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Prasanna S,

Chairman of Institute of Legal Education

No. 08, Arul Nagar, Seera Thoppu,

Maudhanda Kurichi, Srirangam,

Tiruchirappalli – 620102

Phone : +91 94896 71437 - info@iledu.in / Chairman@iledu.in

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MECHANISMS OF REGENERATIVE MEDICINE USING PRIMARY NULL NEURAL STEM CELLS.

AUTHOR - Y. O. KOROLOV, CANDIDATE OF LAW SCIENCE, DOCTORAL STUDENT OF THE DEPARTMENT OF LAW, PRIVATE HIGHER EDUCATIONAL ESTABLISHMENT, EUROPEAN UNIVERSITY, SPACE INTERNATIONAL COMPANY, INVIDUAL BUSINESS Y.O.KOROLOV. EMAIL: SOAHCIT@GMAIL.COM

ORCID ID: 0009-0000-2037-3419

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Introduction.

The potential use of neural stem cells (NSCs) in human clinical therapy has led to rapid progress in research into the properties of NSCs, their innate and directed differentiation potential, and induced reprogramming of differentiated cells. Cells revert to a preferential NSC-like state.

The purpose of this review is to provide an overview of our current operational definitions of NSCs, the growing understanding of extrinsic and intrinsic mechanisms, including heritable but reversible epigenetic modifications of cells, and to highlight pioneering efforts to reprogram cells to generate patient-specific stem cells for cell replacement therapy.

This is compared to the current practical procedures for the application of NSCs, as well as to the current state of the art of human regenerative medicine based on NSCs. Both of these lay the groundwork for translating recent findings into innovative clinical applications with the hope of improving the safety, efficacy, and ethical acceptability of NSC-based therapies in the near future.

Keywords: Industrystem, cellsgene, therapycellular, therapyregenerative, medicine.

Materials and Methods.

Currently, four primary methods are used to enrich neural stem cells (NSCs) in vitro. The first method involves the use of cell surface markers, with Lewis-X (LeX) and CD133 (Prominin-1) being the most widely recognized. Other surface proteins, such as Syndecan-1, Notch-1, and integrin β1, also contribute to NSC enrichment. The second method is dye exclusion, which isolates NSCs based on their ability to expel the fluorescent DNA-binding dye Hoechst 33342, a feature characteristic of side population cells. The third approach is based on morphological selection, as larger and more granular cells have been shown to contain higher numbers of NSCs. The fourth method enhances NSC survival through the addition of specific factors, such as chondroitin sulfate proteoglycans and apolipoprotein E, to the culture medium. While numerous markers, including transcription factors, are associated with NSCs, none can achieve complete purity. As a result, accurately identifying and quantifying NSCs, as well as distinguishing them from neural progenitors (NPs), remains a challenge in vitro.

Results.

So, the most almighty cell that is capable of forming the entire organism, including auxiliary structures such as the placenta, the umbilical cord is totipotent (lat. Totus – whole, whole). Egg cells fertilized by sperm (zygote) and cells resulting from the first several rounds of division of the zygote (blastomeres) are considered totipotent. With the subsequent divisions of blastomeres, a blastocyst is formed – a

hollow layer containing 150-200 cells inside, the so-called «inner cell mass». It is the cells isolated from the blastocyst that are embryonic stem cells and have the ability to form all 200+ cell types of an adult organism, except for extraembryonic structures, that is, pluripotent (Latin Plures – many). Embryonic stem cells are immortal and can reproduce endlessly, which allows them to be obtained in large quantities in the laboratory.

Prospects.

Research conducted on embryonic stem cells is important from the point of view of understanding the early stages of human development, which cannot be done in any other way, studying diseases and establishing strategies that can ultimately lead to therapy. Stem cells with pluripotent potential are absent in an adult organism. All stem cells of an adult organism, or they are also called somatic stem cells, are already somewhat specialized and can give rise to only a few types of cells within a specific tissue or organ.

Analysis of Recent Research and Publications.

