In Vitro Differentiation of Human Mesenchymal Stem Cells to Epithelial Lineage

Thesis

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By

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(M.B.B.Ch.)

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Abstract

Mesenchymal stem cells (MSCs) are a group of cells present in bone-marrow stroma, cord blood, Wharton's jelly and the stroma of various organs with the capacity for mesoderm-like cell differentiation. The aim of the present work is to isolate mesenchymal stem cells (MSCs) from human umbilical cord; Wharton's jelly and to examine the differentiation potential of these (MSCs) toward the epithelial lineage. Human MSCs, derived from Wharton's jelly of umbilical cord (≈31cm) after a caesarian section in full term delivery of 10 cases, were localized and isolated. Then primary culture was done to enrich and enhance their proliferation. These MSCs were detected after an average of 21-30 days not only morphologically, as a uniform spindle fibroblast like cells, and reached 70%-80% confluence with a good cellular yield but also via their immunophenotypic analysis which showed positivity for CD29, CD90, CD105, CD166 and negativity for CD34. Later, their differentiation potential was determined by co-culture with conditioning medium that include bone morphogenetic protein-4 (BMP-4), ascorbic acid, and human epithelium growth factor in a complete medium. These sets of conditions resulted in the expression of keratinocyte markers, namely pancytokeratin (mean value 92%) and cytokeratin19 (mean value 74%) using immunohistochemistry. In conclusion, these findings may have a significant impact on studies of human epithelial differentiation, functional genomics, pharmacological testing, cell therapy and tissue engineering by helping to eliminate worrying ethical and technical issues.

Keywords:

Mesenchymal Stem Cells, Wharton's Jelly, immunophenotyping, immunohistochemistry, cytokeratin

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Nomenclature

5-FU	5-fluorouracil
AGM	Aorta-gonad-mesonephros
ALS	Amyotrophic lateral sclerosis
ASCs	Adult Stem Cells
BDNF	Brain-derived neurotrophic factors
bFGF	Basic fibroblast growth factor
BSC	Biological Safety Cabinet
BM	Bone Marrow
BMP	Bone Morphogenetic Protein
CCR	Chemokine /chemokine receptor
CFU-f	Colony-forming unit-fibroblasts
CK	Cytokeratin
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
DPBS	Dulbecco's Phosphate buffered saline
EGF	Epidermal growth
ESCs	Embryonic stem cells
FCS	Fetal calf serum
FGF	Fibroblast growth factor
G-CSF	Granulocyte colony-stimulating factor
GVHD	Graft versus host disease
H-CAM	Homing-associated cell adhesion molecule
HLA	Human leukocyte antigens
hMSCs	Human mesenchymal stem cells
HMGB1	High-mobility group protein B1
HSCs	Hematopoietic Stem Cells
I/R	Ischemia/ Reperfusion
IFN-β	Interferon beta

IGF-1	Insulin-like growth factor-1
IP	Intraperitoneal
iPS	Induced pluripotent stem cell
ISCT	International Society for Cell Therapy
MDS	Myelodysplastic syndromes.
MEM	Modified Eagle's medium
MHC-I	Major histocompatibility complex-I
MI	Myocardial infarction
MS	Multiple sclerosis
MSA	Multiple system atrophy
MSCs	Mesenchymal Stem Cells/ Multipotent Stem Cells
NGF	Nerve growth factor
OA	Osteoarthritis
PD	Parkinson's disease
SS	Sjogren's syndrome
RA	Rheumatoid arthritis
SCID	Severe combined immune deficiency
SDF-1	Stromal cell-derived factor-1
SGCs	Sweat gland cells
SLE	Systemic lupus erythematosus
SSEA	Stage-specific embryonic antigen
TGF-b	Transforming growth factor-beta
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
UC	Umbilical cord
UCB	Umbilical cord blood
UCMSCs	Umbilical cord Mesenchymal Stem Cells
VEGF	Vascular endothelial growth
WJSCs	Wharton’s Jelly Stem Cells

