Aus dem Zentrum Physiologie und Pathophysiologie

der Universität zu Köln

Institut für Neurophysiologie

Geschäftsführender Direktor: Universitätsprofessor Dr. med. J. Hescheler

Neurale Differenzierung embryonaler Stammzellen

Inaugural-Dissertation zur Erlangung der Würde eines

Doctor rerum medicinalium

der Hohen Medizinischen Fakultät der Universität zu Köln

vorgelegt von

Almahde Matuq Ali

aus Alqileb/Libyen

promoviert am: 19. Januar 2011

Dekan: Universitätsprfessor Dr. med. J. Klosterkötter

1.Berichterstatter: Professor Dr. rer. nat. A. Sachinidis

2.Berichterstatter: Universitätsprfessor Dr. rer. nat. G. Plickert

Eidesstattliche Erklärung

Ich erkläre hiermit, dass ich die vorliegende Dissertationsschrift ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskriptes habe ich keine Unterstützungsleistungen erhalten:

Weitere Personen waren an der geistigen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich nicht die Hilfe einer Promotionsberaterin/ eines Promotionsberaters in Anspruch genommen. Dritte haben von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertationsschrift stehen.

Die Dissertationsschrift wurde von mir bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Köln, den 10. Dezember 2009 Almahde Matuq Ali

Acknowledgements

With my God‘s grace and through His directives, I completed this dissertation.

I am very grateful to my supervisor, Prof. Dr. Agapios Sachinidis, Institute of Neurophysiology, Medical Faculty, University of Cologne, Cologne, Germany, for his hospitality to give me the opportunity to realize my thesis work as a member of his laboratory, for providing excellent facilities and for his inexhaustible encouragement and critical comments throughout my thesis work. I appreciate his vast knowledge and skill in stem cell research.

I especially thank, Prof. Dr. Günter Plickert, Institute of Zoology, University of Cologne, Cologne, Germany, for his instruction and help in understanding theoretical aspects of molecular biology, his advice in designing experimental protocols and help performing analysis, for his patience, trust, and friendship during these years as well as his helpful comments on my work. Without his expert support carrying through, this study would have been much harder and taken longer.

I wish to thank Dr. Conny Gissel, Dr Y. Liyeing, and Isaia Sotiriadou, Institute of Neurophysiology, Medical Faculty, University of Cologne, Cologne, Germany, for their help in many aspects of my work and preparation of this thesis.

I would also like to thank the former and present members of our exceptional research group, I enjoyed the atmosphere, their friendship, their support, and the times we have worked together.

I wish to thank my family for encouraging me to study abroad their eager expectations, particularly my brothers, sisters, and my late mother.

I would like to express my overwhelming gratitude to my wife Gabiss Almabruk Mohamed without her endless love and support this work could not have been done. My Childern Aisha, Jood, Hadel, and Esraa are my constant inspiration and source of happiness that have made everything worthwhile.

My thanks are due to the Ministry of Higher Education and Bureau of the Great Socialist Peoples Libyan Arab Jamahiria for their assistance and financial supports for my studies.

Dedicated

TO MY LATE MOTHER AND MY BELOVED FAMILY

TABLE OF CONTENTS

Glossary

ASCs	Adult Stem Cells
bFGF	basic Fibroblast Growth Factor
cDNA	complementary Deoxyribonucleic Acid
CSCs	Cancer Stem Cells
DA	Dopaminergic
DAT	Dopaminergic Transporter
DG	Dentate Gyros
DDC	DOPA decarboxylase
DNA	Deoxyribonucleic Acid
DOPA	Dihydroxyphenylalanine
EBs	Embryoid Bodies
ESCs	Embryonic Stem Cells
Exp4	Exportin 4
EGF	Epidermal Growth Factor
Foxa2	Forkhead box a2
hESCs	human Embryonic Stem Cell
HMG	High-Mobility Group
hNSCs	human Neural Stem Cells
HSC	Haematopoietic Stem Cells
ICM	Inner Cell Mass
LIF	Leukemia Inhibitory Factor
mDA	midbrain Dopaminergic
mESCs,	mouse Embryonic Stem Cells
MSCs	Mesenchymal Stem Cells
Ngn2	Neurog2

NT	Nuclear Transfer
NPC	Neural Progenitor Cells
NSCs	Neural Stem Cells
PD	Parkinson's Disease
PCR	Polymerase Chain Reaction
RT-PCR	Real Time-Polymerase Chain Reaction
SAGE	Serial analysis of Gene Expression
SCNT	Somatic cell nuclear transfer
SCs,	Stem Cells
SDIA	Stromal Cell-Derived Inducing Activity
SVZ	Sub Ventricular Zone
SCC	Squamous Cell Carcinomas
Sox2	Sry-box containing gene 2
RBCs	Red Blood Cells
TCF	T Cell Factor
TH	Tyrosine Hydroxylase
VM	Ventral Midbrain
WBCs	White Blood Cells

1. INTRODUCTION

Neural stem cells (NSCs) exist in the mammalian developing and adult nervous system during the neural differentiation of the embryonic stem cells (ESCs)[Bentz et al.,2006]. Stem cells (SCs) are important cells for replacement therapy diseases. The interest in the potential of NSCs 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 SCs, their properties and characteristics that distinguish SCs from other cells, the classification, and biological disorders causing neurodegenerative diseases. The literature, data and arguments that are dealing with, how the SCs 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.*

1.1. The history of 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 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. On the scientific front, it is clear that 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, 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 [Thomson et al., 1998].

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 [Turksen and Troy, 2006].

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, 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. 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. In case we cut our finger, the wound is able to heal because cells in the skin divide to fill the gap. When liver cells die, or a portion of the organ is removed surgically, the cells will divide and grow to repair the damage. RBCs, with a life span of only 120 days, have to be replaced on a daily basis. In this case, SCs, located in the bone marrow where blood cells are made, divide and differentiate into both RBCs and WBCs, thus replacing them as they wear out. 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 [Gordon, 2008].

SCs have been used experimentally to form the haematopoietic cells of the bone marrow and heart, blood vessel, muscle, and insulin-producing tissue. Embryonic germ line cells have been used to help paralyzed mice regain some of the ability to move.

Since the 1990s umbilical cord blood SCs 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 [Kurtzberg, 2009]. 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 [Cogle et al., 2003].

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, end-stage kidney disease, liver failure, and cancer [Trounson, 2009].

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.

1.2. Origins of research dealing with SCs

Many of the terms used to distinguish SCs are based on their origins and the cell types of their progeny. These cells are able to divide indefinitely, forming hundreds of copies of themselves. However, this is not the unique property which makes them so important. SCs are undifferentiated cells generally characterized by their functional capacity to both self-renew and to generate a large number of differentiated progeny cells. Conventionally, SCs are either classified as those derived from embryo or adult tissues [Kuci et al., 2009].

SCs found in adult organisms are present in most tissues and are referred to as adult SCs, such as mesenchymal stem cells (MSCs), haematopoietic stem cells (HSCs), and NSCs [Akala and Clarke, 2006]. They are considered multipotent, since they can produce mature cell types of one or more lineages, but cannot reconstitute the organism as a whole.

SCs potency largely depends on intrinsic properties of SCs as well as extrinsic cues provided by the niche (microenvironment where SCs reside). Because of their exceptional properties, SCs have the potential to be used for developmental biology and drug screening [Trujillo et al., 2009].

ESCs have the potential to differentiate into cells from all germ layers, which makes them an attractive tool for the development of new therapies. In general, the differentiation of ESCs follows the concept to first generate immature progenitor cells, which then can be propagated and differentiated into mature cellular phenotypes.

ESCs-derived neurogenesis, in which the development of neural cells follows two major steps: First, the derivation and expansion of immature neuroepithelial precursors and second, their differentiation into mature neural cells. ESCs were first isolated in the 1980s by several independent groups. These investigators recognized the pluripotential nature of ESCs to differentiate into cell types of all three primary germ lineages [Srivastava et al., 2008].

Genetic modification of the murine genome by ESCs technology is a seminal approach to understand the function of mammalian genes *in vivo*. ESCs have been reported for other mammalian species (i.e. hamster, rat, mink, pig, and cow); however, only murine ESCs have successfully transmitted the ESCs genome through the germ line. Recently, interest in SCs technology has intensified with the reporting of the isolation of primate and hESCs. ESCs cells are isolated from the inner cell mass (ICM) of the blastcyst stage embryo and, if maintained in optimal conditions, will continue to grow indefinitely in an undifferentiated diploid state. ESCs are sensitive to pH changes, temperature changes, and making it imperative to care for these cells daily. In addition, healthy cells growing in log phase are critical for optimal transformation efficiency in gene targeting experiments [Suter and Krause, 2008].

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].

Laboratory studies of SCs enable scientists to learn about the cells essential properties and what makes them different from specialized cell types. Scientists are already using SCs in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects. Research on SCs continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. SCs research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on SCs raises scientific questions as rapidly as it generates new discoveries.

In recent years there has been an increase in the need for tissue replacement in the head and neck region. The disadvantages of classical reconstructive procedures are donor site morbidity for autologous transplants and the immunogenity of allogenous transplants. Tissue engineering is a promising method for the generation of autologous cartilaginous transplants for plastic and reconstructive surgery for closure of large defects by the use of minimal amounts of material for reconstruction. For this purpose, material must be cultivated in suitable culture/carrier systems. One obstacle is the loss of phenotype and function once the cells are detached from their environment (dedifferentiation). Adult MSCs are a valuable cell source for tissue engineering. The underlying strategy of using SCs is the replacement of functionally compromised cells either by *in vitro* expanded SCs or activation of SCs in the tissue. However, there are still problems regarding valuable markers for cellular differentiation and the controlled differentiation towards a specific phenotype.

1.3. Biological properties of stem cells

Investigating the biological properties of the SCs in the laboratory can provide information about their essential properties and what makes them different from specialized cell types. It is possible to use the cells not just in cell-based therapies, but also for screening new drugs, toxins and understanding birth defects [Krtolica et al., 2009].

However, hESCs have only been studied since 1998. Therefore, in order to develop such treatments scientists are intensively studying the fundamental properties of SCs, which include:

1) Determining precisely how SCs remain unspecialized and self renewing for many years.

2) Identifying the signals that cause SCs to become specialized cells. One of the fundamental properties of a SCs is that it does not have any tissue-specific structures that allow it to perform specialized functions. SCs cannot work with other cells to pump blood through the body, like a heart muscle cell, it cannot carry molecules of oxygen through the bloodstream, like RBCs, and it cannot send electrochemical signals to other cells that allow the body to move, like a nerve cell. However, unspecialized SCs can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells. A starting population of SCs that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent SCs, the cells are lead to be capable of long-term self-renewal.

The signals in a mature organism that cause a SCs population to proliferate and remain unspecialized until the cells are needed for repair to replace a worn out cells.

Such information is critical to be able to grow large numbers of unspecialized SCs in the laboratory for further experimentation. The external signals for cell differentiation include chemicals secreted by other cells, and physical contact with neighbouring cells [Baron 2001].

1.4. Characteristics that distinguish stem cells from other cells

1. Under certain conditions SCs can be induced to become cells with special functions, such .as cells of the heart muscle or insulin-producing cells of the pancreas.
2. SCs give rise to specialized cells. When this occurs, the process is called differentiation. Signals from both inside and outside the cell may trigger this differentiation. External signals include signaling factors secreted by other cells, physical contact with other cells, and certain molecules called growth factors.
3. SCs are responsible for replacing blood and tissues on a regular basis.
4. Embryonic germ cells are similar to ESCs except they are collected from the fetus later in development. The cells come from a region known as the gonadal ridge, which will later develop into the sex organs. Because the cells are farther along in the developmental process, they are slightly limited in their ability to give rise to organs of the body [Looijenga et al., 2007].

ESCs have been reported to serve as an excellent source for obtaining various specialized cell types and could be used in cell replacement therapy. It is demonstrated the potential of ESCs to differentiate along retinal ganglion cells (RGCs) lineage. Thus, Jagatha et al., [20099] suggested that ESCs can serve as an excellent renewable source for generating RGCs that can be used to treat neurodegenerative diseases like glaucoma.