NSCs (neural stem cells) and neural progenitor cells (NPs) are cultured in vitro as floating three-dimensional spheroid structures known as neurospheres. The neurosphere culture system was pioneered by Reynolds and Weiss in the early 1990s, when they discovered that embryonic and adult cortical cells could proliferate in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) 2–4. Neurospheres are composed of a heterogeneous population of cells, including subclasses at various developmental stages 5. Studying NSCs specifically within neurospheres is challenging due to the presence of NPs. Therefore, enriching NSCs from neurospheres is a critical step for focused investigations.

Problem Statement and Relevance.

Neural stem cells (NSCs) are cells that can self-renew and differentiate into three neural lineages. Here we describe a protocol for determining the frequency of NSCs in a given cell population using neurosphere formation and differentiation under clonal conditions.

Neural stem cells (NSCs) have the ability to self-renew and generate three main neural progenitors – astrocytes, neurons, and oligodendrocytes. NSCs, and neural progenitor cells (NPS), are usually cultivated in a test tube as neurospheres. This protocol describes in detail how to determine the frequency of NSCs in a given population of cells under clonal conditions. The protocol begins with the seeding of cells at a density that allows generating clonal neurospheres. The neurospheres were then transferred to the chamber cover and differentiated in accordance with clone conditions in a conditioned environment that maximizes the differentiation potential of neurospheres. And, finally, the frequency of NSC is calculated on the basis of neurosphere formation and multipotency of opportunities. Utilities of this protocol include evaluation of candidate NSC markers, purification of NSCs, and ability to distinguish NSCs from NPs. This method takes 13 days to complete, which is significantly shorter than current methods for calculating the frequency of NSC.

Formulation of the goals of the article (statement of the task).

Neural stem cells (NSCs) are cells of the central nervous system (CNS) that can self-renew and are multipotent. NSCs are first identified during embryonic forebrain development and persist in the adult brain in specific regions, such as the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus (DG) of the hippocampus. A number of NSC lines are used in clinical trials for cell regeneration.

