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Original Article

Sox2gene experssion on mouse embryonic stem cells in the neural diffrentiation

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Abstract:

Stem cells (SCs) are important cells for replacement therapy diseases.The interest in the potential of neural stem cells (NSCs) for the treatment of neurodegenerative diseases and brain injuries has substantially promoted research on neural stem cell self-renewal and differentiation.The geneissry-box containing gene 2 (Sox2) has been associated with a SC phenotype that predicts for poor outcomes. Sox2 is a transcription factor that regulates embryonic stem cell pluripotency and drives commitment of airway precursor cells to basal-type and neuroendocrine cells in the developing lung. Sox2gene involved will be investigated in practical pirogue according to the expression patterns.Sox2 has been associated with a SC phenotype that predicts for poor outcomes. Subsequently, information has been compiled on the question how Sox2 in neural differentiation is controlled on the molecular level, and controlled in vivo.

Keywords:
SCs- Stem cells, NSCs- neural stem cells, Sox2- geneissry-box containing gene 2, ESCs- Embryonic stem cells

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Introduction

Sox2 expression was examined in pulmonary neoplasms with respect to tumour type, differentiation and in comparison. In the mid of 1800s,andat the same timestem cells (SCs) 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 SC 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.There are also reports on the identification of genes whose expression is strictly regulated during the commitment step. Furthermore, many studies identified sub-networks of genes with a restricted expression in pluripotent ESCs, whose down regulation occurs while the OCT4, and Sry-box containing gene 2 (Sox2) might be down-regulated for driving cells towards differentiation [Trouillas et al., 2009].

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 [Till and Mc, 1961]. 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 1997: Leukaemia origin found as haematopoietic stem cell, was 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 [Thomson et al., 1998].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. 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, Parkinson's disease (PD), 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. 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 [Gordon, 2008].

A new genetic model addresses the role of Sox2 in the adult brain and provides evidence that it is involved in the maintenance of neurons in specific regions, in the proliferation , maintenance of neural stem cells and in neurogenesis. (Episkopou V.2005).

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.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, 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, liver failure, and cancer [Trounson, 2009].

The study of neuronal differentiation of ESCs has raised major interest over recent years. It allows a better understanding of fundamental aspects of neurogenesis and, at the same time, the generation of neurons as tools for various applications ranging from drug testing to cell therapy and regenerative medicine. Since the first report of hESCs derivation, many studies have shown the possibility of directing their differentiation towards neurons [Vescovi and Snyder, 1999].

Classification of stem cells

SCs can be classified into list of the sources:

-ESCs are derived from ICM of the blastocyst 7-10 days after fertilization [Das et al., 2008].

- Fetal SCs are taken from the germ line tissues that make up the gonads of aborted fetuses.

- Cord SCs - Umbilical cord blood contains SCs similar to those found in bone marrow.

- Placenta derived SCs can be harvested from a placenta as from cord blood.

- Adult SCs - Many adult tissues contain SCs that can be isolated.

Embryonic stem cells

Specifically, ESCs are derived from embryos that are developed from eggs that have been fertilized *in vitro* and then donated for research purposes with informed consent of the donors. The embryos from which hESCs are derived are typically 4 or 5 days old. ESCs are pluripotent, this means they are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes ESCs from multipotent progenitor cells found in the adult; these only form a limited number of cell types. [Bhattacharya et al., 2009]. They can form muscle cells, nerve cells, and many other cell types (Fig.1).

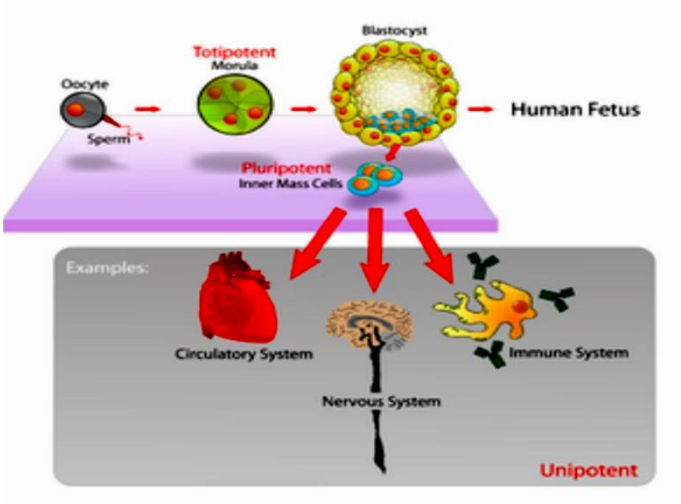


Figure 1: Pluripotent ESCs originate as IMCs within a blastocyst. The SCs can become any tissue in the body, excluding placenta. Only the morale's cells are totipotent, able to become all tissues and a placenta [Foundation, Inc, 2009].

Resources for cell replacement therapy in PD

For cell replacement therapy of neurodegenerative diseases such as PD, methods for efficiently generating midbrain Dopaminergic (mDA) neurons from ESCs cells have been investigated [Kriks and Studer, 2009]. Two aspects of DA neuron generation are considered: genetic modification and manipulation of culture conditions. A transcription factor known as critical for development of DA neurons is Nurr1 [Kim, 2004].

Stem cells differentiation

SCs can be found at different stages of fetal development and are present in a wide range of adult tissues. Many of the terms used to distinguish SCs are based on their origins and the cell types of their progeny. Today, intensive research is done the fundamental properties of SCs that are determining precisely how SCs remain unspecialized, self renewing for many years and identifying the signals that cause SCs to become specialized cells.