1.5. Classification of stem cells

SCs can be classified into three broad categories, based on their ability to differentiate. Totipotent SCs are found only in early embryos. Each cell can form a complete organism. Pluripotent SCs exist in the undifferentiated ICM of the blastocyst and can form any of the over 200 different cell types found in the body [Ulloa-Montoya et al., 2005]. Multipotent stem cells are derived from fetal tissue, cord blood and adult stem cells (ASCs). Although their ability to differentiate is more limited than pluripotent SCs, they already have a track record of success in cell-based therapies.

Here is a current list of the sources of SCs:

-ESCs are derived from the ICM of the blastocyst 7 to 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 - up to ten times as many SCs can be harvested from a placenta as from cord blood.

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

1.5.1. 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.

As long as the ESCs in culture are grown under certain conditions, they can remain undifferentiated. But if cells are allowed to clump together to form so-called embryoid bodies (EBs), they begin to differentiate spontaneously [Bhattacharya et al., 2009]. They can form muscle cells, nerve cells, and many other cell types (Fig.1). Although spontaneous differentiation is a good indication that a culture of ESC is alive, it is not an efficient way to produce cultures of specific cell types. So, to generate cultures of specific types of differentiated cells, heart muscle cells, blood cells, or nerve cells, for example, scientists try to control the differentiation of ESCs. They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes [Boheler et al., 2002].

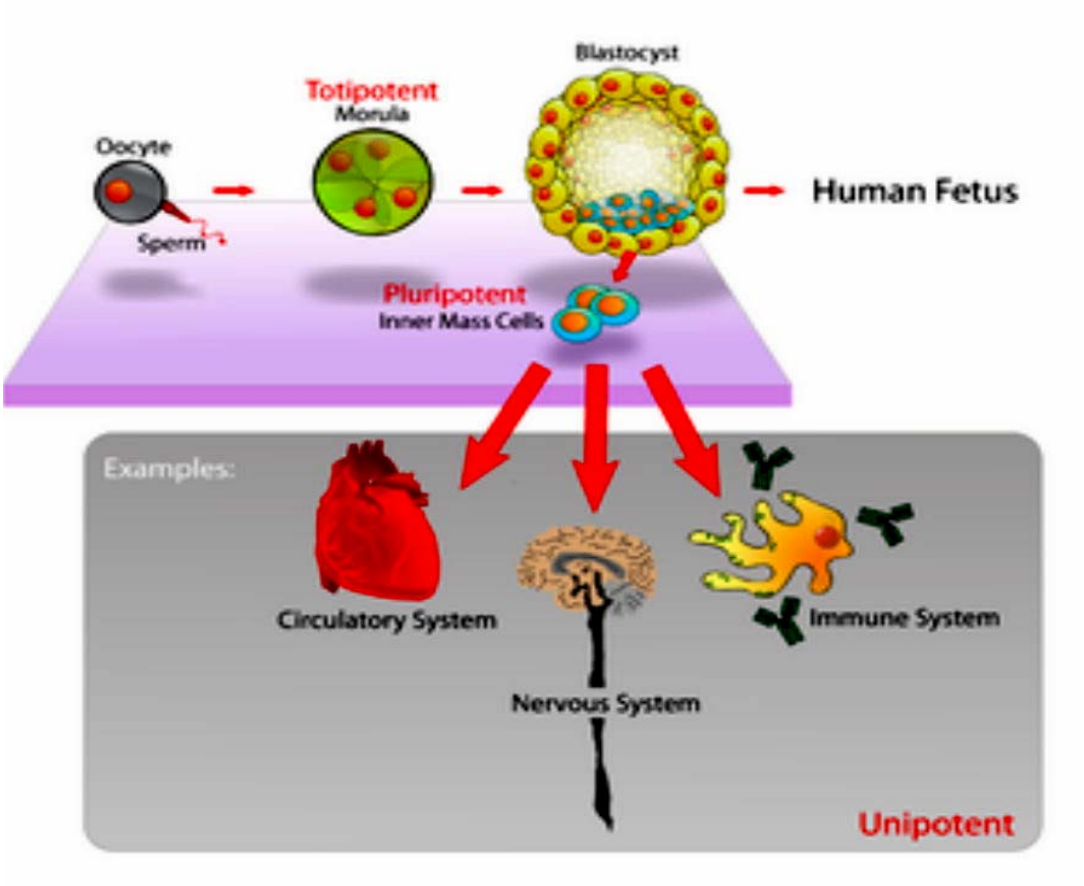

Figure 1: Pluripotent ESCs originate as IMCs within a blastocyst. The SCs can become any tissue in the body, excluding placenta. Only the morale's cells are totipotent, able to become all tissues and a placenta [Foundation, Inc, 2009].

Pluripotent cells are derived from developing mouse blastocysts *in vitro* that maintain long-term self renewal and the capacity to give rise to all cell types in the adult body, when subjected to the appropriate conditions [Ulloa-Montoya et al., 2005]. Furthermore, derivation of mouse ESCs has allowed the generation of thousands of gene-targeted mouse mutants. Culture of mouse ESCs as EBs has provided a convenient system for studying early mouse developmental processes, including several aspects of extra embryonic lineage and axis formation associated with the pre- and peri-gastrulating mouse embryo. Relatively little is known regarding the corresponding development of the early human embryo due to limitations associated with the acquisition of relevant tissue material for study.

The transfer of methods such as EBs formation to human systems should, by association, facilitate a more advanced understanding of similar processes associated with early human development. [Conley et al., 2005].

Mouse embryonic stem cells (mESCs) remain pluripotent *in vitro* when grown in the presence of the cytokine Leukemia inhibitory factor (LIF). Identification of LIF targets and of genes regulating the transition between pluripotent and early differentiated cells is a critical step for understanding the control of ESCs pluripotency. The following factors have been identified:

I. LIF-dependent genes, highly expressed in pluripotent cells, whose expression level decreases sharply upon LIF withdrawal (Puri genes).

II. LIF induced genes whose expression is differentially regulated depending upon cell context (Lifind genes).

III. Genes specific to the reversible or irreversible committed states, in addition by hierarchical gene clustering. Computer based analyses led to the characterization of different sub-types of Pure and Lifind genes, and revealed their differential modulation by Oct4 genes.

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].

ESCs, which are derived from the ICM of mammalian blastocysts, have the ability to grow indefinitely, while maintaining pluripotency and the ability to differentiate into cells of all three germ layers. hESCs might be used to treat diseases, such as PD, spinal cord injury, and diabetes [Thomson et al., 1998].

1.5.2. Adult stem cells

ASCs are found throughout the body after embryonic development that multiply by cell division to replenish dying cells and regenerate damaged tissues. Also known as somatic SCs, they can be found in juvenile as well as adult animals and humans. Interestingly in ASCs have centred on their ability to divide or self-renew indefinitely, and generate all the cell types of the organ from which they originate, potentially regenerating the entire organ from a few cells. Unlike ESC, the use of ASCs in research and therapy is not considered to be controversial as they are derived from adult tissue samples rather than destroyed human embryos. Experiments have demonstrated that ESCs can give rise to a broad range of specialized cells, such as cardiomyocytes, insulin-producing beta cells, dopaminergic (DA) neurons and others [Kriks and Studer, 2009]. These differentiated cells exhibit phenotypic properties comparable to corresponding adult cells and can be successfully used for the replacement of damaged cells in several diseases.

Very promising results have also been obtained from one type of ASCs such as MSC. MSC can be found in almost any adult organ. They can be isolated and expanded to up to hundreds of millions of cells within several weeks. New cell isolation methods may significantly enrich for the desired cell population and reduce the time required for cell expansion. MSC have got both unique biological properties and a unique molecular signature, which clearly discriminate them from other SC types. They express a strong immunomodulatory activity and secrete a variety of growth factors and cytokines. The therapeutic potential of MSC has been evaluated and they were found to be useful in both preclinical animal models and clinical trials [Spitkovsky and Hescheler, 2008].

ASCs typically generate the cell types of the tissue in which they reside. Blood-forming ASCs in the bone marrow, for example, normally gives rise to many types of blood cells such as RBCs, WBCs and platelets.

An ASCs is an undifferentiated cell found among differentiated cells in a tissue or organ that can renew it and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of ASCs in a living organism are to maintain and repair the tissue in which they are found. The term somatic stem cell can also be used instead of ASCs, where somatic refers to cells of the body. Unlike ESCs, which are defined by their origin (the ICM of the blastocyst), the origin of ASC in some mature tissues is still under investigation. The history of research on ASCs began about 50 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of SCs. The first population, called HSCs, forms all the types of blood cells in the body. A second population, called mesenchymal stem cells (MSCs), was discovered a few years later. MSCs make up a small proportion of the cells in the bone marrow, and can generate bone, cartilage, fat, and fibrous connective tissue. In the 1960s, studying on rats discovered two regions of the brain that contained dividing cells that ultimately become nerve cells. Despite these reports, most contributors believed that the adult brain could not generate new nerve cells. Up to 1990s it was agreed, that the adult brain does contain SCs that are able to generate the brain's three major cell types astrocytes and oligodendrocytes, which are non-neuronal cells, and neurons, or nerve cells [Trujillo et al., 2009].

ASCs have been found in many body tissues, and this finding has led researchers to ask whether ASCs could be used for transplants. If the differentiation of ASCs can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies. ASCs have been identified in many organs and tissues. Typically there is a very small number of SCs in each tissue, and these cells have a limited capacity for proliferation, thus making it difficult to generate large quantities of these cells in the laboratory. SCs are thought to reside in a specific area of each tissue, where they may remain non-dividing for many years until they are activated by a normal need for more cells, or by disease or tissue injury.

Among adult tissues reported to contain SCs are brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. Remarkably, in many laboratories attempts have been trying to find ways to grow large quantities of ASCs in cell culture and manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include reformation of decayed insulin-producing cells in type I diabetes and repair of the damaged heart muscle [Pal, 2009].

1.5.3. Experimental sources of SCs

SCs derived from embryos created for research by somatic cell nuclear transfer (SCNT) technique raise major ethical objections from certain parts of society, arguing from religious and other moral perspectives.

The discovery of hESCs has been one of the most exciting developments in the biological sciences in the past decade. The medical community has become very interested in the potential applications of SCs in regenerative medicine. These potential applications may involve tissue engineering, genetic engineering, and other techniques to repair, replace, or regenerate failing tissues and organs. There is little controversy regarding the application of human ASCs, but hESCs have raised a number of ethical controversies. The extent of these controversies is partly dependent on the source of ESCs

There are three currently used sources of ESCs:

1. Already existing ESCs.

2. Embryos that are left unused after *in vitro* fertilization procedures (the so-called “spare” embryos).

3. Embryos created by means of somatic cell NT technique (the same technique that was used when Dolly was created) for the purpose of conducting research [Riazi et al., 2009].

If SCs therapies became routine treatments, human embryos would become a source of therapeutic materials, and using them as merely means to achieve the ends decrease the respect for human life. The currently used sources of hESCs and research methods raise ethical objections in certain sectors of society, based on the arguments for the need of respect for the human embryo [Hug, 2005].

To realize this potential, it is essential to be able to control ESCs differentiation and to direct the development of these cells along specific pathways. Embryology has offered important insights into key pathways regulating ESCs differentiation, resulting in advances in modelling gastrulating in culture and in the efficient induction of endoderm, mesoderm, and ectoderm and many of their downstream derivatives. This has led to the identification of new multipotential progenitors for the haematopoietic, neural, and cardiovascular lineages. Development of protocols for the efficient generation of a broad spectrum of cell types including haematopoietic cells, cardiomyocytes, oligodendrocytes, dopamine neurons, and immature pancreatic beta cells. The next challenge will be to demonstrate the functional utility over these SCs, both *in vitro* and in preclinical models of human disease [Murry and Keller, 2008].

1.6. Biological disorders causing neurodegenerative diseases

Neurodegenerative diseases are the abnormal accumulation and processing of mutant or damaged intra- and extracellular proteins; this leads to selective neuronal dysfunction.

Recent advances in molecular neuroscience have begun to provide the tools to detect diseases like PD, brain disorders, neuroendocrine tumors and others early in their course and

potentially even before the development of clinical manifestations of are disease. These genetic, imaging, clinical, and biochemical tools are being validated in a number of studies.

1.6.1. 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].