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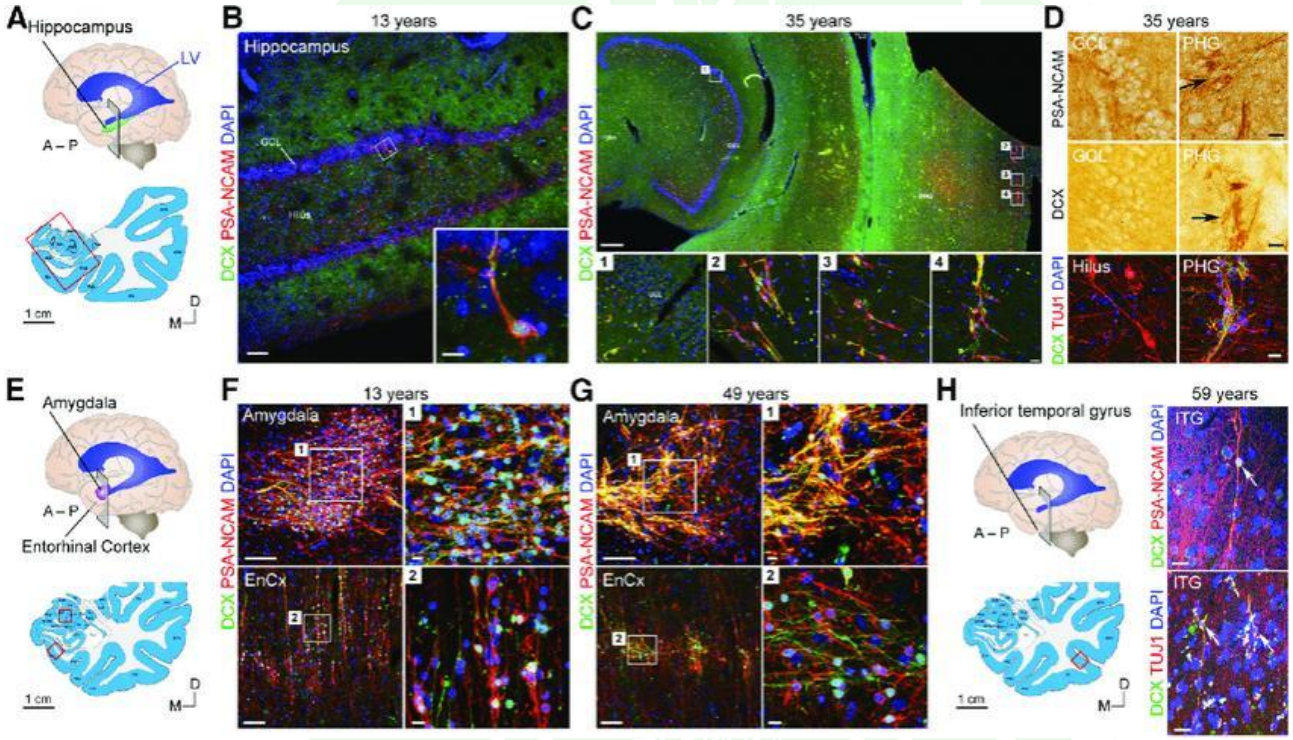
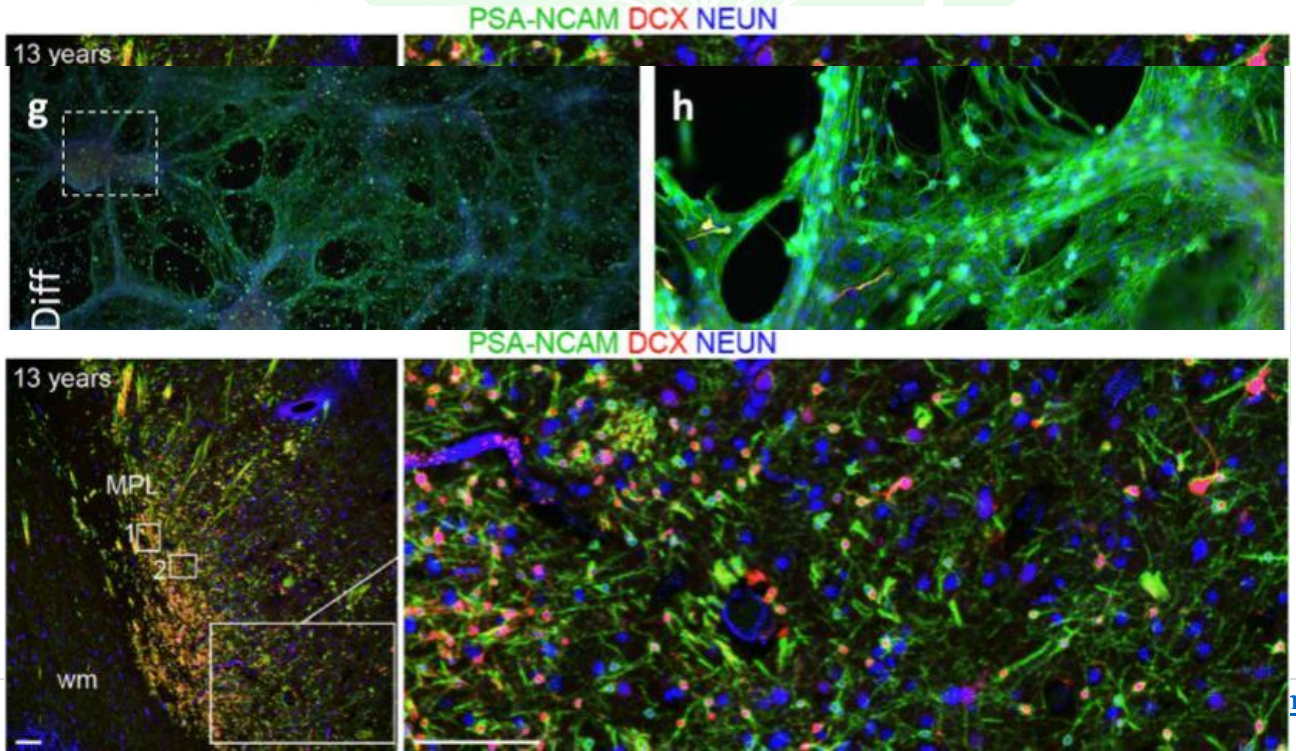
Presentation of the main research material.