Embryoid bodies as model systems to study neural differentiation

NSCs are a topic of intense interest at the moment for two major reasons. First, they provide models for neural development that are easily manipulated and analyzed *in vitro*. Second, they are candidates for cellular and gene therapy of many intractable neurological disorders, EBs formed from murine ESCs recapitulate many aspects of a developing embryo[Tarasenko et al., 2004]

Sisgnaling that controls Sox2 expression-importance in neurogenesi

Neural progenitors of the vertebrate CNS are defined by generic cellular characteristics, including their pseudo epithelial morphology and their ability to divide and differentiate. SoxB1 transcription factors, including the three closely related genes Sox1, Sox2, and Sox3, universally mark neural progenitor and stem cells throughout the vertebrate CNS.It is shown that constitutive expression of Sox2 inhibits neuronal differentiation and results in the maintenance of progenitor characteristics. (Graham V,.et al 2003).

Materals and Methods

SOX2;

-Vector name pyx-ASC
- promoter T3 and T7
- Restriction enzymes T7 for sense line with Not I T3 for antisense with ECOR
- isolated Sox2 gene:

The Sox2 gene clone used in our study were isolated from different sources which is requested from the RZPD, and these cloneis,IRAVp968C04116D6(Sox2).

-Resources for colonie Sox2 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 and make plasmid preparation.

- Sox2 plasmid preparation:
Thiswas done according Birnboim Doly-protocol (Birnboim Doly) with some modifications. So, in essential with according with Birnboim Doly after that purification,
precipitation with Ammouniumacetate.
-Linearization Sox2 gene by suitable restriction enzymes :
In situ-hybridization probes were generated according to protocol which can s-tart our
preparation which published protocol. Plasmid prepared from alkaline phosphates mini-
preparation were linearization by the probe the end 5′ overhong 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.
Preparation of embryos:

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

Resuts and Discussion

A strong Sox2 gene expression investigation that there is identical signal in 10 D EB mouse
is expression ectoderm area, CNS different ion and the neuroepthelium of the
the CNS (Fig. 2). In the ohter brain of the 10 D EB mouse patrens resultexperssion
anti sense,and it is clear from the literature thathere must be at this time because it known that

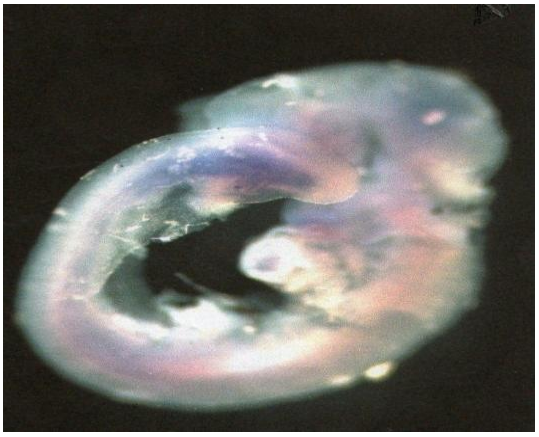
this time Sox2 gene are controlling the expression of other genes.Sox2 is one of the earliest

known transcription factors expressed in the developing neural tube. Although it is expressed
throughout the early neuroepithelium, it is show that its later expression must depend on the
activity of more than one regionally restricted enhancer element. Conversely, inhibition of

Sox2 signaling results in the delimitation of neural progenitor cells from the ventricular zone
and cause exit from the cell cycle. Which is associated with a loss of progenitor markers and

the onset of early neuronal differentiation. The phenotype elicited by inhibition of Sox2
signaling can be rescued by co expression of Sox1, providing evidence for redundant SoxB1
function in CNS progenitors.(Graham V,.et al 2003).A new genetic model addresses the role

of Sox2 in the adult brain and provides evidence that it is involved in the maintenance of neurons in specific regions, in the proliferation , maintenance of neural stem cells and in
neurogenesis. (Episkopou V.2005).
Sox2 is highly expressed in concert in most squamous cell carcinomas (SCC), but may also
influence tumour differentiation in both non-small cell lung carcinomas and pulmonary
neuroendocrine tumours [Sholl et al., 2009].



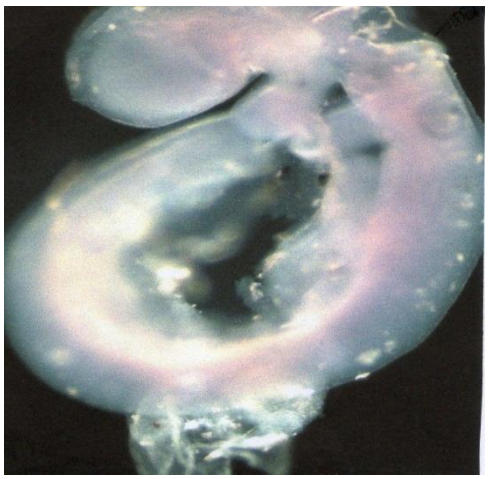


Figure 2: Sox2 gene 10 D EB mouse patrens experssion antisense.

Summary

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 Sox2, and their contribution in neurogenesis is emphasized. Primitive ESCs are an ideal starting cell population for studies of gene expression and lineage segregation during development.Sox2 is expressed highly in the neuroepithelium of the developing CNS and has been shown to function in neural stem cells. Because Sox2-null mutant mice fail to develop beyond implantation, the role of Sox2 in the CNS has lacked validation

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 Sox2 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.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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