Evaluation of human stem cell role in treatment of different neurological diseases.

Submitted for partial fulfillment of the master degree in neuropsychiatry

By:

Ahmed Mohammed Mahmoud Mohamed

M.B.B.CH

Prof. Dr/ Hany Mohammed Aref

Professor of neuropsychiatry

Ain shams university

ASS. Prof. Dr/ Salma Hamed Khalil

Assistant Professor of neuropsychiatry

Ain shams university

ASS. Prof. Dr/ Hany Mahmoud Zaki Eldine

Assistant Professor of neuropsychiatry

Ain shams university

2012

Acknowledgement

Im very grateful to my professor dr/**hany mohamed aref** for inspiring me with the idea of this work, I would like to thank my professor dr/**salma hamed khalil** for all the effort she supplied me with.

Hereby I can not deny all the care, time and help my professor dr/**hany mahmoud zakieldine** pledjed me with.Many thanks to my family for their help and support.

Lists of content

STEM CELLS

- # Introduction.

Stem cells are defined as cells that can proliferate and self-renew, differentiate into the major lineages of the tissue. They are derived from and, theoretically, regenerate the tissue and/or organ they were derived from.

The hematopoietic stem cells (HSC) were the first stem cells to be described in the early 1900's.

The zygote and the cells produced by its first few divisions (comprising the morula) are totipotent cells, as they can produce any tissue, including placental tissue.

The morula then turns into a blastocyst, which will give rise to major three types of pluripotent stem cells:

(1)Embryonic stem (ES) cells.

(2)Umbilical cord blood stem cells.

(3)Adult stem cells**.(Farin A,2009).**

Types of stem cells:

Embryonic Stem Cells.

Embryonic stem cells (ESCs) were first derived in the early 1980's simultaneously by two independent groups.Because of their plasticity and potentially unlimited capacity for self-renewal, they were predicted to transform research in mammalian development, genetics, stem cell biology and regenerative medicine. **(Katja S, 2009).**

Embryonic stem cells are characterized by two main features:

(1)A limitless self-renewal capacity while maintaining undifferentiated state.

(2)The ability to differentiate into cells of the three primary germ layers, the embryonic ectoderm, endoderm and mesoderm.

They can further produce progenitor or mature cells deriving from these layers under specific culture conditions.Embryonic stem cells were isolated first from the mouse and then from other species including the human then from pluripotent cells harvested from the inner cell mass of the pre-implantation embryo**. (Alexandros A,2009).**

Owing to their potential for generating a multitude of cell types and their consideration for clinical applications,Embryonic stem cell have received great attention over the past decade. **(Rowayda Peters, 2010).**

Neurons, cardiomyocytes, endothelial cells, ß-Langerhans cells, hematopoietic precursors, keratinocytes and hepatocytes had all been generated using Embryonic stem cell**. (Rowayda Peters, 2010).**

To avoid the ethical and potential practical (immunological rejection) short comings of the use of Embryonic stem cells, major efforts have been put into the development of patients-specific cell therapy in recent years, by the use of autologous adult stem cells. In the adult, stem cells exist in special niches in many tissues and their main role is to replace cells lost by normal turnover or owing to disease or injury. **(Walker MR, 2009).**

Umbilical cord stem cells.

Umbilical cord stem cells represent a special category of cells that may potentially be used for personalized cell therapy after long-term storage.

Subpopulations of cells from both the cord blood and the cord stroma, although they are of mesodermal origin, have demonstrated a potential to differentiate into neural (ectodermal) cells in vitro. They seem in some cases to exert their beneficial effects through paracrine mechanisms or through suppression of inflammation. **(Low CB, 2008).**