Initial studies used the neurosphere formation assay (NFA) to quantify NSCs+. In this analysis, dissociated cells are cultured with the formation of neurospheres and the number of neurospheres is generated for every 100 seeded cells 100. This value is called neurosphere block formation (NFU). NFU is equal to the frequency of NSC+ if all neurospheres arise from NSCs+. However, it was shown that NFU increased the frequency of NSC+, as neurospheres are formed as NSCs+ and early NPs. It may be possible to give a quantitative assessment of NSCs+ based on their ability to self-renew, proliferate intensively and generate multipotent neurospheres.





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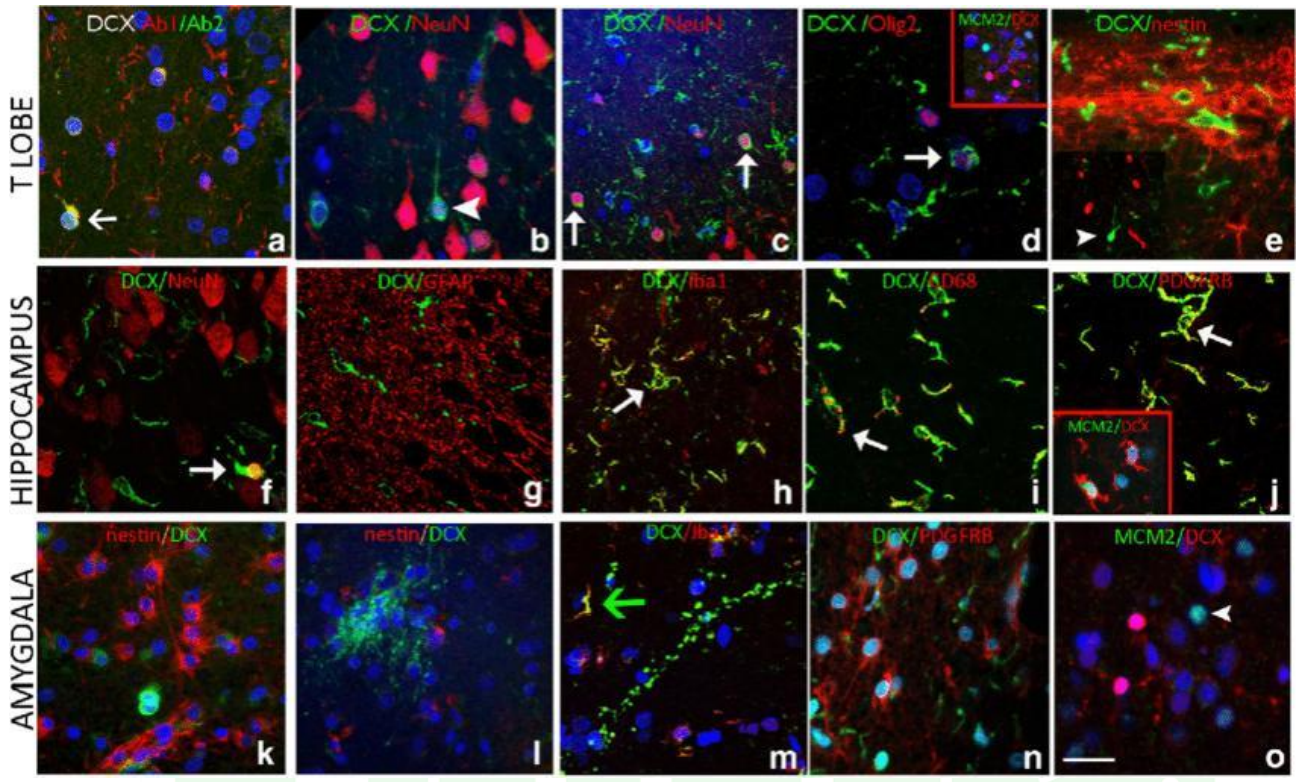
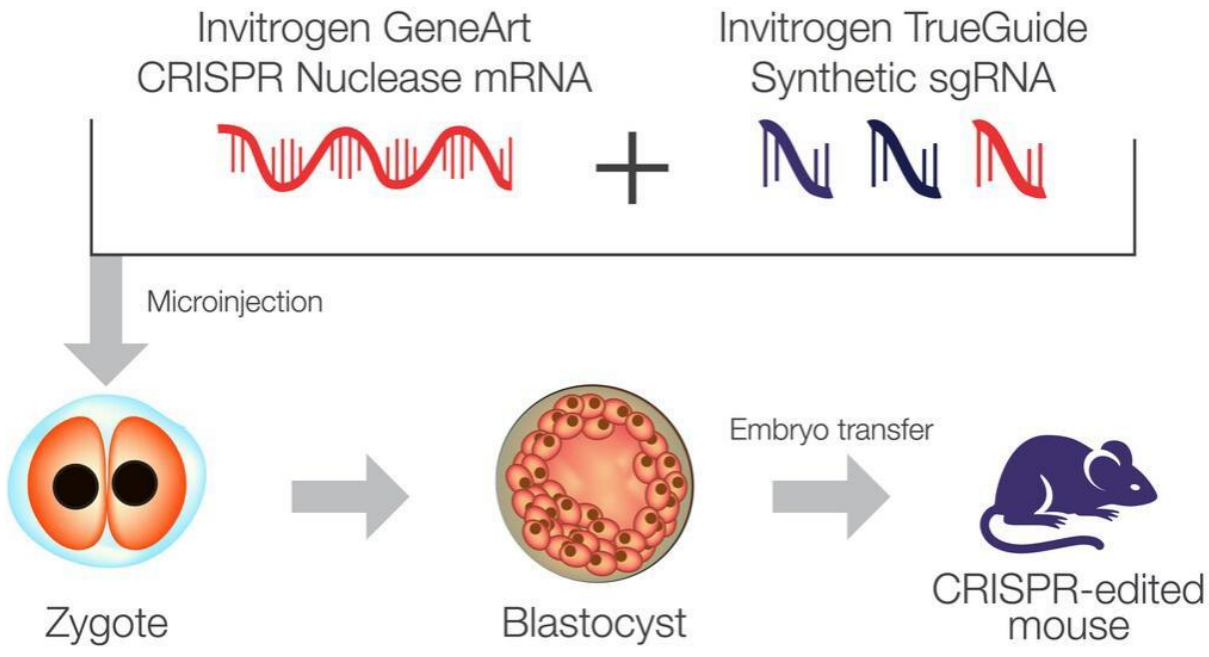


