Neural differentiation of embryonic stem cells

Almahdi M Abdalmolah
Department of Biology, Faculty of Science, Traghen, Sebha University, Libya

Abstract: Neural stem cells exist in the mammalian developing and adult nervous system during the neural differentiation of the embryonic stem cells. Stem cells are important cells for replacement therapy diseases. The interest in the potential of for the treatment of neurodegenerative diseases and brain injuries has substantially promoted research on neural stem cell self-renewal and differentiation. General chapters of this review will deal with the history and origin of, their properties and characteristics that distinguish from other cells, the classification, and biological disorders causing neurodegenerative diseases. The literature, data and arguments that are dealing with, how the differentiate into neural cells, and how could this process can be handled *in vitro* are reviewed. The unique capability of these cells to form various tissues under definite signals received from the body, it makes this cell an object of extensive research. Subsequently, information has been compiled on the question how neural differentiation is controlled on the molecular level, and controlled *in vivo.* Finally one major gene involved will be investigated, which are may be investigated in practical pirogue according there expression patterns. And this gene is tyrosine hydroxylase.

Keywords*:* Stem cells, Neural stem cells, Embryonic stem cells, Tyrosine hydroxylase, Parkinson's disease.

Introduction

Stem cells (SCs) have an interesting history that has been tainted with debate and controversy. In the mid of 1800s. At the same time it was discovered that cells are basically the building blocks of life and have the ability to generate other cells that play a key role to understand the human development and medical research. Researchers, in the early 1900s, realised that a particular SCs can give rise in various cell types for example white and red blood cells (RBCs). SCs are able to divide indefinitely, forming hundreds of copies of themselves, and to repair damaged body tissues. SCs are less likely than other foreign cells To be rejected by the immune system when they are implanted in the body. On the scientific front, it is clear that, embryonic stem cells (ESCs) have already generated new possibilities and stimulated development of new strategies for increasing our understanding of cell lineages and differentiation. The first quantitative descriptions of the self-renewing activities of transplanted mouse bone marrow cells were documented by Canadian researchers (1). Other key events in SCs research include: 1978: SCs were discovered in human cord blood, 1981: First *in vitro* SCs line developed from mice, 1988: Embryonic SCs lines created from a hamster, 1995: First embryonic SCs line derived from a primate, 1997:

Cloned lamb from SCs and 1997: Leukaemia origin found as haematopoietic stem cell, indicating possible proof of cancer SCs.

A scientist at the University of Wisconsin in Madison successfully removed cells from spare embryos at fertility clinics and grew them in the laboratory. He launched SCs research into the limelight, establishing the world's first human embryonic stem cells (hESCs) line which still exists today (2). During the initial phase of regeneration it has been found that cells in the area of the injury can help repair defects and become SCs again. Known as dedifferentiation the process produces cells that will later grow and dedifferentiate to form the new part or organ. The process is unique and is not observed in animals that lack regenerative powers, such as mammals. Scientists are looking for those genes that regulate regeneration and are investigating on the cells transfer to mammals even to humans. Clearly, ESCs models are already providing opportunities for the establishment of limitless sources of specific cell populations (3). SCs are special cells that have the ability to divide for an indefinite period and can give rise to a wide variety of specialized cell types.

This ability, known as totipotency, is a common feature of fertilized eggs and early ESCs. As development progresses, individual cells become multipotent before assuming their final form as a specialized cell that can only give rise to other cells of its kind. SCs may be isolated from embryos, umbilical cords, and adult tissues. SCs isolated from adult tissue possess a wide range of plasticity that varies from pluripotent to multipotent. When placed in culture, SCs grow and divide indefinitely. Stem cell therapies may be able to treat cardiovascular disease, spinal cord disorders, embryonic stem cells (ESCs), Alzheimer's disease, and cancers. Leukaemia, a cancer affecting white blood cells (WBCs), is already being treated by replacing the cancerous cells with SCs programmed to differentiate into live WBCs. Diseases that affect the brain, spinal cord, or heart are ideal candidates for stem cell therapy because these organs have lost the abilities, to retain; in particular, the ability to proliferate (grow and reproduce) and differentiate. All eukaryote cells, at some point in their lives, possess the powers of reproduction and differentiation, but those powers become a liability when cells are trying to live.