Introduction

Mesenchymal stem cells have attracted the attention of most scientists and researches since the early nineties because of their multipotent capacity and their high differentiation potential. In other words, they are capable of generating, both in vitro and in vivo, numerous types of tissue cells including blood, bone (Bruder et al, 1997; Jaiswal et al, 1997), cartilage (Johnstone et al, 1998), fat (Dennis et al, 1999), muscle(Ferrari et al, 1998), skeleton (Young et al, 1998), kidney, heart (Chin et al, 2010), endothelium (Al-Khaldi et al, 2003; Xu et al, 2010), nerve (Choi et al, 2010), liver (Wang et al, 2009), skin (Wu et al, 2007) and pancreatic cells (Chao et al, 2008). Bone marrow has always been considered the major source of MSCs as embryonic cells were always associated with ethical and religious limitations. However, advanced research has proved the availability of other sources as adipose tissue removed during plastic surgery, umbilical cord blood and umbilical cord including different compartments such as the Wharton's jelly (Troyer et al, 2008), the perivascular regions (Sarugaser et al, 2005), the subendothelium and endothelium areas of UC vessel which are removed during delivery (Convas et al, 2003). The latter represents a very good source of MSCs for a number of reasons. First of all, the umbilical cord (Wharton's Jelly) represents no ethical or invasive procedure but rather it is a medical waste in the delivery room (Troyer et al, 2008). Second, it offers a highly sterile source of MSCs as the placenta represents a barrier form both bacterial and viral contamination. Third, it has much more cellular yield and better results when isolated and expanded than umbilical cord blood (UCB). Fourth, being devoid of HLAII surface markers and the presence of some cells devoid of HLAI (Marmotti et al, in press), this represents an immunosuppressive property (Fan C-G et al, 2011) which allows not only their autologous transplantation but also their allogenic one (Weiss et al, 2008) and still reduces the possibility of graft versus host disease (GVHD) or any immunological problem (Fan C-G et al, 2011). Last but not least, they enjoy faster rate of division, absence of tumorigenic transformation or cytogenetic abnormality (Capelli et al, 2011; Weiss et al, 2006). Therefore, the

umbilical cord represents a very reliable source of MSCs as it retains all the MSCs'charactaristics; plastic adherence, morphological appearance, cell surface markers and differentiation potential typical to other sources of MSCs but with added privileges as mentioned earlier.

This current study focuses on the use of such very attractive sources of MSCs, umbilical cord tissue mainly and umbilical cord blood. However it uses a very simple protocol that mainly focuses on mincing and grinding the umbilical cord as is without any additional manipulations as cord vessel dissection or enzymatic digestion (Marmotti et al, in press). Hence, it is based on isolating and expanding a sufficient number of multipotent MSCs and later culturing them in a selectively enriched medium containing the bone morphogenetic protein-4 and epithelium growth factor to enhance and direct their differentiation to epithelial cell lineage (Sasaki, et al, 2008) which is again tested for by a selective epithelial marker cytokeratin 19 (Wu et al, 2005; Păunescu et al, 2007). This work intends to identify the transdifferentiation potential of these cells which could later be of great use for not only degenerative but also regenerative medicine (Salem & Thiemermann, 2010).

Review of Literature

Chapter I: Mesenchymal Stem Cells

Definition

Mesenchymal stem cells (MSCs) are adult stem cells traditionally found in the bone marrow. However, mesenchymal stem cells can also be isolated from other tissues including cord blood, peripheral blood, fallopian tube, adipose tissue, amniotic fluid, fetal liver and lung. Multipotent stem cells, MSCs differentiate to form fat, cartilage, bone, tendons & ligaments, nerve, muscle, and skin as in Figure 1. (Nardi et al, 2006).

