Review on Ethical Issues in Human Embryonic Stem Cell Research and the Use Amniotic Fluid Cells in the Stem Cell Therapy

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Abstract: *Stem cell research is a very fascinating and promising field that provides hopes to treat many disease. It is evident that the stem cell can be able to renew, replicate and can be differentiate itself into 200 cell types. The very first isolation technique of embryonic stem cells was carried out by James Thomson and his team at the university of Wisconsin.(Kaufman, D.S. et al., 1999; Barclay, E et al., 2009.) Stem cell research is very useful in understanding the development of human organ and helps in treating many diseases such as diabetes, spinal cord, Parkinson's disease and Myocardial infarction (Committee on the Biological and Biomedical Applications of Stem Cell Research et al., 2002). The embryonic stem cell research is highly controversial because they are derived from blastocyst. To date, the human embryonic stem cells are derived from In vitro fertilisation. (Guido de Wert et al., 2003)A major goal in this area review is to identify potential new sources for the isolation of progenitor cells or stem cells, without raising the ethical issues involved in embryonic stem cell research. Amniotic fluid cells (AFC) are widely used in routine Prenatal genetic testing , and it is evident that these AFC can be a potent source of pluripotent stem cell in the stem cell research (Andrea-RomanaPrusa et al., 2002)*

1. Embryonic Stem Cell

Stem cells are capable to differentiate into any specific cells in the body. There are two types of stem cells.Embryonic stem are derived from Pre implantation embryo and have ability of pleuripotency. Adult stem cells are multipotent with impotant function such as bone repair. Pluripotent stem cell lines are derived from the inner cell mass of the 5- to 7-d-old blastocyst(Kish SJ, Shannak et al., 1988).

2. Stem cell based therapies

Parkinson's disease (PD)
Parkinson's disease (PD) is caused due to loss of dopaminergic neurons (DA) in the substantianigra of brain and it is reported that it affects more than 500, 000 people in the United States.(Kish SJ et al., 1988, Agid Yet al., 1991)The cause of idiopathic PD is unknown, Although it is suggested that the loss of the dopaminergic neurons results in defect of programmed cell death and viral infection. The L-dihydroxyphenyl alanine (L-DOPA), a precursor of dopamine have been given for the treatment for PD, but long-term administration of L-DOPA produces many side effects (Lang AE et al., 1998).The successful therapy that transplants the human fetal ventral mesencephalic tissues into the striatum of PD patients has been adopted (Lindvall O et al., 1990, Olanow CW et al., 1996, Kordower JH et al., 1997).

Huntington's disease
Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder which shows involuntary choreiformic movements, cognitive impairment, and emotional disturbances (Greenmayre JT et al., 1994, Harper PS et al., 1996,). Transplantation of fetal human brain tissue may serve as a useful strategy for reducing neuronal damage in HD brain, and a study has documented improvements in motor performance in HD patients following fetal cell transplantation (Whitehouse PJ et al 1981).

Alzheimer's disease
AD is characterized by degenerative loss of neurons and synapses throughout the brain, particularly in the basal forebrain, amygdala, hippocampus, and cortical area which forms papez circuit. Memory and cognitive function of patients progressively decline, and patients become demented and die prematurely (Whitehouse et al., 1981; Bartus et al., 1982; Coyle et al., 1983. No effective treatment is currently available except for acetyl cholinesterase inhibitors, which augment cholinergic function, but this is not curative and is only a temporary measure. Nerve growth factor (NGF) prevents neuronal death and improves memory in animal models of aging, toxicity and degeneration (Hefti, 1986; Fischer et al., 1987; Tuszynski et al., 1990; Emerich et al., 1994; Tuszynski, 2002), suggesting that NGF may be used for treating neuronal degeneration and cell death in AD. However, delivery of NGF into the brain is not possible via peripheral administration. Because of its size and polarity, NGF does not cross the blood–brain barrier.