The human brain, for example, is an intricate assemblage of 10 billion neurons that is constructed during embryonic development. The neurons in our brain can form new associations with other neurons throughout our life, but they become post mitotic (lose the ability to divide) soon after an individual is born. Organs, such as the spinal cord, heart, kidneys, and muscles, adhere to the same developmental pattern: active cell division during embryogenesis, loss of cell division in the adult. But if a person suffers a disease or trauma such as heart attack, the post mitotic cells, in this case myocytes (cardiac muscle cells), are unable to repair the damage. When the damage is extensive, the heart muscle cannot contract properly, and the patient dies or has to receive a transplant. However, some of our tissues and organs, such as skin, liver, and bone marrow, retain the power of division throughout the life span of the individual. Many observations indicate that SCs can replace worn out RBC; it might be possible to "train" them to repair organs, such as the brain or heart

that are incapable of repairing themselves. However, many investigators believe that these results can only be obtained by using ESCs (4).

Since the 1990s umbilical cord blood SCs cells have sometimes been used to treat heart and other defects in children, who have rare metabolic diseases and to treat children with certain anaemia's and leukaemia. It has been shown in the cord SCs and from this cells can migrate to damaged tissues and repair them (5). Stem cell research is a new field with unlimited scope. SCs hold the key role on replacing cells lost in many diseases that are caused by loss of functioning cells. The unique capability of SCs to form various tissues under definite signals received from the body, makes them an object of extensive research. SCs are unspecialized cells that can divide and renew themselves for long periods of time and become specific specialized cell types of the body (6).

Scientists are intensively studying the fundamental properties of SCs that are determining precisely how SCs remain unspecialized and self renew for many years; and identifying the signals, that cause SCs to become specialized cells. SCs research has been found beneficial in diseases like Alzheimer's, Parkinson's disease (PD), myocardial infarction, stroke, spinal cord injuries, chronic liver cirrhosis, sickle cell anaemia, leukaemia, Non-Hodgkin's lymphoma and some other cancers, auto-immune diseases, multiple sclerosis, diabetes, chronic heart disease, end-stage kidney disease, liver failure, and cancer (7). Recently there has been great interest in the SC research finding a creative treatment for many diseases, including the cancers, spinal injuries, limb ischemia, myocardial infarction, and Parkinsonism and many more. The field requires dedicated team of basic researchers and clinicians to fully understand the cell physiology, modulators, their potentials and scope of applications more so in the diseases where presently there is no cure.

Materials and methods

The clone used in our study were isolated from source which is requested from the RZPD, and these clone is: RZPDp471B0753D2, it is entry (TH)
The clones contain DNA from the following library:

Library No: 47
Creator: Minoru Ko
Source: embryo-derived stem cells
Vector: pSPORT1
Host: E.coli DH10B
Growth Conditions: Unconfirmed antibiotics ampicillin

Resource for clone gene: For preparing the bacterial culture, I grow bacteria in LB media, a colonies a propagated according to the colonies properties and transformed bacterial culture in to the LB medium and ampincilin for make plasmid preparation.

Plasmid: This was done according Birnboim Doly-protocol (Birnboim Doly) with some modifications. So, in essential with according with Birnboim Doly after that purification, precipitation with ammonium acetate.

Linearization by suitable restriction enzymes: In situ-hybridization probes were generated according to protocol which can start our preparation which published protocol. Plasmid prepared from alkaline phosphates mini-preparation were linearization by the probe the end 5′ overhung copies enzymes and in vitro transcript to label

diogoxygenin rib probes using the promoters T3,T6 and sp6. In the original vector survival from the library, there is known T7 or T3 promoter in order to allow rib probes there will sub cloning in the blue script.

TH;

-vector name pSPORT1
-promoter T7 and sp6
-Restriction enzymes T7 for sense line with Not I
Sp6 for antisense line with Sal I

Preparation of embryos: Dissect embryos (129V mice type) in cold PBS, change solution often. Punch a hole in brain cavities for embryos older than 9 dpc. Transfer after dissecting a few embryos to a 5 ml screw cap flat bottomed glass vial containing 4% paraformaldehyde. When all the embryos of the same mother are dissected, renew the 4% paraformaldehyde and incubate at 4 °C for 4 hrs for 7.5 d embryos or overnight for older embryos (9.5d), (or over day if dissection is done in the morning). The next day, wash 2x with PBSw (PBSw=PBS with 0.1% Tween-20) Dehydrate with methanol series (25%, 50%, 75%, and 100% in PBSw). Change 2 x in 100% methanol. Store the embryos at -20 °C (up to 2 months).

