Effect of Mesenchymal Stem Cell Culture on Differentiation Potential

Essay

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بحث تأثير مزرعة الخلايا الجذعية المزنكيمية على قدرة الخلايا على التمييز

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List of Contents

List of figures

List of Abbreviations

AGEs :Advanced Glycation End Product

alloHS :Allogenic Human Serum

AS :Autologous Serum

β-Catenin/LEF :β-Catenin lymphoid enhancer binding factor

B-GAL :Beta Galactosidase

BDNF :Brain-derived Neurotropic factor

b-FGF :Basic Fibroblast Growth Factor

bHLH :Basic helix-loop-helix

BME :Eagle′s Basal Medium

BMMSCs :Bone Marrow Mesenchymal Stem Cells

BMP :Bone Morphogenetic Protien

CFU-F :Fibroblast Colony Forming Units

DMEM :Dulbecco's Modified Eagle's medium

DNA :Deoxyribonucleic Acid

ECM :Extracellular Matrix

EGF :Epidermal Growth Factor

FBS :Fetal Bovine Serum

FCS :Fetal Calf Serum

FGFs :Fibroblast Growth Factor

GDF :Growth and Differentiation Factor

GMP :Good Manufacturing Practice

HATs :Histone Acetyltransferases

HGF :Hepatocyte Growth Factor

HIF :Hypoxia Induced Factor

HSCs :Haematopoietic stem cells

hUCMSCs :Human Umbilical Cord Mesenchymal Stem Cells

IGF :Insulin-Like Growth Factor

IL :Interleukin

IMDM :Iscove's Modified Dulbecco's Medium

INF :Interferon

IPS :Induced pluiripotency of somatic cells

LIF :Leukemia Inhibitory Factor

MAPC :Multipotent Adult Progenitor cell

MAPK :Major Mitogen-Activated Protien kinase

MEM :Minimum Essential Medium

MSCs :Mesenchymal Stem Cells

NGF :Nerve Growth Factor

NO :Nitric Oxide

NSCs :Neural stem cells

PDGF :Platelet Derived Growth Factor

PL :Platelet Lysate

PPAR □ :Peroxisome Proliferator Activated Receptor Gama

RNA :Ribonucleic Acid

ROS :Reactive Oxygen Species

SCs :Stem Cells

SFM :Serum Free Medium

SMA :Smooth muscle Actin

TCF :T cell factor

TGF- β :Transforming Growth Factor Beta

TIPs :Tension-induced/inhibited proteins

UCB :Umbilical cord blood

VEGF :Vascular Endothelial Growth Factor

VSEL :Very small embryonic like stem cells

List of Tables

Chapter 1

Stem Cell Biology

Stem Cell Biology

Introduction

Stem cells (SCs) have recently generated more public and professional interest than almost any other topic in biology. One reason stem cells capture the imagination of so many is the promise that understanding their unique properties may provide deep insights into the biology of cells as well as a path toward treatments for a variety of degenerative illnesses. And although the field of stem cell biology has grown rapidly, there exists considerable confusion and disagreement as to the nature of stem cells. This confusion can be partly attributed to the sometimes idiosyncratic terms and definitions used to describe stem cells. Although definitions can be restrictive, they are useful when they provide a basis for mutual understanding and experimental standardization (Baksh et al., 2004).

Definition of Stem Cells

Stem cells are defined functionally as cells that have the capacity to self-renew as well as the ability to generate differentiated cells. More explicitly, stem cells can generate daughter cells identical to their mother (self-renewal) as well as produce progeny with more restricted potential (differentiated cells). This simple and broad definition may be satisfactory for embryonic or fetal stem cells that do not perdue for the lifetime of an organism. But this definition breaks down in trying to discriminate between transient adult progenitor cells that have a reduced capacity for self-renewal and adult stem cells. It is therefore important when describing adult stem cells to further restrict this definition to cells that self-renew throughout the life span of the animal.