Also, two culture procedures, the 5-stage method and stromal cell-derived inducing activity (SDIA) method, were used for ESCs differentiation into therapeutic DA neurons. Furthermore, using the SDIA method with treatment by signaling molecules, found Nurr1-overexpression. ESCs can differentiation to DA neurons with the highest efficiency ever reported. Importantly, the semi-quantitative and real-time polymerase chain reaction (RT-PCR) analyses demonstrate that all known DA marker genes (e.g., TH, AADC and dopaminergic transporter [DAT]) were up-regulated in Nurr1-over expressing ESCs when compared to the native ESCs. These cells produced increased dopamine compared to native D3 cells after differentiation. In the *in vivo* context after transplantation, the genetically modified ESCs also showed the highly increased DA neuronal phenotypes. Thus, the combination of genetic engineering and appropriate culture conditions provides a useful tool to generate a good cell source from ESCs for cell replacement therapy of degenerative diseases such as PD [Kim et al., 2007].

There are four primary symptoms of PD: (i) tremor, or trembling in hands, arms, legs, jaw, and (ii) face rigidity, stiffness of the limbs, (iii) impaired balance and (iv) coordination. As these symptoms become more pronounced, patients may have difficulties in walking,

talking, or completing other simple tasks. PD usually affects people over the age of 50. Early symptoms of PD are subtle and occur gradually. In some people the disease progresses more quickly than in others. As the disease progresses, the shaking, or tremor, which affects the majority of PD patients may begin to interfere with daily activities. Other symptoms may include depression and other emotional changes; difficulty in swallowing, chewing, and speaking; urinary problems or constipation; skin problems; and sleep disruptions. There are currently no blood or laboratory tests that have been proven to help in diagnosing sporadic PD [Shagam, 2008].

Therefore the diagnosis is based on medical history and a neurological examination. The disease can be difficult to diagnose accurately. Doctors may sometimes request brain scans or laboratory tests in order to rule out other diseases. Therapy study is investigating DA enzyme gene therapy and neurotrophic gene therapy in animal models of PD. The investigators are comparing different genes and testing different gene delivery approaches, and developed a viral gene vector with a special modification that allows the introduced gene to be temporarily, when a small dose of a specific antibiotic is given to the patient. The development of this vector should permit researchers to better control the delivery of genes once the vector is in the host. The researchers are now conducting safety and toxicity studies of this new vector with the hope that it will be proved safe enough for testing in humans [Chen and Le, 2006].

For many years, it was recognized that brain and spinal cord tissues could not be regenerated once they were damaged. Recently, this concept has been challenged and many basic and clinical studies regarding neural regeneration and transplantation have been reported. The problems result from the death of specialized brain cells called dopamine neurons. These cells produce dopamine, a compound that helps control muscle movements.

The effects of the disease can be treated with drugs that help increase dopamine in the brain, but there is no known cure.

Projects aim for the PD knew that the problem was to replace the dead dopamine neurons with healthy dopamine-producing cells, or the treatment of PD and cerebral ischemia. The biological features for various types of SCs have been widely investigated and applied to the treatment of neurological disorders through cell transplantation [Date and Yasuhara, 2009].

Derivation of midbrain dopamine DA neurons from hESCs has been of particular interest because of the clinical potential for DA neuron transplantation in patients with PD. Several protocols for DA neuron differentiation from mESCs and hESCs have been reported: however, protocols involving hESCs have yet to be improved [Sonntag et al., 2007]. A slightly modified SDIA method, consisting four different culture stages, was developed to show that KHES-1 cells differentiate into TH-positive DA neurons. These results provide valuable information that will assist in efficient DA neuron differentiation from hESCs and for future transplant application. Dopamine replacement therapy is useful for treating motor symptoms in the early phase of Parkinson's disease, but it is less effective in the long term.

1.6.2. Integration of transplanted therapeutic cells into functional context during brain disorder therapy

Neurogenesis occurs throughout adulthood in the mammalian brain by coordinated proliferation and differentiation of adult NSCs. Newborn neurons are incorporated into the functional networks of both the olfactory bulb [Imayoshi et al., 2009].

Neurogenesis in mammals was considered to occur only during embryonic and early post-natal development. It was believed to play no significant role in the adult nervous system. However, it is now accepted that neurogenesis occurs in two brain regions in adult mammals, namely, the hippocampus and the olfactory bulb.

In both regions new neurons arise from a resident population of neural progenitor cells (NPCs) that are maintained throughout adult life. Hippocampus neurogenesis is required for some types of hippocampus-dependent learning. Many factors enhance hippocampus neurogenesis including hormones, growth factors, drugs, neurotransmitters, and physical exercise as well as learning a hippocampus-dependent task. Other factors suppress hippocampus neurogenesis; these include aging, stress, glucocorticoids and stimuli that activate the pituitary/adrenal axis. Indeed all major pharmacological and non-pharmacological treatments for depression enhance hippocampus neurogenesis and suppressing hippocampus neurogenesis in mice blocks behavioural responses in some antidepressant-sensitive tests. Altered hippocampus neurogenesis may also play a path physiological role in neurodegenerative disorders such as Alzheimer's disease. Neural progenitors are found throughout the neuritis including both neurogenic and non-neurogenic regions. When cultured *in vitro* or isolated and transplanted back into neurogenic brain regions, these cells can differentiate into neurons although in their *in situ* location they seem to behave as lineage-restricted glial progenitors. The environmental cues that limit the potential of progenitor cells in non-neurogenic brain regions are unknown. However, an emerging view is that astrocytes, a subset of which also functions as NPCs, are critical in regulating the local environment. After transplantation into adult brain, NSCs are capable of surviving and differentiating into both neurons and glial cells, offering hope that stem cell therapy may be utilized to treat a variety of neurological and perhaps psychiatric disorders. The widespread existence of endogenous neural progenitors even in non-neurogenic brain regions also offers hope that the potential of these cells may be harnessed to repair cellular injuries caused by injuries such as stroke, trauma or neurodegenerative diseases [Elder et al., 2006].

Generation of new neurons persists in the two restricted regions of adult brain, the dentate gyros (DG) of the hippocampus and the sub ventricular zone (SVZ) [Zappone et al., 2000].

Newly generated neurons are functionally integrated into the neuronal circuits, which is involved in regulation of brain plasticity. Endogenous neuronal production in the DG and SVZ is expected to provide a continuous source of new neurons that replace degenerated neurons in the injured brain. Recent studies indicate that adult neurogenesis is modified by various brain insults including stroke, epilepsy and neurodegenerative disorders. While up-regulation of neurogenesis in these situations may partially contribute to restoration and regeneration of damaged neural tissues, inadequate cell differentiation and/or excessive supply of new neurons should disturb existing neural circuits. For the development of successful regenerative medicine for injured brain, it is one has to understand more precisely and comprehensively the mechanisms that regularities adult neurogenesis [Kaneko and Sawamoto, 2009].

1.6.3 Brain tumor stem cells are still explored by the same approach?

A major drawback to the use of SCs remains the demonstrated tendency of such cells to grow into a specific kind of tumor, called teratoma, when they are implanted in laboratory experiments into mice. It is assumed that this tumorigenic feature will be manifested upon transplantation to human patients as well. The development of tumors from ESCs is especially puzzling given that these cells start out as completely normal cells.

A study of neoplastic tissues has provided evidence of self-renewing, stem-like cells within tumors, which have been called cancer stem cells (CSCs). CSCs constitute a small minority of neoplastic cells within a tumor and are defined operationally by their ability to seed new tumors. CSCs were first identified in the hematopoietic system more recently, however, also have been discovered in solid tumors, including those arising in the breast, colon, and brain [Aleckovic and Simon, 2008].

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. Sox2 expression was examined in pulmonary neoplasms with respect to tumour type, differentiation and in comparison. A strong Sox2 expression was detected in 23% of low-grade and 72% of high-grade neuroendocrine carcinomas. 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].

1.7. 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.

SCs hold the key to a number of cellular processes from development, tissue regeneration, and can change into virtually any type of specialized cell. These cells are helpful in treating diseases because they can help repair any type of damaged cells, help repair defects in tissue, and regenerate tissues. They are unspecialized cells with abilities that have made them a great focus of medical research. These cells are able to divide indefinitely, forming hundreds of copies of themselves. However, that is not a unique property. What makes them so important is that within them, SCs hold immense potential and categorized as pluripotent. SCs are undifferentiated cells generally characterized by their functional capacity to both self-renew and to generate a large number of differentiated progeny cells [Boheler, 2009]

1.7.1. In vivo differentiation in the course of neurogenesis

Differentiation *in vivo* engraftment pattern of unapparent ICM cells in fetal brains are not primarily due to limitations in the proliferation or differentiation properties of unapparent NPCs. Attempts have been made a difficult task as they try to realize the therapeutic potential of SCs and neurons. To better understand how to manipulate these cells, they need to monitor the gene-expression patterns, as well as working out how these genes are controlled.

1.7.1.1. Neurogenesis of stem cells *in vivo*

NSCs are the most primordial and least committed cells of the nervous system, the cells that exist before regional specification develops. In contrast to a "progenitor" or "precursor “a single neuroectodermally-derived cell must fulfill an operational definition that is essentially similar to that used in hematopoietic. The functional properties which are as follows:

1. "Multipotency" i.e. the ability to yield mature cells in all three fundamental neural lineages throughout the nervous system neurons (of all subtypes) and astrocytes (of all types).
2. The ability to populate a developing region and/or repopulate an ablated or degenerated region of the nervous system with appropriate cell types.
3. The ability to be serially transplanted.
4."Self-renewal" i.e. the ability to produce daughter cells (including new NSCs) with identical properties and potential. Having identified a murine neural cell clone that fulfills this strict operational definition, then it could be subjected to be examined by gene expression profiles:

(a) A multipotent somatic stem cell from another organ system the hematopoietic SCs.

(b) A pluripotent stem cell derived from the ICM and hence without organ assignment.

ESCs, hSCs, and operationally-defined NSCs all of which have been identified not only by markers but by functional assays in their respective systems and whose state of differentiation could be synchronized shared in a large number of genes. Although, as expected, the most stem-like genes were expressed by ESCs, NSCs and HSCs shared a number of genes. CNS-derived neurospheres, on the other hand, expressed fewer "stem-like" genes held in common by the other operationally-defined stem cell populations. Rather they displayed a profile more consistent with differentiated neural cells. Furthermore, when operational definitions are employed, a common set of stem-like genes does emerge across both embryonic and somatic SCs of various organ systems, including the nervous system [Ahmed, 2009].

ESCs transplantation offers new therapeutic strategies for neurodegenerative diseases and injury. However, the mechanisms underlying integration and differentiation of engrafted ESCs are poorly understood. Some study elucidates the influence of exogenous signals on ESCs differentiation using in vitro modeling of non-stem/stem cell interactions. Under these conditions, ESCs differentiation was predominantly directed towards a glial fate. Treatment of ESCs with endothelial cell-or astrocyte-conditioned medium promoted neuronal as well as glial differentiation. This indicates that ESCs fate is determined by endothelial and glial cells that comprise the environmental niche of these SCs *in vivo*. The direction of differentiation processes appears to depend on humeral factors secreted by adjacent cell lines [Bentz et al., 2006].

The potential to generate virtually any differentiated cell type from ESCs offers the possibility to establish new models of mammalian development and to create new sources of cells for regenerative medicine. To realize this potential, it is essential to be able to control ESCs differentiation and to direct the development of these cells along specific pathways.

Embryology has offered important insights into key pathways regulating ESCs differentiation, resulting in advances for modeling gastrulating in culture and in the efficient induction of endoderm, mesoderm, ectoderm and many of their downstream derivatives. This has led to the identification of new multipotential progenitors for the hematopoietic, neural, and cardiovascular lineages. Development of protocols for the efficient generation of a broad spectrum of cell types including hematopoietic cells, cardiomyocytes, oligodendrocytes, dopamine neurons, and immature pancreatic beta cells. The next challenge will be to demonstrate the functional utility of these cells, both *in vitro* and in preclinical models of human disease [Murry and Keller, 2008].