Situ hybridization protocol:

Day 1: 4% paraformaldehyde fresh in PBS. About 5 ml will be needed for each sample after proteinase K treatment. hybridization solution dissolve. Rehydrate the embryos through 75, 50 and 25% methanol series in PBSw. Incubate each step for 5 min. on ice. Wash 3 times for 5 min. with PBSw on ice. Staining for highly expressed gene requires less digestion, but for low expression genes longer digestion may help to get stronger staining. Make sure to thaw the proteinase K stock completely and vortex to dissolve precipitate at the bottom of the tube. Use aliquots of the proteinase K stock 10 mg/ml, do not thaw-freeze repeatedly. Rinse in PBSw. Wash 2 times with PBSw for 5 min. Refix in 5 ml of 4% paraformaldehyde-0.2% glutaraldehyde in PBSw for 15 min. Rinse in PBSw. Wash 3 times with PBSw for 5 min. each. Wash in 1 ml of 50% PBSw: 50% hybridization solution, followed by 100% hybridization solution for about 3 min each standing. Replace 900 µl of fresh hybridization mix in each Eppendot tubes. Prehybridize samples for 3 hrs at 70ºC. Heat 200 ng of the RNA probe in 100 µl of hybridization mix to 95 °C for 5 min. Add the probe/hybridization mix to the embryos. The final probe concentration should be about 200ng/ml. Hybridize overnight at 70 °C in a incubator.

DAY 2: Remove hybridization solution and add 800µl of prehybridization solution. Wash for 5 minutes at 70 °C.
Add 400µl of 2X SSC, ph 4.5 (without removing prehybridization solution). Repeat the addition of the 2XSSC wash twice more. Remove the mix and wash twice, 30 min each time, in 2XSSC pH7/0.1% CHAPS 70 °C. Wash twice, 10 min each, in Maleic Acid Buffer (MAB; 100 mM maleic acid, 150 mM NaCl; pH 7.5) at room temperature. Wash twice, 30 min each time, in MAB at 70 °C. Wash twice 10 min each in PBS at room temperature.
Wash 5 min in PBSw at room temperature. Incubate the embryos in 1 ml antibody buffer for at least 2 hours at 4 °C with rocking. BM block-mouse antibody buffer 2.5ml needed for each sample: 10% Goat serum (heat inactivated 30 min at 56 °C) 1% boehringer blocking reagent in PBSw
Heat the mixture at 65 °C until total dissolution, filter through 4.5 micron

filters (several may be needed), then cool on ice. During the blocking step, preabsorbe the antibodies.

The dilution for the Alkaline phosphatase conjugate (AP) is 1/10000 from a stock of 150 units/200 µl (Boehringer). Use this solution to replace the blocking solution. Replace buffer with diluted antibody and incubate overnight at 4 °C.

Results

TH gene: In the 9.5 EB mouse the expression in my pattern is exactly supporting this hypothesis is there expression in this regions in the head, endothelial cells, ectoderm and digestive system (fig. 1).

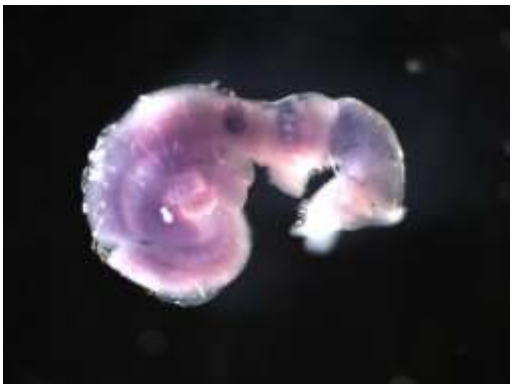

Figure 1: TH gene 9.5 EB mouse expression antisense

The expression pattern is exactly supporting this hypothesis is there expression in this regions. There are some processes that accruing during the embryoid development and one except some stages of cell differentiation because in the bone marrow point there are multiple stem cells whish can get in to different pathway in the differentiation. During the postnatal development, the existence of enteric dopaminergic neurons has been existing. But there is additional report in the literature that expects there is a second dopamine nerves system in the animals and this is around the gut. It is reported that transcripts encoding tyrosine hydroxylase (TH) and the DA transporter (DAT) are present in the marine bowel. Because sympathetic neurons are extrinsic, transcript encoding TH in the bowel is probably derived from intrinsic neurons.