Stemnness refers to the common molecular processes underlying the core stem cell properties of self—renewal and the generation of differentiated progeny. Although stem cells in different cellular microenvironments or niches will by necessity have different physiological demands and therefore distinct molecular programs, there are likely certain genetic characteristics specific to and shared by all stem cells. Through transcriptional profiling, many of the genes enriched in Embryonic Stem cells (ESC), Haematopoietic Stem cells (HSCs), and Neuronal Stem cells (NSCs) populations have been identified (Park et al., 2002). By extending this approach to other stem cells and more organisms, it may be possible to develop a molecular fingerprint for stem cells. This fingerprint could be used as the basis for a molecular definition of stem cells that, when combined with their functional definition, would provide a more comprehensive set of criteria for understanding their unique biology.

At the same time, many would argue that a self-renewing cell that can only produce one type of differentiated descendant is nonetheless a stem cell. A case can be made, for clarity, that a unipotent cell is probably best described as a progenitor. Progenitors are typically the descendants of stem cells, only they more constrained in their differentiation potential (Honczarenko et al., 2006).

Origin of Stem Cells

The origin or lineage of stem cells is well understood for Emberyonic Stem Cells (ES) cells; their origin in adults is less clear and in some cases controversial. It may be significant that ES cells originate before germ layer commitment, raising the intriguing possibility that this may be a mechanism for the development of multipotent stem cells, including some adult stem cells. The paucity of information on the developmental origins of adult stem cells leaves open the possibility that

they too escape lineage restriction in the early embryo and subsequently colonize specialized niches, which function to both maintain their potency as well as restrict their lineage potential. Alternatively, the more widely believed, though still unsubstantiated, model for the origin of adult stern cells assumes that they are derived after somatic lineage specification, whereupon multipotent stem cells - progenitors arise and colonize their respective cellular niches (Ratajckzak et al., 2008).

Types of Stem Cells:

(1) Prenatal Stem Cells: Embryonic & Fetal Stem Cells

An embryonic stem cell is derived from a group of cells called the inner cell mass, which is part of the early (4 to 5 day old) embryo called the blastocyst. Once removed from the blastocyst, the cells of the inner cell mass can be cultured into embryonic stem cells.

Beyond the 8th week of development, the embryo has matured into a fetus and the embryonic stem cells have matured into fetal stem cells, which exist until birth (after which time they are known as "adult" stem cells).

Embryonic and fetal stem cells are known to be pluripotent, which makes them attractive candidates for use in medical therapies. However, from their pluripotency they are also capable of forming teratomas (tumors). (Akiva et al., 2008).

Development of human blastocyst in vitro:

After a human oocyte is fertilized in vitro by a sperm cell, the following events occur according to a fairly predictable timeline. At 18 to 24 hours after in vitro

fertilization of the oocyte is considered day 1. By day 2 (24 to 25hours), the zygote (fertilized egg) undergoes the first cleavage to produce a 2-cell embryo. By day 3 (72 hours), the embryo reaches the 8-cell stage called a morula. It is at this stage that the genome of the embryo begins to control its own development. This means that any material influences due to the presence of mRNA and proteins in the oocyte cytoplasm are significantly reduced. By day 4, the cells of the embryo adhere tightly to each other in a process known as compaction and by day 5, the cavity of the blastocyst is completed. The inner cell mass begins to separate from the outer cells, become the trophectoderm that surrounds the blastocyst. This represents the first observable sign of cell differentiation in the embryo. (Zhao et al., 2003).

Day-5 blastocysts are used to derive ES cell cultures. A normal day-5 embryo in vitro consists of 200 to 250 cells. Most of the cells comprise the trophectoderm. For deriving ES cell cultures, the trophectoderm is removed, either by microsurgery or immunosurgery (in which antibodies against the trophectoderm help break it down, thus freeing the inner cell mass). At this stage, the inner cell mass is composed of only 30 to 34 cells.