A better understanding of fundamental aspects of neurogenesis and, at the same time, the generation of neurons as tools for various applications is rising from drug testing to cell therapy and regenerative medicine. However, there are still many challenges ahead, including gaining a better understanding of the mechanisms involved and developing techniques to allow the generation of homogeneous neuronal and glial subtypes. It was reviewed, that the current state of knowledge of embryonic neurogenesis has been acquired from animal models. Several aspects of current protocols which need to be optimized for generate high-quality ESCs-derived neuronal precursors suitable for clinical applications [Suter and Krause, 2008].

NSCs are present during embryonic development and in certain regions of the adult CNS. Mobilizing adult NSCs to promote repair of injured or diseased CNS is a promising approach. Since NSCs may give rise to brain tumor, they represent *in vitro* models for anti-cancer drug screening. To facilitate the use of NSCs in clinical scenarios, there is the need to explore the biology of these cells in greater details. One clear goal is to be able to definitively identify and purify NSCs. The neurosphere-forming assay is robust and reflects the behavior of NSCs. Neurosphere formation in combination with other markers of NSCs behavior such as active Notch signaling represents the state of the art to follow these cells.

Many issues connected with NSCs biology need to be explored to provide a platform for clinical applications [Ahmed, 2009].

It was indicated that hSCs populations harvested from the adult have low or undetectable telomerase levels, age in culture, and may not be propagated indefinitely. Therefore, shown that SCs age and as such, their properties will have changed depending on the age of the individual from which they are harvested, and the time for which they are propagated in culture. Others have shown that cells maintained in culture may undergo alterations as they are propagated, and that these alterations may alter the predicted behavior of SCs. Researchers in the SCs field focused on translational work need to develop a practical plan that takes into account such difficulties while developing manufacturing protocols, designing animal studies, or developing trial protocols [Rao and Vemuri, 2009].

1.7.1.2 Signaling in neurogenesis and genes controlling differentiation in the mouse

Both SCs and neurons are not easy to maintain in culture, and it is hard to introduce, deoxyribonucleic acid (DNA) or, ribonucleic acid (RNA) molecules into them to target specific genes or pathways. Nonetheless, there is a success in determining the expression of thousands of genes and comparing expression patterns between different cells or cells grown under different conditions. Such work has allowed identifying, for example, master regulators of SC differentiation or neuronal survival. In addition, a wide variety of tools has been designed specifically for use in SCs or neurons to control the expression of a gene of interest and study its function. One useful technique, serial analysis of gene expression (SAGE) allows identifying all the genes involved in a particular process, such as the migration or the differentiation of SCs. SAGE is an open platform for monitoring the expression patterns of thousands of transcripts in one sample and can lead to the discovery of novel genes.

The technique relies on the generation of a library of short complementary DNA (cDNA) ‘tags’ each corresponding to a sequence near the 3 end of every transcript in a cell or tissue sample. The Genome Analysis System uses a tag amplification step on the surface of a glass flow cell. Methods such as SAGE and the ever-popular microarrays can be at the complement of transcripts isolated from a population of cells [Robson, 2004].

A variety of new technologies, such as DNA microarray technology, are revolutionizing the way molecular mechanisms underlying learning and memory can be explored. A genomic approach can be used to dissect and analysis the complex dynamic interactions involved in gene regulation during learning and memory. Illustration of the changes of serotonin receptor subtypes has been observed in different time, domains and behavioral paradigms [Cavallaro, 2008].

1.7.1.3. Importance of Wnt signaling neurogenesis

Regulators of Wnt signaling are involved in several neurodevelopment processes. Recent results indicate that Wnts are key regulators of proliferation and differentiation of DA precursors during ventral midbrain (VM) neurogenesis. Different Wnts have specific and unique activity profiles. Interestingly, chemical inhibitors of glycogen synthesis kinase-3beta stabilize beta-catenin and increase DA differentiation in VM precursor cultures. It has been proposed that Wnts likely contribute in the future to improve stem/precursor cell replacement therapy approaches [Castelo-Branco and Arenas, 2006].

The normal generation of mDA neurons *in vivo* is still rudimentary, despite many attempts to recapitulate the underlying events *in vitro*. Because the loss of these neurons is implicated in PD, this lack of information is one of the major setbacks in the development of better therapies for this severe human neurological disorder.

Recently, substantial advances have been made by demonstrating that the secreted molecule Wnt1 regulates a genetic network, including the transcription factors Otx2 and Nkx2-2.

In addition, Wnt1 appears to regulate the differentiation of the post mitotic progeny of these precursors by initiating the expression of mDA-specific transcription factors. Wnt directed target genes are Wnt proteins form a family of highly conserved secreted signaling molecules that regulate cell-to-cell interactions during embryogenesis (Table 1) Wnt genes are secreted glycoprotein that drives signaling pathways contributing to cell fate determination, spatial-temporal patterning, and cell motility. Insights into the mechanisms of Wnt action have emerged from several systems, from mouse genetics, *Drosophila* and *Caenorhabditis elegance* [Van Hoffelen and Herman, 2008]. Mutations in Wnt genes or Wnt pathway components lead to specific developmental defects, while various human diseases, including cancer, are caused by abnormal Wnt signaling. Through several cytoplasm relay components, the signal is transducer to beta-catenin, which then enters the nucleus and forms a complex with T cell factor (TCF) to activate transcription of Wnt target genes [Vendrell et al., 2009].

The Wnt\beta-catenin pathway is the best understood Wnt signaling pathway, and its core components are highly conserved during evolution. As the amount of beta-catenin rises, it accumulates in the nucleus, where it interacts with the transcription factors, leading to regulation of target genes.

In the nucleus, before Wnt signaling, LIF and TCF homology's bind to DNA with sequence specificity in promoter and enhancer regions of target genes .

One of the most reliable criteria for the genes to be target gene is the present of TCF/LEF finding site of the regulatory region of the gene. In addition further criteria are found in data of Wnt target gene characters including further arguments that ingest a gene to be a Wnt-target are:

I. The prospective TCF/LIF sites actually bind these factors *in vitro*.

II. The enhancer/promoter region driving expression of a reporter such as luciferase is activated by B-catenin *in vivo*.

III. Beta-catenin-dependent reporter activity can be blocked with a dominant-negative TCF/LIF protein.

IV. The ability of the reporter to be induced above basal levels by B-catenin is abrogated by mutation of the consensus TCF/LIF sites. In some but not all cases, mutation of the TCF/LIF sites increases basal expression of the reporter construct.

In the absence of a Wnt signal, the beta-catenin turnover complex enhances beta-catenin, N-terminal phosphorylation by CK1 and GSK-3 (Fig.2). This generates a recognition signal for components of the ubiquity ligase pathway leading to the destruction of beta-catenin. In the presence of Wnt ligands, the function of the beta-catenin turnover complex is impaired through the action of the dishevelled protein leading to the accumulation of beta-catenin which then translocates to the nucleus and acts as a co-transcription factor with members of the TCF DNA binding protein family. Mutations to Wnt, Axin, APC, beta-catenin and TCF family members have been shown to induce tumours and activate TCF-dependent transcription. Other signalling pathways that are independent of beta-catenin are activated by Wnt signalling. As a consequence of the increased nuclear accumulation of beta-catenin, the ability to activate transcription of Beta-catenin-TCF/LIF target genes that are required for osteoblastic differentiation was up regulated [Zhou et al., 2009].

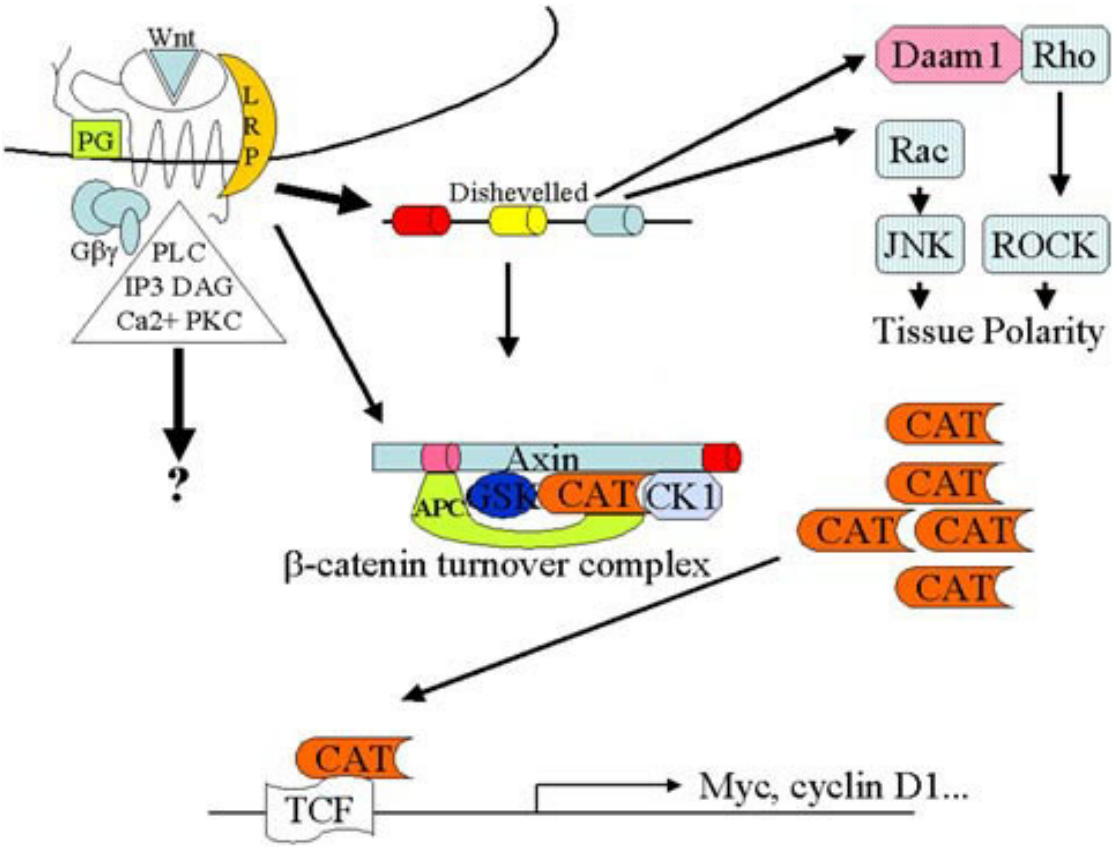

Figure 2: In the absence of a Wnt signal, the beta-catenin turnover complex enhances beta-catenin, N-terminal phosphorylation by CK1 and GSK-3. This generates a recognition signal for components of the ubiquitin ligase pathway leading to the destruction of B-catenin. In the presence of Wnt ligands, the function of the beta-catenin turnover complex is impaired through the action of the dishevelled protein leading to the accumulation of beta-catenin which then translocates to the nucleus and acts as a co-transcription factor with members of the TCF DNA binding protein family. Other signalling pathways that are independent of B-catenin are activated by Wnt signalling [Dale, 2008].