The use of Umbilical cord stem cells has provided a number of encouraging results; however, the exact mechanism of action, the degree of cell differentiation and the degree of incorporation in the lesion sites are still debated. Human umbilical cord blood infusions could potentially benefit animals modeling various neurodegenerative diseases. Human umbilical cord blood cells introduced by intravenous infusion into rats lead to improved functional recovery. Either infused or transplanted into animal spinal cord injury models, improved behavioral recovery, although only a small number of transplanted cells were found to express neuronal or glial markers. It has also been suggested that oligodendrocyte-like cells generated by differentiation of cord blood stem cells could enhance locomotor function recovery after moderate spinal cord injury by remyelination of injured axons. **(Dasari VR,2007).**

with some of the human cord blood cells having entered the site of the stroke and expressing neuronal and astroglial markers. Even delayed cord blood infusion (48 h) was demonstrated to help reduce secondary events of inflammation and apoptotic cell death. **(Newcomb JD, 2006).**

Bone marrow stem cells.

Postnatal bone marrow is an easily accessible source of adult stem cells for autologous transplantation and contains:

(1)Hematopoietic stem cells.

Hematopoietic stem cells are multipotent stem cells that give rise to all of the blood-cell types including myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocyte,megakaryocytes,platelets and dendritic cells) and lymphoid lineages (T cells, B cells and natural killer cells).These hematopoietic stem cells, although they have not been proven to be able to produce cells of neural lineages, can prove very helpful in the treatment of Autoimmune demyelinating diseases by using high-dose immunosuppression followed by reconstitution by hematopoietic stem cells**.(Van Wijmeersch B,2008).**

Allogenic hematopoietic stem cells can be used as well as autologous hematopoietic stem cells, which have a potentially increased efficiency in treating the source of the demyelinating reaction. Owing not only to the replacement of the reactive autoimmune compartment by healthy allogeneic cells, but also to the development of a graft versus autoimmunity effect**.(Sprangers B,2009).**

(2)Mesenchymal Stem Cells.

Mesenchymal stem cells received considerable attention as a potential source of cell-based therapies and as a cell type that support the engraftment of haematopoietic stem cells. The usual source of mesenchymal stem cells is the bone marrow, which is not easy to obtain from healthy donors as well as umbilical cord blood. The advantages of umbilical cord blood as the source of

mesenchymal stem cells are the availability of units and the primitive nature of umbilical cord blood derived mesenchymal stem cells. **(Rowayda Peters, 2010).**

Bone marrow and umbilical cord blood derived mesenchymal stem cells are presumably highly similar precursors as they share the following features:

(1)Capacity of self-renewal.

(2) Multipotency, allowing in vitro differentiation into mesenchymal tissues (bone, cartilage, tendon, muscle, adipose tissue, stroma) and possibly non-mesenchymal tissues (neuronal, endothelial and hepatic).

(3) Formation of colonies of fibroblastic cells (CFU-F).

(4) Expression of mesenchymal stem markers,cluster differentiation (CD29, CD44, CD73, CD105) and lack of haematopoietic markers (CD14, CD34, CD45).

(5) Migration to inflammatory sites, stimulation of proliferation/differentiation of resident progenitor cells and promotion of recovery of injured cells through growth factor secretion and matrix remodeling.

Although the frequency of mesenchymal stem cells referred here as undifferentiated cells is much higher in bone marrow (0.001–0.1%) than in umbilical cord blood (0.00003%) and some reports have even doubted the presence of mesenchymal stem cells in umbilical cord blood, umbilical cord blood derived mesenchymal stem cells have a better potential to expand and can give rise to up to 10^{15} cells.However, the scarcity of mesenchymal stem

cells in umbilical cord blood and the lack of a robust protocol to reproducibly expand,mesenchymal stem cells from umbilical cord blood units have hampered clinical applications. **(Rowayda Peters, 2010).**

It can not be excluded that the low numbers of mesenchymal stem cells in cord blood actually derive from placental mesenchymal stem cells that were released into cord blood due to mechanical stress during umbilical cord blood isolation procedure.Several studies reported that mesenchymal stem cells can be isolated and established from only 20–63% of the cord blood units, questioning the feasibility of mesenchymal stem cells isolation and cultivation from umbilical cord blood.