One of the first major events in the embryonic development is the specialization of the three embryonic germ layers (figure 1): **ectoderm** (believed to give rise to skin and neural lineages), **mesoderm** (believed to generate blood, bone, muscle, cartilage and fat), and **endoderm** (believed to contribute tissues of the respiratory and digestive tracts).The participating of embryonic cells into these groups requires the sequential action of multiple gene products, and although the precise moment at which cells become committed to each lineage remains not completely clear, it has been thought that the segregation of cells into particular germ layers during embryogenesis is an enduring event, all subsequently arising cells, including mature cells, progenitor cells and stem cells of each of the resulting tissue lineage,

irreversibly maintain this specification into and throughout adulthood (Wagers et al., 2004)

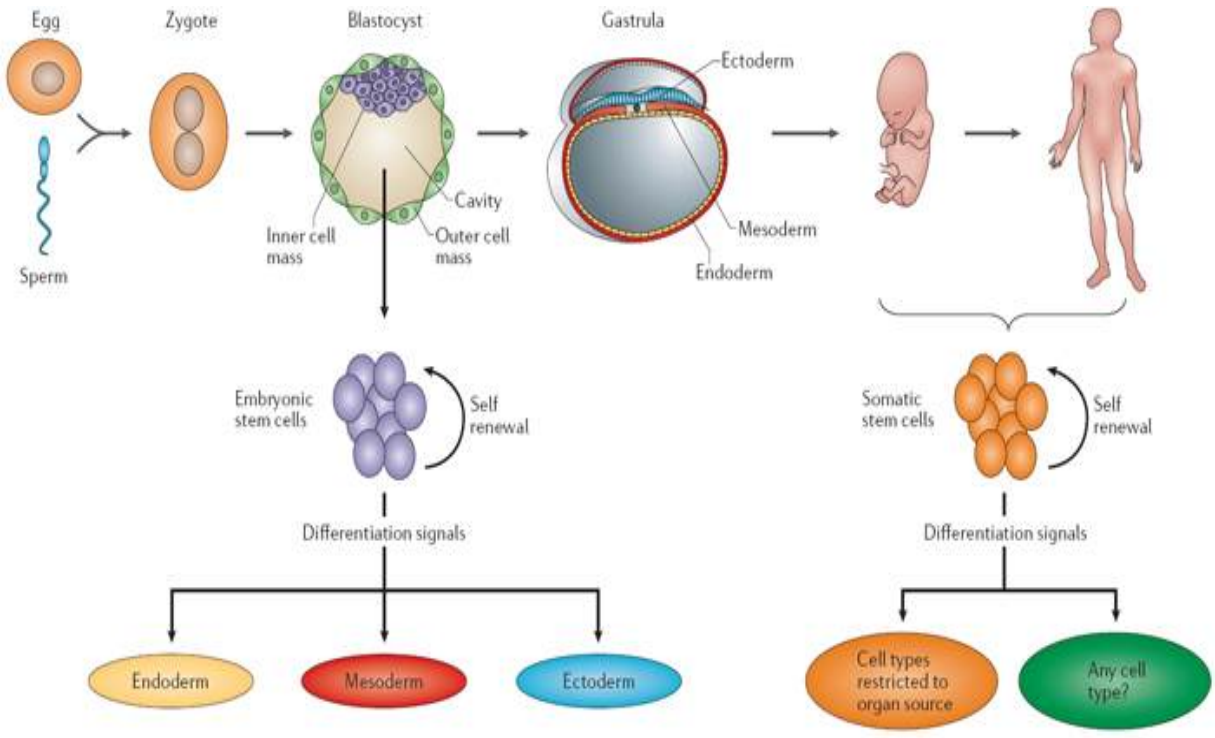

Figure (1) Development of human blastocyst. (Akiva, 2008)

(2) Postnatal Stem Cells: Placental and Umbilical Cord Stem Cells

Placental stem cells are isolated from placentas, and umbilical cord stem cells are isolated from umbilical cords, at the time of a healthy birth. In both cases, the material from which the stem cells are derived would have otherwise been discarded. Unlike with embryonic stem cells, the destruction of an embryo is not involved in the gathering of placental and umbilical cord stem cells, yet the placental and umbilical cord stem cells exhibit the same pluripotency as do embryonic stem cells.