Table 1: List of target genes of Wnt B/catenin signaling. Direct targets are defined as those with TCf binding sites:

Gene	Organism/system	Direct/Indirect	up/down
Ac-myc	human colon cancer	yes	up
A Cyclin D	human colon cancer	yes	up
Tcf-1	human colon cancer	yes	up
LEF1	human colon cancer	yes	up
PPARdelta	human colon cancer	yes	up
c-jun	human colon cancer	yes	up
fra-1	human colon cancer	yes	up
uPAR	human colon cancer	?	up
matrix metalloproteinase MMP-7	human colon cancer	yes	up
Axin-2	human colon cancer	yes	up
Nr-CAM	human colon cancer	yes	up
ITF-2	human colon cancer	yes	up
Gastrin	human colon cancer	?	up
A CD44	human colon cancer	?	up
EphB/ephrin-B	human colon cancer	?	up/down
BMP4	human colon cancer	?	up
claudin-1	human colon cancer	yes	up
Survivin	human colon cancer		up
VEGF	human colon	yes	up up/down
FGF18	human colon cancer	yes	up
Hath1	human colon cancer		down
Met	human colon cancer		up

Gene	Organism/system	Direct/Indirect	up/down
endothelin-1	human colon cancer		up
c-myc binding protein	human colon cancer	yes	up
L1 neural adhesion	human colon cancer		up
Id2	human colon cancer	yes	up
Tiam1	Colon tumors		
Nitric Oxide Synthase 2	Hepg2 cells		up
Dickkopf	Various cells, tumors		up
FGF9	ovarian endometrioid adenocarcinoma		up
FGF20	Various cells, tumors		
LGR5/GPR49	Intestine	yes	up
Sox9	Intestine		up
Sox9	mesenchyme		down
Runx2	chondrocytes		up
Gremlin	fibroblasts		up
SALL4			
RANK ligand	Osteoblasts		down
CCN1/Cyr61	Osteoblasts		up
Sox2	Xenopus retina		up
Pituitary tumor transforming gene (PTTG)	esophageal squamous cell carcinoma		
Delta-like 1	somites		
FoxN1	thymus	?	yes
matrix metalloproteinase-26	Human		
Oct 4	ESCs		
Frizzled 7	EC cells	yes	up
Follistatin	EC cells, ovary	yes	up
Fibronectin	Mouse lung		up
Islet1	Cardiac cells		up

Gene	Organism/system	Direct/Indirect	up/down
MMP2, MMP9	T cells		
Siamois	Xenopus	yes	up
fibronectin	Xenopus	yes	up
BMP4	Xenopus	?	down
myogenic bHLH	Xenopus	?	up
engrailed-2	Xenopus	yes	up
Xnr3	Xenopus	yes	up
connexin43	Xenopus, Mouse	yes	up
twin	Xenopus	yes	up
connexin 30	Xenopus	?	
retinoic acid receptor gamma	Xenopus	?	
dharma/bozozok	Zebra fish	yes	up
MITF/nacre	Zebra fish	yes	up
Stra6	Wnt-1 transformed mouse cells	? co-induced by Wnt plus RA	up
Wrch-1	Wnt-1 transformed mouse cells	? Not through TCF	up
TNF family 41BB ligand, ephrinB1, Stra6, autotoxin and ISLR	Wnt-1 transformed mouse cells	By Wnt plus retinoic acid	up
Twist	Wnt1 induced mammary cancer		up
Stromelysin	Wnt-1 transformed mouse cells		up
WISP	Wnt-1 transformed mouse cells	yes, but not through TCF	up
Brachyury	Mouse (Wnt-3A)	yes	up
Proglucagon	Mouse	?	up

Gene	Organism/system	Direct/Indirect	up/down
Osteocalcin	Mouse	yes	down
Cdx1	Mouse embryo		
cyclooxygenase-2	mouse (Wnt-1)	?	up
Irx3 and Six3	Mouse brain		
neurogenin 1	Mouse brain	yes	up
SP5	Mouse brain	yes	up
Nkx2.2	Neural tube	yes	down
WISP-1, WISP-2, IGF-II,Proliferin-2,Proliferin-3, Emp, IGF-I, VEGF-C, MDR1, COX-2, IL-6	3T3-L1 Preadipocytes	?	up
A periostin	Mouse Wnt-3	A not through B-catenin?	down
Cdx1	Mouse Wnt-3A	yes	up
Cdx4	Mouse Wnt-3A		
Cdx4	Zebra fish HSC	?	up
A betaTrCP		independent of transcription	up
sFRP-2	Mouse (Wnt-4)	?	up
Pitx2	pituitary	yes	up
EGF receptor	Liver		up
E-cadherin	Mouse hair follicle	yes	down
Keratin	Mouse hair follicle	yes	up
movo1	Mouse hair follicle	yes	up
Jagged1	Mouse hair follicle		up
P16ink4A	Melanocytes	yes	down
CTLA-4	Melanomas	yes	up

Gene	Organism/system	Direct/Indirect	up/down
A mBTEB2	Mouse	A independent beta-catenin	up
FGF4	Mouse tooth bud	yes	up
Interleukin8	Endothelial cells		
ret	rat PC12	?	up
connexin43	Rat cardiomyocytes	?	up
versican	Vascular smooth muscle cells	yes	up
Tnfrsf19	Somitic mesoderm	yes	up
Ubx	Drosophila	yes	up or down
wingless	Drosophila	?	up or down
Â Dpp	Drosophila	yes	down
Engrailed	Drosophila	?	up
Dfrizzled2	Drosophila	?	down
shaven baby	Drosophila	?	down
stripe	Drosophila	yes	down

1.7.1.4. Signaling that controls TH expression-importance of TH in neurogenesis

In particular, Tyrosine Hydroxylase (TH) genes have been investigated in numerous association studies, that have produced contrasting results, suggesting that genomic imprinting may be operating in bipolar disorder [Muglia et al., 2002].

Genetic defect of neurodegenerative diseases such as PD, methods for efficiently generating mDA neurons from ESCs have been investigated. 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, Nurr1, was introduced into ESCs to see how they facilitate the generation of DA neurons from ESCs. Also, two culture procedures, the 5-stage method and SDIA method, were used for ESCs differentiation. Furthermore, using the SDIA method with treatment of signaling molecules, it is found that Nurr1-overexpressing ESCs can differentiate to DA neurons with the highest efficiency ever reported. Importantly, the semi-quantitative and RT-PCR analyses demonstrate that all known DA marker genes were up-regulated in Nurr1- over expressing ESCs when compared to the native ESCs. Thus, the combination of genetic engineering and appropriate culture conditions provides a useful tool to generate a good cell source from ESCs for cell replacement therapy of degenerative diseases such as PD [Kim et al., 2007].

There are two non-allelic genes encoding TH from the diploid teleosts, *Barramundi* species. TH1 is the homologue of the higher vertebrate TH genes and encodes a protein of 489 amino acids that shares 90% sequence identity to the THs of other teleost species. A second non-allelic TH2 gene encodes a protein of 472 amino acids and shares 62% identity with TH1 and the vertebrate THs.TH1 mRNA is found in the brain and kidney of *Barramundi*, while TH2 mRNA is found only in brain [Candy and Collet, 2005]. It is reported that transcripts encoding TH and the DA transporters are present in the murine bowel [Date and Yasuhara, 2009].

The DA system has been previously associated to behavioural facilitation and aggression. There were studied gene variants in the TH and DOPA decarboxylase (DDC) genes. In the genetic variation is known for a sample of 571 individuals consisting of 167 German suicide attempters. TH variants were not associated with suicide and related traits. There as DDC variants could mediate some features related to suicide and be involved in violent suicidal behaviour [Giegling et al., 2008].

Personality influences several characteristics of normal and pathologic behaviours and it is associated with neurotransmitter systems that are under genetic control. The DA system has been proposed to play a role in the modulation of personality traits. Variants of the TH and DDC genes were investigated in 111 suicide attempters and 289 healthy subjects to assess the involvement of the DA synthesis pathway in personality traits. No strong evidence was found for the associations between personality and TH or DDC in overall tests. An interaction effect of genotype and diagnosis was present, with TH and DDC SNPs having a greater effect on the respective personality dimensions in the group of suicide attempters. These findings should be interpreted with highest caution. Direct replication attempts within independent groups of suicide attempters will help to resolve this question [Giegling et al., 2009].

1.7.1.5. Signaling that controls Sox2 expression-importance of Sox2 in neurogenesis

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 SCs throughout the vertebrate CNS.

It is shown that constitutive expression of Sox2 inhibits neuronal differentiation and results in the maintenance of progenitor characteristics.

Conversely, inhibition of Sox2 signaling results in the delimitation of NPCs from the ventricular zone and cause exit from the cell cycle. 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 et al., 2003].

Sox2 is expressed highly in the neuroepithelium of the developing CNS and has been shown to function in NSCs. Because Sox2-null mutant mice fail to develop beyond implantation, the role of Sox2 in the CNS has lacked validation. 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 NSCs and in neurogenesis [Episkopou, 2005].

The identification of NSCs in situ has been prevented by the inability to identify a marker consistently expressed in all adult NSCs and is thus generally accomplished using the *in vitro* neurosphere-forming assay. Transcription factor Sox2 is expressed in embryonic neural epithelial SCs. These cells are thought to give rise to the adult NSC population. It is hypothesized that Sox2 may continue to be expressed in adult NSCs. Furthermore, all neurospheres derived from these neurogenic regions express Sox2, suggesting that Sox2 is indeed expressed in adult NSCs. It demonstrates that NSCs are heterogeneous within the adult brain, with differing capacities for cell production. *In vitro,* all neurospheres express Sox2, but the expression of markers common to early progenitor cells within individual neurospheres varies; this heterogeneity of NSCs is mirrored *in vivo*. The expression of Sox2 is a unifying characteristic of NSCs in the adult brain, but that not all NSCs maintain the ability to form all neural cell types *in vivo* [Brazel et al., 2005].

Other studies have shown that Sox2 and Oct-3/4 work together cooperatively to stimulate the transcription of their own genes as well shown being an important regulator in complete network of genes required for embryogenesis. Small changes in the levels of Sox2 and Oct-3/4 target genes alter the fate of SCs.

Although positive feed forward and feedback loops have been proposed to explain the activation of these genes, little is known about the mechanisms that prevent their over expression [Boer et al., 2007].

Sox2 is a key transcription factor that maintains the proliferation of neuron SCs and inhibits neuronal fate commitment. Moreover, it was recently found that brain tumors contain Scs that resemble normal neuroglial SCs in many respects. The kinetics of Sox2 expression was investigation in to brain tumour by different methods including in cytochemistry (special staining methods to identify composition of cells, constituents and products). To describe Sox2 expression in various brain tumors and to determine whether Sox2 expression is a universal feature of brain tumors, or whether its expression is limited to a specific lineage of brain tumors. Sox2 immunohistochemistry was performed on 194 brain tumour tissues of various kinds. Fetal and adult normal brain tissues obtained by autopsy and brain tissues of epilepsy patients with cortical dysplasia were used as controls [Phi et al., 2008].

The use of Immunohistochemistry are:

- Categorisation of undifferentiated tumors. E.g., presence of keratin indicates epithelial cell tumor whereas desmin, and presence indicates neoplasia of muscle.
- Determination the site of origin of metastasis spread.
- Detection the molecules of prognostic importance. E.g., hormone receptors
- Categorisation of Leukemia's and lymphomas Semi quantitative reverse transcription polymerase chain reaction was used to confirm the immunohistochemical results.

It is often useful to be able to stain for two or more antigens in one common tissue section. This can be achieved by immunofluorescence method using different fluorescent dyes [Li et al., 2004].

Double immunofluorescence was performed to characterize the lineage of Sox2-positive cells. Sox2 was found to be expressed in various glial tumors, including many glial components of mixed neuroglial tumors, regardless of pathologic grade.

It has been suggested that Sox2 may be a tumor marker of glial lineages rather than a universal brain tumor stem cell marker, because its expression pattern was found to correspond to differentiation pathways [Phi et al., 2008].

SRY and other Sox-type transcription factors are important developmental regulators with various implications in human disease. Sox2 in mESCs and neural progenitors can be an interaction with exportin 4 (Exp4). The side function is established in nuclear export, Exp4 acts as a bona fide nuclear import receptor for Sox2 and SRY. Thus, Exp4 is an example of a nuclear transport receptor carrying distinct cargoes into different directions. Import signals for the three pathways overlap and include conserved residues in the Sox2 high-mobility group (HMG) box domain that are also critical for DNA binding. This suggests that nuclear import of Sox proteins is facilitated by several parallel import pathways [Gontan et al., 2009].

In brain tumors of embryonic origin, supratentorial primitive neuroectodermal tumors showed robust Sox2 expression, whereas medulloblastomas and pineoblastomas did not. The majority of Sox2-positive tumor cells co- expressed glial fibrillary acidic protein, and most Sox2-negative cells in medulloblastomas and pineoblastomas showed neuronal differentiation. On the other hand, the aberrant co expressions of Sox2 and of a neuronal marker were widely observed in glioblastomas, which reflects a disorganized differentiation pattern that characterizes highly malignant tumors [Phi et al., 2008].

1.7.1.6. Signaling that controls Foxa2 expression-importance of Foxa2in neurogenesis

The Forkhead box a2 (Foxa2) subfamily of winged helix/forkhead box transcription factors have been the subject of genetic and biochemical studies for over 15 years. During this time its three members, Foxa1, Foxa2, and Foxa3 have been found to play important roles in multiple stages of mammalian life. Beginning with early development, continuing during organogenesis, and finally in metabolism and homeostasis in the adult. Foxa2 is required for the formation of the node and notochord, and in its absence severe defects in gastrulating, neural tube patterning, and gut morphogenesis result in embryonic lethality. Foxa1 and Foxa2 cooperate to establish competence in foregut endoderm and are required for normal development of endoderm-derived organs such as the liver, pancreas, lungs, and prostate. In post-natal life, members of the Foxa family control glucose metabolism through the regulation of multiple target genes in the liver, pancreas, and adipose tissue. Insight into the unique molecular basis of Foxa function has been obtained from recent genetic and genomic data, which identify the Foxa proteins as 'pioneer factors' whose binding to promoters and enhancers enable chromatin access for other tissue-specific transcription factors [Friedman and Kaestner, 2006].