To overcome this difficulty, gene therapy approach could be adopted. By using an ex vivo gene therapy approach (genetically modifying cells), NGF can be given directly to the brain and diffuse for a distance of 2–5 mm (Tuszynski et al., 1990). Recently, a phase 1 clinical trial of ex vivo NGF gene delivery was performed in eight mild-AD patients, implanting autologous fibroblasts genetically modified to express human NGF into the forebrain. After a mean follow-up of 22 months in six subjects, long-term adverse effects were not found. Evaluation of Mini-Mental Status Examination and Alzheimer's Disease Assessment suggested improvement. Serial PET scans showed significant increases in cortical fluorodeoxyglucose after treatment (Tszynski et al., 2005). Because stem cells can be genetically modified to carry new genes and have high migratory capacity after brain transplantation (Flax et al., 1998; Kim, 2004; Lee et al., 2007), they could be used in place of fibroblasts that are known for their immobility following transplantation (Kang et al., 1993) for delivery of NGF to prevent degeneration of basal forebrain cholinergic neurons.

Stroke

There are two major types of stroke: ischemia (infarct) and intracerebralhemorrhage (ICH). Ischemic stroke caused by abrupt and near-total interruption of cerebral blood flow produces ischemic changes in the striatum and cortex, leading to a long-term sensorimotor deficit. (seung et al).

There are several reports of cell therapy in the brain in ischemia animal models (Savitz et al., 2002), and various sources such as rodent bone marrow mesenchymal stem cells (Sinden et al., 1997; Chen et al., 2001, 2003; Zhao et al., 2002; Modo et al., 2002), human umbilical cord cells (Chen et al., 2001), mouse neuronal precursor cells (Veizovic et al., 2001), and human carcinoma NT2-derived neurons (Borlongan et al., 1998; Saporta et al., 1999) were transplanted into the ischemic brain, reducing the defects.

Spinal cord injury

Traumatic SCI causes many neurological defects. There is no effective therapeutic option to improve function. By employing a rodent model of contusive injury that closely mimics human SCI, have proved that the white matter occured by injury is closely associated with the human etiology (Noble and Wrathall, 1989; Fehlings and Tator, 1995; Basso et al., 1996). The important pathological processes in the white matter causes progressive demyelination of spared axons (Bunge et al., 1993; Reier, 2004; Guest et al., 2005), which occurs primarily as a result of the delayed apoptosis of oligodendrocytes (Crowe et al., 1997; Springer et al., 1999).Recent advances in stem cell biology have opened up an avenue to therapeutic strategies to replace lost neural cells by transplantation of stem cells in various disorders in the CNS. For the therapy for SCI various cell types such as fibroblasts stem cells have been used to promote axonal regeneration (Tuszynski et al., 1994; Xu et al., 1995; Li et al., 1997; Liu et al., 1999; Teng et al., 2002).

Lysosomal storage disorder

Lysosomal storage diseases are caused by inborn error genetic defects, affecting 1 in 7, 700 births, and most affected babies show a diffuse CNS involvement (Meikle et al., 1999). There is no effective treatment is available for most of the lysosomal diseases, because the blood–brain barrier prevents the entry of enzyme penetration into the brain (Sly and Vogler, 2002). In animal models of lysosomal diseases, therapeutic administration of enzymes could be achieved in the brain by direct inoculation of genetically cultivated mouse NPCs (Snyder et al., 1995), fibroblasts (Taylor and Wolfe, 1997), or amniotic epithelial cells (Kosuga et al., 2001)

Bone repair

In 1999, three children with severe deforming type III OI were transplanted in the second or third year of life (13-32 months of age) with bone marrow MSCs derived from HLA-matching or single-antigen-mismatched siblings (5.7-6.2 x108 cells/kg), following ablative conditioning. Opportunistic bone biopsies were collected from two of the transplanted children and engraftment of donor cells was estimated around 2%. Despite the low level of engraftment, an increase in bone mineral content was observed in all three children, ranging from 45% to 77% gain of total body mineral content in the first 6 months after transplantation. The three patients showed a 2-2.5 cm growth in the first 3 months and two children grew in line with the normal median growth velocity for their age in the first 6 months. A dramatic decrease in fracture rate was also reported. In the year before transplantation, two children reported more than 20 fractures, which decreased to 2-3 in the first 6 months after the procedure and none were reported in the following 6 months. In terms of toxicity, one child experienced pulmonary insufficiency, sepsis and developed anhygroma, all of which were resolved (Horwitz et al., 1999a).