There is a novel, simple and reproducible method, which is based on stroma-free liquid culture, to expand substantial numbers of multipotent mesenchymal stem cells from only a small number of umbilical cord blood derived mononuclear cells (MNC).

This method allows an extensive expansion of non-adherent haematopoietic stem cells plus a marked increase in adherent mesenchymal stem cells.

Mesenchymal stem cells produced in vitro by this novel culture method maintain their stem cell properties of self-renewal and multi-lineage differentiation for a long-time (up to passage 24), even following cryopreservation.

(Rowayda Peters, 2010).

(3)Multipotential adult progenitor cells.

It is a rare cell type within bone marrow mesenchymal stem cells cultures that can be obtained by long-term propagation of marrow cells under selective conditions.

They have been demonstrated to undergo a molecular program of neural differentiation, similar to that for neural stem cells. However, the extended passaging period required to harvest these cells would render them problematic for human clinical applications owing to the accumulation of genetic errors. **(Phinney DG, 2007).**

Adult Neural Stem Cells.

Adult neural stem cells is the self-renewing, multipotent cells that generate the main phenotypes of the nervous system.They are attractive candidates for central nervous system(CNS)-cell replacement therapy.

They can be maintained in vitro in a proliferating state and can be led to differentiate into mature neurons and glia. It is now accepted that what are treated as neural stem cells populations are in fact mixtures of true neural stem cells and early precursors with some limitations in their lineage potential or self-renewal.

Difficulties related to the harvesting of adult neural stem cells are expected to be resolved through derivation from other cell types. **(Louis SA, 2008).**

- **Derivation of autologous pluripotent stem cells from adult somatic cells by nuclear programming.**

Ideally, stem cells to be used for therapy should be: (1)Autologous. (2)Easily accessible.(3)Expandable. (4)Having broad developmental potential.(5)Having a capacity to consistently differentiate into the desired cell types.

To this end, considerable attention has been given to the prospect of reprogramming the nucleus of terminally differentiated adult cells towards pluripotency and, eventually, towards the desired differentiation which can be very different to that followed in their tissue of origin. **(Matthias S, 2010).**

The discovery of induced pluripotency represents the synthesis of scientific principles and technologies that have been developed over the last six decades.

These are:

(1) The demonstration by somatic cell nuclear transfer in which differentiated cells retain the same genetic information as early embryonic cells.

(2) The development of techniques that allowed researchers to derive, culture, and study pluripotent cell lines.

(3) The observation that transcription factors are key determinants of cell fate whose enforced expression can switch one mature cell type into another. **(Matthias S, 2010).**

Rapid progress has been made towards improving the efficiency of induced pluripotent stem cell generation, development of integration-free strategies or substitution of some reprogramming factors with other proteins or chemical compounds.Though initial reports relied on the use of retroviral or

lentiviral delivery systems to introduce the reprogramming transcription factors.

induction of pluripotency can now be achieved with:

(1) Plasmid transfection.(2) Non-integrative episomal vectors.(3) Self-excisable vectors.(4) Delivery of reprogramming proteins.

Successful reprogramming has been achieved recently without the use of viral or plasmid vectors at all.

Specifically, induced pluripotent stem cell (IPSCs) have been derived from both mouse and human fibroblasts by delivering the reprogramming factors as purified recombinant proteins. **(Zhou W,2009).**

Or as whole-cell extracts isolated from embryonic stem cells. **(Cho HJ, 2010).**

The universality of the process has been demonstrated by the generation of induced pluripotent stem cells from different species, as well as from different sources of somatic cells including fibroblasts, adipose cells, keratinocytes, neural stem cells or hepatocytes. **(Sergio R,2010).**

- ## Mechanism of reprogramming.

Given that all cells within an organism have the same genome, the functional characteristics of different cell types are defined by specific patterns of gene expression. Epigenetic molecular mechanisms control gene transcription by inducing stable changes in gene expression. These changes favor the