The role of transcription factors in regulating the development of mDA neurons is intensively studied owing to the involvement of these neurons in diverse neurological disorders. During specification, Foxa1 and Foxa2 regulate the extent of neurogenesis in mDA progenitors by regulating Neurog2 (Ngn2) expression. Interestingly, genetic evidence indicates that these functions require different gene dosages of Foxa1 and Foxa2 [Ferri et al., 2007].

Forkhead transcription factors are critical regulators of survival and longevity of the ESCs.

The role of Fox transcription factor has been studied by knockout overxpression in the mouse. Foxa2 gene has the function to generate dopamine neurons during fetal development and from ESCs. Mice carrying only one copy of the Foxa2 gene show abnormalities in motor behaviour in old age and an associated progressive loss of dopamine neurons. Fox genes have evolved to acquire a specialized function in many key biological processes. Mutations in Fox genes have a profound effect on human health, disease-related phenotypes as varied as cancer, glaucoma and language disorders [Hannenhalli and Kaestner, 2009].

Functions of can be summarized as follows:

-Foxa2 regulates a complex pulmonary program of epithelial cell maturation required for transition to air breathing at birth.

-Foxa2 regulates multiple pathways of insulin secretion.

-In preadipocytes Foxa-2 inhibits adipocyte differentiation by activating transcription of the Pref-1 gene.

- Foxa2 gene expression, it is controls in pancreatic beta-cells.

-Foxa2 plays an integral role in the formation of axial mesendoderm, which is required to maintain the specification of the forebrain and the anterior definitive endoderm.

A novel role for Foxa2 has been reported in bile acid metabolism. The winged helix transcription factor Foxa2 is required to prevent intrahepatic cholestasis and liver injury in mice fed a colic acid enriched diet. Functional genomics were used to study how Foxa2 regulates its targets in a colic acid-dependent manner. This suggests that the deletion of Foxa2 in the hepatocyte affects the liver on a large scale. It was discovered distinct feed-forward regulatory loops controlling Foxa2-dependent targets in a colic acid dependent. It shows that Foxa2 interacts with different transcription factors to achieve gene expression responses appropriate for each physiologic state [Bochkis et al. 2009].

In the mammalian CNS an important contingent of dopaminergic neurons are localized in the substantia nigra and in the ventral segmental area of the VM.

They constitute an anatomically and functionally heterogeneous group of cells involved in a variety of regulatory mechanisms. mDA primary cultures represent a useful tool to study molecular mechanisms involved in their development and maintenance. Considerable information has been gathered on mDA neurons development and maturation *in vivo,* as well as on the molecular features of mDA primary cultures [Greco et al., 2009].

Foxa2 is specifically expressed in adult dopamine neurons and their precursors in the medial floor plate. Experiments show that Foxa2, is required to generate dopamine neurons during fetal development and from ESCs. Also show the abnormalities in motor behaviour in old age and an associated progressive loss of dopamine neurons. Manipulating forkhead function may regulate the birth of dopamine neurons and their spontaneous death, two major goals of regenerative medicine. Targeting the survival function of Foxa2 gene was proved important in SCs-based and pharmacological approaches to dopamine neuron disease. SCs biology, embryology, and animal model of PD suggest that Foxa2 is a critical gene at multiple times during the "molecular biography" of dopamine neuron.

1.7.2. *In vitro* differentiation to neural cell types

The derivation of specific neuronal or glial cell types from ESCs invariably includes the production of NSCs. The basic mechanisms of neural induction during vertebrate embryogenesis gave proceeded cues for the pure development protocols which are used to generate NSCs from ESCs [Brazel et al., 2005].

Model system has been development because murine ESCs are easily available inside of hESCs. The neuroepithelial cells generate radial glia that produces fetal and adult NSCs within the CNS. Adult NSC and restricted progenitors are found in the several regions of the CNS throughout life [Singec et al., 2007].

1.7.2.1. 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].

When mESCs are placed in suspension cultures to promote the formation of aggregates, known as EBs. The adherence of EBs or cells from dissociated EBs in serum-free conditions with appropriate supplements supports the development of neural lineage cells. Manipulations of culture conditions and cell selection strategies have been used to increase the efficiency of mESCs differentiation into neural precursors, then to neurons, and astrocytes. mESCs-derived neural cells can be detected *in vivo* following implantation, and characterized *in vitro* by immunostaining for the presence of neural markers and analysis of morphology and protein or gene expression [Wobus et al., 2001, Bain et al., 1995].

Human neural stem cells (hNSCs) can be expanded *in vitro* by mitogens or growth factors, such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and/or LIF. Their effects on proliferation rate and differentiation pattern of hNSCs, however, have not been fully characterized. Particularly, significant generation of neuron cells was observed only in hNSCs expanded with EGF/bFGF or EGF/bFGF/LIF, but not with other treatment regimens, even when they are exposed to the same priming procedure [Tarasenko et al., 2004].

1.7.2.2. Mouse ESCs derivatives in EBs

Mouse ESC *in vitro* differentiation provides a unique and powerful system to examine cellular and molecular processes, perform drug screening, and to investigate potential tissue engineering and cellular therapy applications. Most of the methods utilized for EBs differentiation studies involve several steps: 1) production of EBs from suspension culture,

2) exposure of EBs to agents designed to induce differentiation of a specific lineage, and 3) growth of EBs on tissue culture plates coated with biological molecules such as gelatin or laminin [Boheler et al., 2002].

During mouse embryogenesis, the primitive ectoderm of the epiblast forms three primary germ layers: the ectoderm, the mesoderm, and the definitive endoderm. These germ layers interact to form all tissues and organs of the developing embryo. Moreover, coculture with stromal cell line activity, and recently, even adherent monolayer cultures in the absence of LIF have been used to differentiate mESCs *in vitro*. Differentiation is induced by culturing ESCS as aggregates in the absence of the self-renewal signals provided by feeder layers or LIF, either in hanging drops, in liquid "mass culture", or in methylcellulose.

Initially, an outer layer of endoderm-like cells forms within the EBs, followed over a period of a few days by the development of an ectoderm and subsequent specification of mesoderm cells (Fig. 3). Transfer of these EBs to tissue culture plates allows continued differentiation into a variety of specialized cell types including cardiac, smooth, and skeletal muscle as well as hematopoietic, pancreatic, hepatic, lipid, cartilage, or neuronal and glial cells [Guan et al., 1999].

Current techniques for EBs studies include hanging drop and suspension methodologies.

The suspension culture method gives rise to none uniformly sized cells. The hanging drop method provides uniform sizes of EBs however, this technique is challenging to perform and not amenable to through put screening strategies.

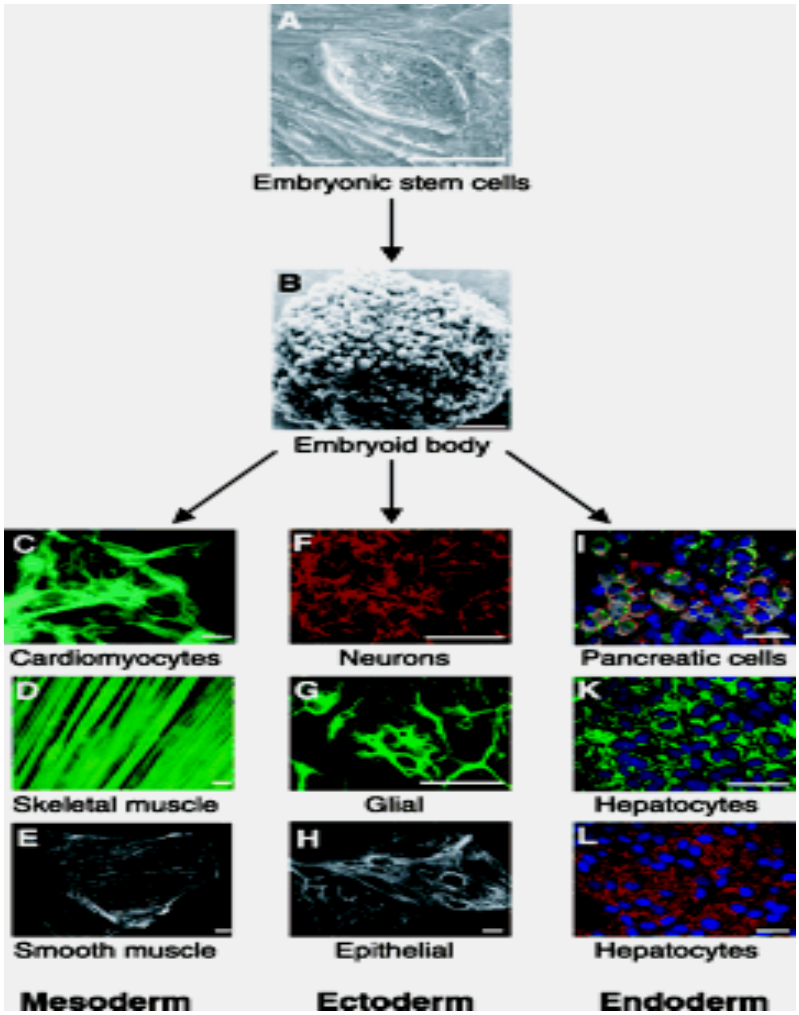

Figure 3: In *vitro* differentiation of ESCs. Undifferentiated mouse ESCs, (*A*) develop in vitro via three-dimensional aggregates EBs, (*B*) into differentiated cell types of all three primary germ layers. *C, F, I, D, G, K, E, H, L* represent examples different a variety of specialized cell types [Guan et al., 1999].

1.7.2.3 Human ESCs current technologies and applications

hESCs lines, which have recently been derived, may additionally serve as an unlimited source of cells for regenerative medicine. Ethical issues surround the derivation of hESCs from *in vitro* fertilized blastocysts. The number of human ESCs lines available for research also is insufficient to adequately determine their therapeutic potential. Recent molecular and cellular advances with mouse ESCs, however, the successful use of these cells in therapeutics. Mouse and human ESCs are respect to *in vitro* propagation and differentiation as well as their use in basic cell and developmental biology. The establishment of hESCs lines from *in vitro* fertilized embryos (Fig.4) and the demonstration of their developmental potential *in vitro* have discussions concerning future applications of human EBs Primordial Germ (PG) cells, which form normally within the developing genital ridges, represent a third embryonic cell type with pluripotent capabilities. In most respects, these cells are indistinguishable from blastocyst-derived ESCs and are characterized by high proliferative and differentiation capacities *in vitro,* and the presence of stem cell markers typical of other ESCs. These cell lines showed multilineage development *in vitro,* but have a limited proliferation capacity, and currently can only be propagated as EBs derivatives.

During cloning of Dolly sheep in 1997, a technique has been used called SCNT or, nuclear transfer (NT) provided a means of generating ESCs with defined genetic makeup. The advantage of using NT to derive hESCs is that the nuclear genomes of the resulting hESCs would be identical with those of the donors for the somatic cells. One obvious benefit is that this would avoid the problem of rejection if cells generated from the hESCs were to be transplanted into the donor. More imminent, however, is the employment of SCs technologies for drug discovery and development.

Novel improved *in vitro* models based on physiologically relevant human cells will result in better precision and more cost-effective assays [Ameen et al. 2008]. Defined tissue for specific cells types are crucial for optimizing the generation of somatic cells *in vitro* for therapeutic approaches. However, experimental models are required allowing rapid and "easy-to-handle" parallel screening of chemical libraries to achieve this goal [Sachinidis et al., 2008].