Two years later, the authors reported a 36-month follow-up of two patients and an additional child, which also included clinical data from two non-transplanted age matched OI children as controls. The transplanted children showed a showed a median 7.5-cm growth in the first 6 months, compared to the 1.25 cm observed in control children in the same period of time. After this initial improvement, however, growth reached a plateau. The number of fractures decreased from a median of 10before treatment to a median of 2 at 12 months after transplantation, while in control children it remained constant at 4. Six children, who had previously undergone bone marrow transplantation as part of previous studies, were enrolled in a study to assess the therapeutic effects of repeated MSC transplantations. Two infusions (1-5 x 106 cells/kg) with the same MSC donor used for the previous study were performed 1834 months after the original treatment. Five children showed engraftment of donor cells in bones. In the six months after transplantation, growth rates showed a marked acceleration (60-94% of the predicted median). No adverse effects were observed apart from one child suffering from a skin rash (Horwitz et al., 2002).

Ethical concerns

Research into the development of cell-replacement therapy requires the instrumental use of pre-implantation embryos from which hES cells are derived since current technology requires lysis of the trophectoderm and culture of the ICM; the embryo disintegrates and is thus destroyed. There is extensively high debate in the embryo-research debate, considerable differences of opinion exist with moral status of the pre-implantation embryo (Hursthouse, 1987). On the other side of the spectrum the embryo is a person and because of the potential of the embryo to develop into a person, it ought to be considered as a person.(Guido de Wert and Christine Mummery (2003)).

Proportionality

There is a different opinions differ on the interpretation and made operational. In a number of countries, research on preimplantation embryos is permitted provided it is related to human reproduction. Internationally, however, such a limitation is being increasingly regarded as too restrictive (De Wert et al., 2002). It is difficult to argue that research into hES cells is disproportional. If embryos may be used for research into the causes or treatment of infertility, then it is inconsistent to reject research into the possible treatment of serious invalidating diseases as being not sufficiently important.

Fetalist perspective

Instrumentalization of the embryo is regarded as use of embryo for researches. Ought to this the use of embryo instrumentally is regarded as degradation of the moral status and ethical status. It is a misconception to think that in the context of regular IVF treatment every embryo is created as a `goal in itself': the goal is the solution of involuntary childlessness and the loss of some embryos is a calculated risk beforehand.

Feminist perspective

From a feminist perspective, the creation of embryos for research should be evaluated critically in as far as it may require hormone treatment of a woman to obtain oocytes for research purposes: An objection is that women themselves become objects of instrumental use. Relevant considerations concern whether or not the research serves an important goal, whether the burdens and risks to the subjects are proportional, and whether valid informed consent of the research subject/donor is given. The second objection is that the health risks to the women themselves are too high and the degree of discomfort disproportional.

To overcome the ethical dilemma

Laboratory evidence has revealed the capacity of amniotic fluid-derived stem cells (AFSCs) to differentiate along multiple cell fate lineages. Including the ability to produce cell types of all three germ layers. Human amniotic fluid, which can be safely extracted during amniocentesis, contains amniotic fluid cells (AFCs) which originate from embryonic and nonembryonic tissue sources. (Moraghebi et al)

3. Laboratory Evidence

The collection of Term amniotic fluid

The Healthy women who were selected undergoing caesarean section deliveries were informed about the study and the concern was obtained. Twenty women were accepted for this research and the research was carried out in Moraghebi et al., The operation procedures for caesarean section delivery was carried out as per SOP (Standard Operating protocol) .when the amniotic membrane was seen, A soft catheter was used to penetrate the amniotic membrane and the collecting tube was inserted into the amniotic cavity for amniotic fluid collection. The amniotic fluids were collected into a sterile expandable collection vessel through a sterile tubing procedure. The processing of fluid must be started within four hours. The filtration of fluid was done using a sterile mesh gauze pad to remove vernix and other substances, and then sent through a 100-μm nylon cell strainer for obtaining clear filtrate.

