific growth conditions to become muscle cells, liver cells and/or nerve cells. Moreover, stem cells isolated from other organs may be induced to differentiate into mature blood cells. In a mouse model of myocardial infarction, mouse hematopoietic stem cells injected directly into damaged myocardium have been observed to develop into cardiomyocytes and vascular endothelial cells, indicating that these adult stem cells have the potential to replaced damaged heart tissues (Orlie et al, 2001). In the same animal model, similar results were observed when human adult hematopoietic stem cells were injected directly into the damaged myocardium (Kocher et al, 2001). Recently, a news item from an Australian newspaper, featured the first attempt at injecting human adult hematopoietic stem cells into a man who had suffered a myocardial infarction. No follow-up news item, however, on the outcome of this maneuver could be found.

More recently, genetically modified hematopoietic stem cells have been tapped for use in gene therapy (Kaji and Leiden, 2001; NBAC, 1998; Sadelain, 2000). There are more than 400 clinical trials employing the transplant of genetically modified cells. Most of these studies, however, have been met with a number of technical challenges involving gene delivery and appropriate gene expression within the cell. To date, only one clinical trial involving the therapeutic correction of the gene responsible for X-linked severe combined immune deficiency has reported improvement in the immune status of transplanted patients with correction of the disease (Cavazzana-Calvo et al., 2000). A better success rate is anticipated as more efficient methods of gene delivery are developed, alternative sources of stem cells are tested, and regulatory mechanisms involved in self-renewal are identified.

FUTURE STUDIES

Significant progress has been made in the development of stem cell-based therapies over the past decade. However, more studies are needed to determine the long-term effects of these therapies. In particular, areas that need to be addressed include the immunogenicity and safety of stem cell-derived transplants. Ethical issues involving the derivation and use of embryonic and fetal tissue stem cells also need to be resolved.

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