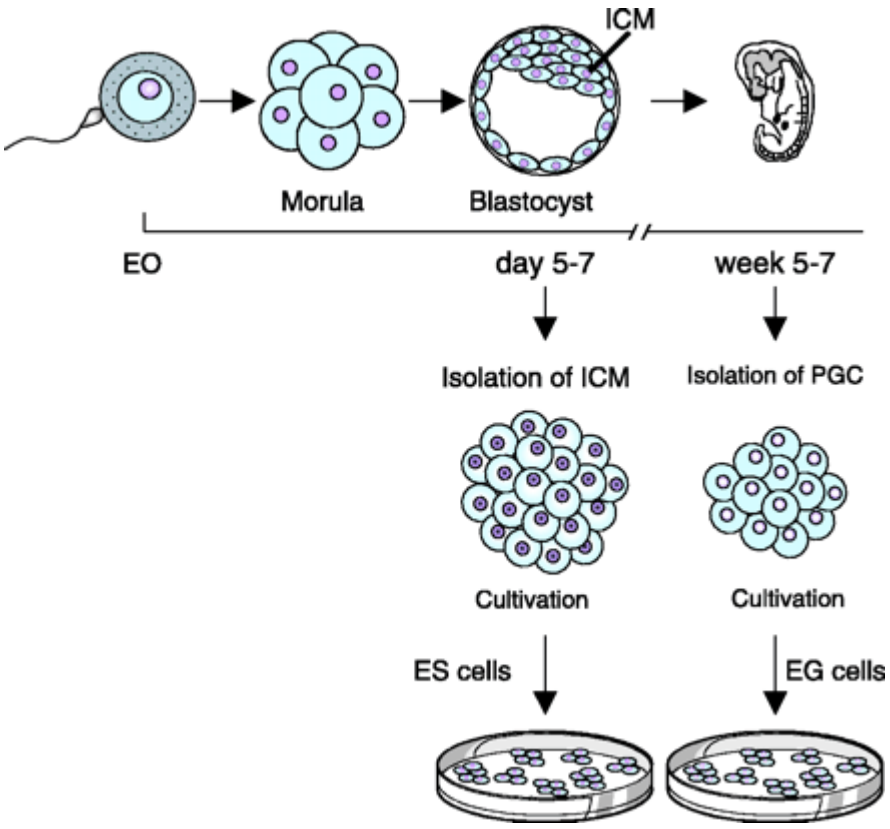

Figure 4: Human pluripotent ESCs and EGCs have been derived from *in vitro* cultured ICM cells of blastocysts (after in vitro fertilization) and from primordial germ cells (PGC) isolated from aborted fetuses, respectively [Thomson et al., 1998].

1.7.2.4. Side view: the generation of cardiomyocytes for replacement therapy in humans

It is clear that for successful SCs-based therapy several obstacles have to be overcome; other opportunities lay ahead for the use of human SCs. A more immediate application would be the development of human models for cell-type specific differentiation and disease *in vitro*. Because of the high *in vitro* differentiation potential, ESCs have been used as model system in cell and developmental biology. Cardiomyocytes can be generated from SCs, which have been shown to follow similar molecular events of cardiac development *in vivo*. Furthermore, several monogenic cardiovascular diseases have been described, for which *in vitro* models in SCs could be generated.

Identification of signaling cascades involved in cardiomyogenesis is crucial for optimizing the generation of cardiomyocytes from ESCs *in vitro* [Sachinidis et al. 2002]. Several monogenic cardiovascular diseases have been described, for which *in vitro* models in SCs could be generated.

Recent clinical studies revealed that positive results of cell transplantation on cardiac function are limited to the short- and mid-term restoration phase following myocardial infarction. These transient effects may depend on the transplanted cell-type or its differentiation state [Smits et al., 2009].

2. 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 markers and important genes controlling the neural differentiation of SCs, such as TH, Sox2, Foxa2 and their contribution in neurogenesis are emphasized. Primitive ESCs are an ideal starting cell population for studies of gene expression and lineage segregation during development.

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.

3. ZUSAMMENFASSUNG

Untersuchungen zur neuralspezifischen Differenzierung embryonaler Stammzellen erfahren in jüngster Vergangenheit zunehmend Interesse und gewinnen an Bedeutung, weil neural differenzierte Stammzellen als Grundlage der Therapie zahlreicher humaner Erkrankungen in Betracht kommen könnten.

Es wird ein Überblick gegeben, welche Fortschritte im Gebiet der neuralen Stammzellforschung erzielt werden konnten. Ausgehend von einer historischen Würdigung der Stammzellforschung und einer Beschreibung des Ursprungs von Stammzellen und des Stammzellbegriffes werden im Detail Strategien diskutiert, neurodegenerative Erkrankungen mit Hilfe transplantierter Zellen zu therapieren. Es wird dargelegt, inwieweit Neurogenese aus Stammzellen kontrolliert *in vitro* modelliert werden kann und welche Rolle dabei der kombinatorischen Interaktion intrinsischer mit extrinsischen Faktoren in der Mikroumgebung des *in vitro* Systems zukommen. In besonderem Maße werden Gene wie TH, Sox2 und Foxa2, die Differenzierungszustände anzeigen oder aber maßgeblich neurale Stammzelldifferenzierung kontrollieren und ihre Rolle in der Neurogenese gewürdigt. Es wird aufgezeigt, daß embryonale Stammzellen ein ideales Ausgangsmaterial für Untersuchungen von differenzieller Genexpression im Zuge der Spezifikation von Differenzierungsschicksalen darstellen.

Neurale Stammzellen zeichnen sich durch wechselnde und sich anpassende Genaktivitätssmuster, Zellmorphologien und durch ein veränderliches Zellverhalten aus, was ihre bedeutende Mitlerfunktion für embryonale Musterbildung und für den Fortgang der Entwicklung nahelegt. Differenzierungsmarker ermöglichen die Isolation solcher Zellen aus unterschiedlichen Entwicklungsstadien und aus verschiedenen Regionen. In Folge lassen sich ihre Entwicklungspotentiale und Änderungen ihrer Genexpression nach Transplantation in andere Embryonen oder in erwachsene Organsimen untersuchen.

Biologische Störungen, die auf irregulären Anhäufungen oder falschen Prozessierungen mutierter oder geschädigter intra- oder extrazellulärer Proteine beruhen und letztendlich neurodegenerative Erkrankungen wie etwa PD oder auch Hirntumore verursachen, werden im Detail diskutiert.

Ein künftiger Schwerpunkt der Forschung besteht in der Untersuchung der Signalwege, die Neurogenese steuern. Jüngsten Befunden zufolge besteht Forschungsbedarf, entsprechende Signalbedingungen *in vivo* und/oder *in vitro* zu erzeugen, um geeignetes Zellmaterial für Transplantationen bereitzustellen. Darüber hinaus wird deutlich, daß Aktionsspläne für klinische Studien zur Behandlung focaler und multifocaler Erkrankungen des ZNS zu entwerfen sind. Unmittelbar anstehende Aufgaben bestehen in der Untersuchung und Ermittlung des rechten Timings einer Zelltherapie, sowie der Bestimmung der optimalen Anzahl der zu transplantierenden Zellen.

4. REFERENCES

1 Ahmed S (2009). The culture of neural stem cells. J Cell Biochem. 106:1-6.

2 Akala OO, Clarke MF (2006). Hematopoietic stem cell self-renewal. Curr Opin Genet Dev. 16:496-501.

3 Aleckovic M, Simon C (2008). Is teratoma formation in stem cell research a characterization tool or a window to developmental biology? Reprod Biomed Online. 17:270-280.

4 Ameen C, Strehl R, Bjorquist P, Lindahl A, Hyllner J, Sartipy P (2008). Human embryonic stem cells: Current technologies and emerging industrial applications. Crit Rev Oncol Hematol. 65:54-80.

5 Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI (1995). Embryonic stem cells express neuronal properties in vitro. Dev Biol. 168:342-357.

6 Baron MH (2001). Molecular regulation of embryonic hematopoiesis and vascular development: A novel pathway. J Hematother Stem Cell Res. 10:587-594.

7 Bentz K, Molcanyi M, Hess S, Schneider A, Hescheler J, Neugebauer E, Schaefer U (2006). Neural differentiation of embryonic stem cells is induced by signalling from non-neural niche cells. Cell Physiol Biochem. 18:275-286.

8 Bhattacharya B, Puri S, Puri RK (2009). A review of gene expression profiling of human embryonic stem cell lines and their differentiated progeny. Curr Stem Cell Res Ther. 4:98-106.

9 Bochkis IM, Schug J, Rubins NE, Chopra AR, O'Malley BW, Kaestner KH (2009). Foxa2-dependent hepatic gene regulatory networks depend on physiological state. Physiol Genomics. 38:186-195.

10 Boer B, Kopp J, Mallanna S, Desler M, Chakravarthy H, Wilder PJ, Bernadt C, Rizzino A (2007). Elevating the levels of sox2 in embryonal carcinoma cells and

embryonic stem cells inhibits the expression of sox2:Oct-3/4 target genes. Nucleic Acids Res. 35:1773-1786.

11 Boheler KR (2009). Stem cell pluripotency: A cellular trait that depends on transcription factors, chromatin state and a checkpoint deficient cell cycle. J Cell Physiol. 221:10-17.

12 Boheler KR, Czyz J, Tweedie D, Yang HT, Anisimov SV, Wobus AM (2002). Differentiation of pluripotent embryonic stem cells into cardiomyocytes. Circ Res. 91:189-201.

13 Brazel CY, Limke TL, Osborne JK, Miura T, Cai J, Pevny L, Rao MS (2005). Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. Aging Cell. 4:197-207.

14 Candy J, Collet C (2005). Two tyrosine hydroxylase genes in teleosts. Biochim Biophys Acta. 1727:35-44.

15 Castelo-Branco G, Arenas E (2006). Function of wnts in dopaminergic neuron development. Neurodegener Dis. 3:5-11.

16 Cavallaro S (2008). Genomic analysis of serotonin receptors in learning and memory. Behav Brain Res. 195:2-6.

17 Chen S, Le W (2006). Neuroprotective therapy in parkinson disease. Am J Ther. 13:445-457.

18 Cogle CR, Guthrie SM, Sanders RC, Allen WL, Scott EW, Petersen BE (2003). An overview of stem cell research and regulatory issues. Mayo Clin Proc. 78:993-1003.

19 Conley BJ, Denham M, Gulluyan L, Olsson F, Cole TJ, Mollard R (2005). Mouse embryonic stem cell derivation, and mouse and human embryonic stem cell culture and differentiation as embryoid bodies. Curr Protoc Cell Biol. Chapter 23:Unit 23 22.

20 Das S, Bonaguidi M, Muro K, Kessler JA (2008). Generation of embryonic stem cells: Limitations of and alternatives to inner cell mass harvest. Neurosurg Focus. 24:E4.

21 Date I, Yasuhara T (2009). Neurological disorders and neural regeneration, with special reference to parkinson's disease and cerebral ischemia. J Artif Organs. 12:11-16.

22 Elder GA, De Gasperi R, Gama Sosa MA (2006). Research update: Neurogenesis in adult brain and neuropsychiatric disorders. Mt Sinai J Med. 73:931-940.

23 Episkopou V (2005). Sox2 functions in adult neural stem cells. Trends Neurosci. 28:219-221.

24 Ferri AL, Lin W, Mavromatakis YE, Wang JC, Sasaki H, Whitsett JA, Ang SL (2007). Foxa1 and foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. Development. 134:2761-2769.

25 Friedman JR, Kaestner KH (2006). The foxa family of transcription factors in development and metabolism. Cell Mol Life Sci. 63:2317-2328.

26 Giegling I, Moreno-De-Luca D, Calati R, Hartmann AM, Moller HJ, De Ronchi D, Rujescu D, Serretti A (2009). Tyrosine hydroxylase and dopa decarboxylase gene variants in personality traits. Neuropsychobiology. 59:23-27.

27 Giegling I, Moreno-De-Luca D, Rujescu D, Schneider B, Hartmann AM, Schnabel A, Maurer K, Moller HJ, Serretti A (2008). Dopa decarboxylase and tyrosine hydroxylase gene variants in suicidal behavior. Am J Med Genet B Neuropsychiatr Genet. 147:308-315.

28 Gontan C, Guttler T, Engelen E, Demmers J, Fornerod M, Grosveld FG, Tibboel D, Gorlich D, Poot RA, Rottier RJ (2009). Exportin 4 mediates a novel nuclear import pathway for sox family transcription factors. J Cell Biol. 185:27-34.