TH protein was demonstrated immunocytochemically in neuronal primary, and it is immuno-reactivities were coincident in subsets of neurons in mouse intestines in situ and in cultured guinea pig enteric ganglia. Expression of mRNA encoding TH disappears after embryonic day E 14 mouse (8). These data provide proof of concept that over-expression of multiple transcription factors can drive the fate of NPC first towards neurons, and then towards the DA phenotype. However, further factors may be required to generate fully functional DA neurons (9). Mid-brain dopaminergic (DA) neurons from human embryonic stem (hES) cells have been of particular interest because of the clinical potential for DA neuron transplantation in patients with Parkinson's disease (PD). Several protocols for DA neuron differentiation from mouse embryonic stem cells and hES cells have been reported a slightly modified stromal cell-derived inducing activity method. All the four develop method that hydroxylase (TH)-positive cell differentiation from embryoid bodies. And these are providing valuable information that will assist in efficient DA neuron differentiation from hES cells and for future transplant application (10).

Discussion

The study of neural differentiation of ESCs has raised major interest over recent years, because SCs directed to neural differentiation could be the source for many therapeutic applications in human disorders. The progress in the field of neuronal differentiation of SCs has been reviewed.

The origin of SCs, historical aspects of SCs research and their potential application in neurodegenerative diseases through transplantation offering new therapeutic strategies are in detailed presented. Neurogenesis of SCs *in vitro* is tightly regulated in a specialized microenvironment via combinatorial functions of extrinsic signals and intrinsic factors. Persistent marker and important gene controlling the neural differentiation of SCs, such as TH, and their contribution in neurogenesis is emphasized. Primitive ESCs are an ideal starting cell population for studies of gene expression and lineage segregation during developpment. As regions of the embryo are patterned and development unfolds, neural stem cells may be an essential mediator of developmental signals, acquiring a changing repertoire of gene expression**,** morphology and behavior. Markers for neural stem cells will allow their selection from different stages and regions to examine their potential after transplantation into the embryo or adult, and a comparison of their gene expression.

Biological disorders of the abnormal accumulation and processing of mutant or damaged intra- and extracellular proteins causing neurodegenerative diseases such as PD and brain tumour are in detail discussed. The elements of the signaling pathways that control neurogenesis are under further investigation. Several unexpected consistencies have emerged pointing to important areas for further investigation: the establishment of the most adequate *in vivo* and/or *in vitro* manipulations to obtain the appropriate cells for transplantation, the construction of a detailed map for clinical routes of focal and multifocal CNS disorder and the determination of the right timing for cell transplantation and the appropriate number of cells for transplantation treatments.

References

1. Till JE and Mc CE (1961) A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res. 14: 213-222.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS and Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science. 282: 1145-1147.
3. Turksen K and Troy TC (2006) Human embryonic stem cells: Isolation, maintenance, and differentiation. Methods Mol Biol. 331: 1-12.
4. Gordon MY (2008) Stem cells for regenerative medicine-biological attributes and clinical application. Exp Hematol. 36: 726-732
5. Kurtzberg J (2009) Update on umbilical cord blood transplantation. Curr Opin Pediatr. 21: 22-29.
6. Cogle CR, Guthrie SM, Sanders RC, Allen WL, Scott EW and Petersen BE (2003) An overview of stem cell research and regulatory issues. Mayo Clin Proc. 78: 993-1003.
7. Trounson A (2009) New perspectives in human stem cell therapeutic research. BMC Med. 7: 29.
8. Li ZS, Pham TD, Tamir H, Chen JJ and Gershon MD (2004) Enteric dopaminergic neurons: Definition, developmental lineage, and effects of extrinsic denervation. J Neurosci. 24: 1330-1339.
9. Kim HJ, Sugimori M, Nakafuku M and Svendsen CN (2007). Control of neurogenesis and tyrosine hydroxylase expression in neural progenitor cells through bhlh proteins and nurr1. Exp Neurol. 203: 394-405.
10. Bentz K, Molcanyi M, Hess S, Schneider A, Hescheler J, Neugebauer E, and Schaefer U (2006). Neural differentiation of embryonic stem cells is induced by signalling from non-neural niche cells. Cell Physiol Biochem. 18: 275-286.