The isolation of Amniotic fluid cells

The Filtrate of fluid were centrifuged at 850×g for 5 min and pellet obtained was resuspended in 20–50 ml DMEM+10% FCS. The separation of cells from RBC (red blood cell) was done by centrifugation of the fluid over at 850×g for 20 min at room temperature. The mononuclear cells were isolated and calculated for the by Trypan blue exclusion method, and they were analysed for their clonogenic potential (CFU-F assay) and expansion activity.

AFC cultivation procedure

The MonoNuclearCells were cultured on rat tail collagen I with coated six-well plates in each of four FCS-based media; medium 1 (EM) medium 2 (FM), medium 3 (MSC expansion media), and medium 4 (DMEM+10% FCS).In the days of 11–14, the fibroblastic colony forming units were counted with colony counter and then identified for individual cell colonies. The individual colonies were picked for clonal cell expansion and cultured in all the 4 media. Cells were subcultured every 3 days by separating individual colonies. The colony of epithelial cells was also cultured in a epithelial cell growth medium.

Colony forming unit-fibroblast assays

The culturing cells were counted for colony forming unit-fibroblasts (CFU-F) and were assessed microscopically for morphology after 11-14 days of plating. The cells that shows clear spindle shaped were selected and the other morphological shape was excluded. Colonies were considered significant if the cell count ≥40 cells..

Characterization of TAF-derived cells by flow cytometry

The Single-cell suspensions from cultures of passages in media 1 and 2 were prepared and they were coated with fluorescent-labelled antibodies. As it is intracellular staining of OCT4, cells were fixed with 4% PFA and permeabilizedby 0.5% Triton X-100 before staining. The Monotype antibodies served as control. Quantitative analysis was performed using flow cytometer and FlowJo software.

In-vitro differentiation of TAF-derived cells to osteoblasts and adipocytes

Cells expanded in media were allowed to be differentiated intoosteoblastic and adipogenic lineages. cells were cultured in osteoblast induction medium for 21 days and stained with Alizarin Red to measure calcium mineral content. For adipogenic differentiation, cells were cultured in AdipDiff medium and stained with Oil Red-O to detect lipid vacuoles.

Gene expression analyses

The limma package uses linear models to assess differential expression in the context of multifactor designed experiments. For published data sets, reported lists of significant genes were overlapped with genes found to be significantly enriched in the present analysis ($p < 0.05$).

4. Conclusion

In this review the amniotic fluid cells were found to be potent in replacing it with the stem cells .There are only a limited number of publications characterizing term human amniotic fluid cells. The identified cells with either spindle-shaped or round-shaped morphology from term amniotic fluid, and assessed their growth characteristics. The genetic marker, the induction medium, the morphology, flow cytometry and gene expression analysis were carried out. Conversion of cells from amniotic cells to true pluripotent states may offer an expanded utility of these cells for disease modelling, drug discovery and testing, and regenerative medicine.

The conversion of amniotic cells into human progenitor cells and stem cells used for replacing damaged cells within a

tissue. It is the hope of investigators and patients alike that such cells will have the potential to treat a wide array of diseases, including Parkinson's and Alzheimer's disease, as well as heart disease, diabetes, stroke, spinal cord injuries, and burns. The fact that the most potent stem cells are probably those derived from the inner cell mass during embryonic development raises a number of ethical issues. On the other hand, recent investigations provide evidence that other progenitor cells or stem cells, isolated from a variety of human sources, may also have the potency to differentiate into different cell types. Concerning these questions, we believe that the findings discussed above warrant further investigation of cells in the amniotic fluid cells.

The high potential of amniotic fluid material, and high cell yields of the procedure, make this currently untapped source of cells attractive for further evaluation in bio-banking, cell therapy, disease modelling, and regenerative medicine applications.

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