29 Gordon MY (2008). Stem cells for regenerative medicine--biological attributes and clinical application. Exp Hematol. 36:726-732.

30 Graham V, Khudyakov J, Ellis P, Pevny L (2003). Sox2 functions to maintain neural progenitor identity. Neuron. 39:749-765.

31 Greco D, Volpicelli F, Di Lieto A, Leo D, Perrone-Capano C, Auvinen P, di Porzio U (2009). Comparison of gene expression profile in embryonic mesencephalon and neuronal primary cultures. PLoS One. 4:e4977.

32 Guan K, Rohwedel J, Wobus AM (1999). Embryonic stem cell differentiation models: Cardiogenesis, myogenesis, neurogenesis, epithelial and vascular smooth muscle cell differentiation in vitro. Cytotechnology. 30:211-226.

33 Hannenhalli S, Kaestner KH (2009). The evolution of fox genes and their role in development and disease. Nat Rev Genet. 10:233-240.

34 Hug K (2005). Sources of human embryos for stem cell research: Ethical problems and their possible solutions. Medicina (Kaunas). 41:1002-1010.

35 Imayoshi I, Sakamoto M, Ohtsuka T, Kageyama R (2009). Continuous neurogenesis in the adult brain. Dev Growth Differ. 51:379-386.

36 Kaneko N, Sawamoto K (2009). Adult neurogenesis and its alteration under pathological conditions. Neurosci Res. 63:155-164.

37 Kim DW (2004). Efficient induction of dopaminergic neurons from embryonic stem cells for application to parkinson's disease. Yonsei Med J. 45 Suppl:23-27.

38 Kim HJ, Sugimori M, Nakafuku M, Svendsen CN (2007). Control of neurogenesis and tyrosine hydroxylase expression in neural progenitor cells through bhlh proteins and nurr1. Exp Neurol. 203:394-405.

39 Kriks S, Studer L (2009). Protocols for generating es cell-derived dopamine neurons. Adv Exp Med Biol. 651:101-111.

40 Krtolica A, Ilic D, Genbacev O, Miller RK (2009). Human embryonic stem cells as a model for embryotoxicity screening. Regen Med. 4:449-459.

41 Kuci S, Kuci Z, Latifi-Pupovci H, Niethammer D, Handgretinger R, Schumm M, Bruchelt G, Bader P, Klingebiel T (2009). Adult stem cells as an alternative source of

multipotential (pluripotential) cells in regenerative medicine. Curr Stem Cell Res Ther. 4:107-117.

42 Kurtzberg J (2009). Update on umbilical cord blood transplantation. Curr Opin Pediatr. 21:22-29.

43 Li ZS, Pham TD, Tamir H, Chen JJ, Gershon MD (2004). Enteric dopaminergic neurons: Definition, developmental lineage, and effects of extrinsic denervation. J Neurosci. 24:1330-1339.

44 Looijenga LH, Gillis AJ, Stoop HJ, Hersmus R, Oosterhuis JW (2007). Chromosomes and expression in human testicular germ-cell tumors: Insight into their cell of origin and pathogenesis. Ann N Y Acad Sci. 1120:187-214.

45 Motomura H, Niimi H, Sugimori K, Ohtsuka T, Kimura T, Kitajima I (2007). Gas6, a new regulator of chondrogenic differentiation from mesenchymal cells. Biochem Biophys Res Commun. 357:997-1003.

46 Muglia P, Petronis A, Mundo E, Lander S, Cate T, Kennedy JL (2002). Dopamine d4 receptor and tyrosine hydroxylase genes in bipolar disorder: Evidence for a role of drd4. Mol Psychiatry. 7:860-866.

47 Murry CE, Keller G (2008). Differentiation of embryonic stem cells to clinically relevant populations: Lessons from embryonic development. Cell. 132:661-680.

48 Pal R (2009). Embryonic stem (es) cell-derived cardiomyocytes: A good candidate for cell therapy applications. Cell Biol Int. 33:325-336.

49 Phi JH, Park SH, Kim SK, Paek SH, Kim JH, Lee YJ, Cho BK, Park CK, Lee DH, Wang KC (2008). Sox2 expression in brain tumors: A reflection of the neuroglial differentiation pathway. Am J Surg Pathol. 32:103-112.

50 Rao MS, Vemuri MC (2009). Neural transplantation and stem cells. Methods Mol Biol. 549:3-16.

51 Riazi AM, Kwon SY, Stanford WL (2009). Stem cell sources for regenerative medicine. Methods Mol Biol. 482:55-90.

52 Robson P (2004). The maturing of the human embryonic stem cell transcriptome profile. Trends Biotechnol. 22:609-612.

53 Sachinidis A, Kolossov E, Fleischmann BK, Hescheler J (2002). Generation of cardiomyocytes from embryonic stem cells experimental studies. Herz. 27:589-597.

54 Sachinidis A, Sotiriadou I, Seelig B, Berkessel A, Hescheler J (2008). A chemical genetics approach for specific differentiation of stem cells to somatic cells: A new promising therapeutical approach. Comb Chem High Throughput Screen. 11:70-82.

55 Shagam JY (2008). Unlocking the secrets of parkinson disease. Radiol Technol. 79:227-239; quiz 240-222.

56 Sholl LM, Long KB, Hornick JL (2009). Sox2 expression in pulmonary non-small cell and neuroendocrine carcinomas. Appl Immunohistochem Mol Morphol.

57 Singec I, Jandial R, Crain A, Nikkhah G, Snyder EY (2007). The leading edge of stem cell therapeutics. Annu Rev Med. 58:313-328.

58 Smits AM, van Laake LW, den Ouden K, Schreurs C, Szuhai K, van Echteld CJ, Mummery CL, Doevendans PA, Goumans MJ (2009). Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium. Cardiovasc Res. 83:527-535.

59 Spitkovsky D, Hescheler J (2008). Adult mesenchymal stromal stem cells for therapeutic applications. Minim Invasive Ther Allied Technol. 17:79-90.

60 Srivastava AS, Malhotra R, Sharp J, Berggren T (2008). Potentials of es cell therapy in neurodegenerative diseases. Curr Pharm Des. 14:3873-3879.

61 Suter DM, Krause KH (2008). Neural commitment of embryonic stem cells: Molecules, pathways and potential for cell therapy. J Pathol. 215:355-368.

62 Tarasenko YI, Yu Y, Jordan PM, Bottenstein J, Wu P (2004). Effect of growth factors on proliferation and phenotypic differentiation of human fetal neural stem cells. J Neurosci Res. 78:625-636.

63 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998). Embryonic stem cell lines derived from human blastocysts. Science. 282:1145-1147.

64 Till JE, Mc CE (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res. 14:213-222.

65 Trouillas M, Saucourt C, Guillotin B, Gauthereau X, Ding L, Buchholz F, Doss MX, Sachinidis A, Hescheler J, Hummel O, Huebner N, Kolde R, Vilo J, Schulz H, Boeuf H (2009). Three lif-dependent signatures and gene clusters with atypical expression profiles, identified by transcriptome studies in mouse es cells and early derivatives. BMC Genomics. 10:73.

66 Trounson A (2009). New perspectives in human stem cell therapeutic research. BMC Med. 7:29.

67 Trujillo CA, Schwindt TT, Martins AH, Alves JM, Mello LE, Ulrich H (2009). Novel perspectives of neural stem cell differentiation: From neurotransmitters to therapeutics. Cytometry A. 75:38-53.

68 Turksen K, Troy TC (2006). Human embryonic stem cells: Isolation, maintenance, and differentiation. Methods Mol Biol. 331:1-12.

69 Ulloa-Montoya F, Verfaillie CM, Hu WS (2005). Culture systems for pluripotent stem cells. J Biosci Bioeng. 100:12-27.

70 Van Hoffelen S, Herman MA (2008). Analysis of wnt signaling during caenorhabditis elegans postembryonic development. Methods Mol Biol. 469:87-102.

71 Vendrell V, Summerhurst K, Sharpe J, Davidson D, Murphy P (2009). Gene expression analysis of canonical wnt pathway transcriptional regulators during early

morphogenesis of the facial region in the mouse embryo. Gene Expr Patterns. 9:296-305.

72 Vescovi AL, Snyder EY (1999). Establishment and properties of neural stem cell clones: Plasticity in vitro and in vivo. Brain Pathol. 9:569-598.

73 Wobus AM, Guan K, Pich U (2001). In vitro differentiation of embryonic stem cells and analysis of cellular phenotypes. Methods Mol Biol. 158:263-286.

74 Zappone MV, Galli R, Catena R, Meani N, De Biasi S, Mattei E, Tiveron C, Vescovi AL, Lovell-Badge R, Ottolenghi S, Nicolis SK (2000). Sox2 regulatory sequences direct expression of a (beta)-geo transgene to telencephalic neural stem cells and precursors of the mouse embryo, revealing regionalization of gene expression in cns stem cells. Development. 127:2367-2382.

75 Zhou H, Shang L, Li X, Zhang X, Gao G, Guo C, Chen B, Liu Q, Gong Y, Shao C (2009). Resveratrol augments the canonical wnt signaling pathway in promoting osteoblastic differentiation of multipotent mesenchymal cells. Exp Cell Res. 315:2953-2962.

5. PUBLICATIONS

1. MATOUQ A. A (1998) “Determinate of the *Aeromonas* group in deep frozen food, contaminated environmental water and relationship in humans.” Science et technique du froid 424-423(Note(s): (424 p.) (13 ref.)ISSN 0151-1637.

6. CURRICULUM VITAE

PERSONAL INFORMATION:

Name: Almahdi Matoug Ali

Date of brith: 04.06.1962, Alqulab , Libya

Nationalty: Libyan

Martial status: Married, 4 children

Mob. No: 0049 179 9878825

Email: Matuqali@yahoo.com.

EDUCATION :

1981 High School, graduated at Murzuk High School, Murzuk –Libya.

1985 (B.Sc.) degree in Medical Laboratory Science, at sebha University, Sebha – Libya.

1994 Special Certificate, from the Institute of Clinical and Experimental Laboratory Medicine of the Haynal Imre Medical University, Budapest – Hungary

1999 DIPLOMA (MSc) degree in Microbiology, at Eötvös Lorand University, Department of Microbiology, Budapest – Hungary.

1999 Special Certificate Courses, in advanced food microbiology and safety with the co-operation of National Institute of Public Health Science and Eötvös Lorand University, Budapest - Hungary.

2000 Special Certificate, at the National Center of public health, National of Food Hygiene Nutrition, Budapest – Hungary.

EXPERIENCE

1985 Pathological Laboratory, Central Hospital, Murzuk-Libya 1987.

1987 Organizer, different Camp works and head of a medical laboratory at Libyan Red Crescent.

1987 Attended international health Activator in Greece, and Switzerland as a member of Red Crescent in Libya.

1988 Adviser of Medical Staff, arrangement medical library, purchase for scientific books and journals, Central Hospital. Murzuk-Libya.

2000 Member of teaching at Faculty of Science, University of Sebha-Libya.

2000 Teaching staff in high Medical Institutes, Murzok and Oubari – Libya.

RESEARCH & MEETINGs :

1998 Membership of the Society for General Microbiology in UK.

1998 Attended and participate in IIF-IIR-Commission Conferences entitled presenting a contribution (Determinate of the *Aeromonas* group in deep frozen food, contaminated environmental water and relationship in humans), Nantes-France.

1999 Attended and participate in the 13th International Congress of the Hungarian Society for Microbiology, entitled presenting a contribution (characterization of *Aeromonads* isolated from different sources in Hungary and Libya), Budapest-Hungary.

2000 Participate in the 3rd International Conference on Predictive Modeling in Food, entitled a contribution (model examinations for cultivation of *Aeromonas* strains from deep-frozen Fishes), Leuven- Belgium.

2001 Attended in the 5th Medical Conference&Exhibation organized by High community of Health, Alzawia-Libya.

2004 Zoology, Training in laboratory and molecular methods in the Laboratory of Prof. Dr. Plikert, Cologne – Germany.

2005 Practical work in preparation of thesis, ISH techniques, cloning, and subcloning, Cologne – Germany.

2006 – 2007 Practical section in Neurophysiology Institute, cell culture ESc techniques, and embryoid body techniques *in vitro* differentiation, Cologne – Germany.