Human neural stem cells (NSK+) offer therapeutic potential, enormous prospects in medicine, offering the potential treatment of a number of conditions by enhancing regeneration and replacement of damaged tissues and organs. Due to their unique ability to regenerate and differentiate into specialized cell types, NSK+ cells pave the way for innovative therapies aimed at repairing or replacing diseased or damaged tissues in the body.[15]

Gene editing in NSCs (GE-NSCs) may enhance their therapeutic potential We show that NSCs are amenable to gene targeting at multiple loci using Cas9 mRNA.

With the help of GeneArt CRISPR Nuclease mRNA, you can simultaneously transfect up to four different



gRNAs in one well and evaluate the efficiency of cleavage of many genes simultaneously. You can analyze this approach to determine which gRNA sequence is best suited for a particular target, or edit multiple genomic loci in a single transfection.

Conclusions and prospects for further research.

Considering their ability to regenerate cells, NCS+ stem cells open new perspectives for possible methods of treatment of any cells in the body. Thus, the biology of NCS+ stem cells becomes the driving force of many researches in the field of regenerative medicine. During development, pluripotent embryonic stem cells (ESCs) give rise to all types of cells.

The ability of NSCs to generate new cells in certain niches gives hope that if this process can be strengthened and/or expanded, it can serve as a basis for the body's regenerative strategies, as well as for any diseases. Strengthening of NSC can already be part of standard clinical practice.

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