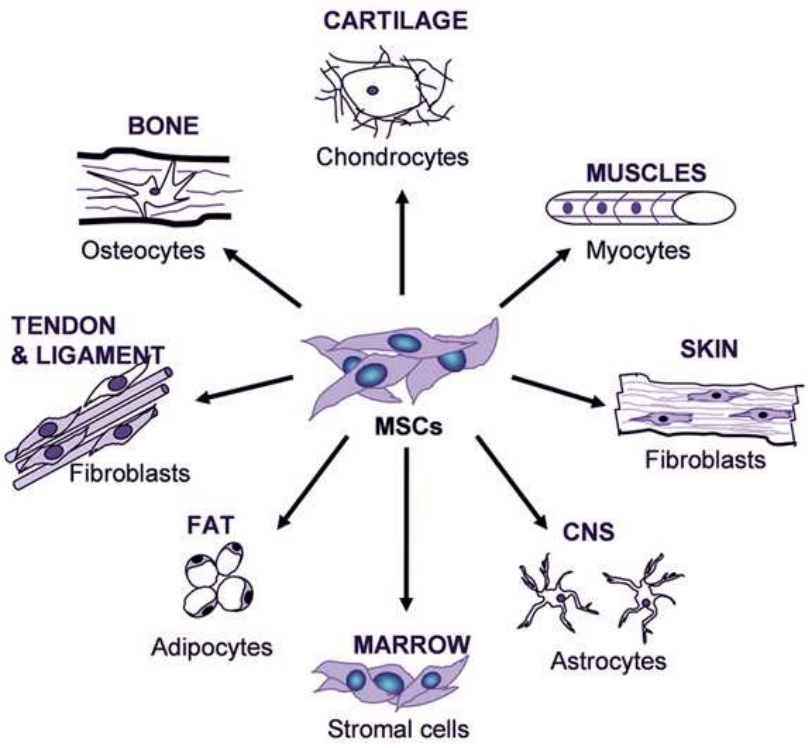

Figure 1 - MSCs Differentiation (Grassel et al, 2007)

While the terms Mesenchymal Stem Cell and Marrow Stromal Cell have been used interchangeably, neither term is sufficiently descriptive as discussed below:

- Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into hematopoietic and connective tissue, whereas MSCs do not differentiate into hematopoietic cells.
- Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSCs, the term fails to convey the relatively recently-discovered roles of MSCs in the repair of tissue.
- Because the cells, called MSCs by many labs today, can encompass multipotent cells derived from other non-marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth, yet do not have the capacity to reconstitute an entire organ, the term Multipotent Stromal Cell has been proposed as a better replacement (Wikipedia the free encyclopedia, 2011).

History

In 1924, Russian-born morphologist Alexander A. Maximow used extensive histological findings to identify a singular type of precursor cell within mesenchyme that develops into different types of blood cells (Becker et al, 1963). The clonal nature of marrow cells was first revealed in the 1960s (Siminovitch et al, 1963). An ex vivo assay for examining the clonogenic potential of multipotent marrow cells was later reported in the 1970s by Friedenstein and colleagues. In this assay system, stromal cells were referred to as colony-forming unit-fibroblasts (CFU-f) (Friedenstein et al, 1974).

Subsequent experimentation revealed the plasticity of marrow cells and how their fate could be determined by environmental cues. Culturing marrow stromal cells in the presence of osteogenic stimuli such as ascorbic acid, inorganic phosphate, and dexamethasone could promote their differentiation into osteoblasts. In contrast, the

addition of transforming growth factor-beta (TGF-b) could induce chondrogenic markers (Friedenstein et al, 1976)

As mentioned earlier, MSCs were originally described as early as the 1960's in animal experiments on embryos, but it was not until the 1980's that the concept of common MSCs in adult tissues was confirmed. Source and availability of MSCs have, however, taken some time to work out. While some MSCs can be found in many organs, those organs are not realistic targets for harvesting them without resulting organ damage. MSCs have also been sourced in bone marrow (1- 4 per 100,000 nucleated cells) or, in a smaller numbers, in the umbilical cord blood itself. They are also present, in much smaller concentrations in many adult human organs, and neonatally in fetal liver and amniotic fluid. Compared to these sources, however, MSCs can be found in more considerable numbers in Wharton's jelly, the matrix of the umbilical cord, and in the placental tissues. The added advantage of Wharton's Jelly is also that there is no risk in the harvesting procedure (Grossi et al, MSC from the Wharton's Jelly).

Characteristics

Morphology

Mesenchymal stem cells are characterized morphologically by a small cell body with a few cell processes that are long and thin, as shown in Figure 2. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance (Netter, 1987). The remainder of the cell body contains a small amount of Golgi apparatus, rough endoplasmic reticulum, mitochondria, and polyribosome. The cells, which are long and thin, are widely dispersed and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils (Brighton et al, 1991).