Therapeutic Uses of Stem Cells in Corneal Diseases Associated with Limbal Stem Cell Deficiency

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٢٠١1

Contents

List of Abbreviations

3T3	**:** 3-day transfer, inoculum 3×10^5 .
5-FU	**:** 5 flourouracil.
ABCG2	**:** The ATP-binding-cassette sub-family G member 2.
AFMS cells	**:** Amniotic fluid mesenchymal stem cells.
AFS cells	**:** Amniotic fluid stem cells.
AM	**:** Amniotic membrane.
AMT	**:** Amniotic membrane transplantation.
APECED	**:** Autoimmune polyendocrinopathy–candidiasis– ectodermal dystrophy.
APS-I	**:** Autoimmune polyendocrine syndrome type I.
ASCs	**:** Adult stem cells.
CD	**:** Cadaver donor.
CK	**:** Cytokeratins.
CLAU	**:** Conjunctival limbal autograft transplantation.
CP	**:** Cicatricial pemphigoid.
DNA	**:** Deoxyribonucleic Acid.
EBs	**:** Embryoid bodies.
ESCs	**:** Embryonic stem cells.
eTAC	**:** Early transient amplifying cell, and early transient amplifying cells.
FCS	**:** Fetal calf serum.
FSCs	**:** Fetal stem cells.
HAM	**:** Human amniotic membrane.
hESCs	**:** Human embryonic stem cells.
HFSCs	**:** Hair follicle-derived epithelial stem cells.
HIV	**:** Human immune deficiency virus.
HLA	**:** Human leucocyte antigen.
HSCs	**:** Hematopoietic stem cells.
iPS cells	**:** Induced pluripotent stem cells.
KLAL	**:** Keratolimbal allograft transplantation.
LECs	**:** Limbal epithelial crypts.

List of Abbreviations (Cont.)

lr-CLAL	**:** Living-related conjunctival limbal allograft transplantation.
LRD	**:** Living related donors.
LSC	**:** Limbal stem cell.
LSCD	**:** Limbal stem cell deficiency.
LSCs	**:** Limbal stem cells.
LSCT	**:** limbal stem cell transplantation.
lTAC	**:** Late transient amplifying cell.
MSCs	**:** Mesenchymal stem cells.
OCP	**:** Ocular cicatricial pemphigoid.
OSR	**:** Ocular surface reconstruction.
P63	**:** Transformation-related protein 63.
PAX 6 gene	**:** Paired box 6 gene.
PMC	**:** Post-mitotic cell.
PMCs	**:** Post-mitotic cells.
SJS	**:** Stevens-Johnson syndrome.
SLT	**:** Sector limbal transplant.
SSCE	**:** Sequential sector conjunctival epitheliectomy.
TAC	**:** Transient amplifying cell.
TACs	**:** Transient amplifying cells.
TDCs	**:** Terminally differentiated cells.
TEN	**:** Toxic epidermal necrolysis.
VSEL	**:** Very small embryonic like.

List of Figures

Introduction

Stem cells have attracted considerable attention not only as means of understanding metazoan development but also as potential therapeutic agents for a spectrum of currently untreatable diseases **(Klassen et al., 2004).**

Stem cells are cells that can proliferate with almost unlimited potential, maintaining a pool of growing and dividing cells, with the added ability that some of the daughter cells can differentiate into specific cell types. It has been suggested that stem cells could be used to replace tissue destroyed by disabling conditions **(Boulton and Albon, 2004).**

In the ophthalmic field, much hope has been placed on the potential use of stem cells to restore sight, particularly in those conditions in which other established treatments have failed and in which visual function has been irreversibly damaged by disease or injury. Stem cell transplantation to treat ocular diseases has triggered enthusiasm across the medical and scientific communities **(Limb et al., 2006).**

The cornea is the primary refractive element at the anterior surface of the eye and represents two thirds of its refractive power **(Levis and Daniels, 2009)** .The corneal epithelium is maintained by a population of stem cells known

as ***limbal stem cells (LSCs)*** due to their location in the basal layer of the outer border of the cornea known as the limbus **(Kolli et al., 2010).**

The limbus is the transition zone between the cornea, conjunctiva and sclera, where the corneal epithelium merges with that of the conjunctiva. Within the limbus, LSCs reside in a stem cell niche, which maintains them in their undifferentiated state. This stem cell niche is represented by ***"the palisades of Vogt"***, which are believed to provide a protective environment for the LSCs **(Ahmad et al., 2010).**

When LSCs are destroyed or the limbal stem cell niche is dysfunctional, a pathological state known as ***limbal stem cell deficiency (LSCD)*** emerges **(Dua et al., 2003).**

LSCD can result from hereditary etiologies such as aniridia **(Holland et al., 2003).** More commonly LSCD occurs as a result of acquired factors including thermal or chemical injuries, Steven-Johnson syndrome, contact lens wear and multiple surgeries and cryotherapies **(Gomes et al., 2003).**

The outcome of LSCD is persistant epithelial breakdown, superficial corneal vascularization, chronic discomfort and impaired vision caused by the migration of the neighboring conjunctival epithelial cells and blood vessels onto the corneal surface **(Daniels et al., 2007a).** So, LSCD is characterized by

a classic triad of signs: conjuctivalization, neovascularization, and decrease in vision **(Vemuganti et al., 2009).**

LSCD can be managed with autologous or allogenic transplantation of LSCs. Systemic immunosuppression is required with allotransplantation. Limbal derived stem cells can be expanded ex vivo on a processed human amniotic membrane (HAM), and transplanted back to the ocular surface with LSCD without the need of immunosupression **(Sangwan, 2001).** More recently, transplantation of alternative sources of epithelium, such as cultivated oral mucosa epithelial stem cells have been reported **(Lim et al., 2009).**

Aim of the work

This review aims to discuss the therapeutic uses of stem cells in corneal diseases associated with limbal stem cell deficiency (LSCD).

Stem Cells Basics

Stem Cells Definition:

Stem cells are undifferentiated cells able to divide indefinitely yet maintain the ability to differentiate into specific cell types. They are able to survive throughout the lifetime of the organism, while maintaining their number, producing populations of daughter cells that can proceed down unique pathways of differentiation **(Levin et al., 2004).**

Stem cells have no particular morphological features, though they have a high nucleus/cytoplasm ratio. These cells provide a renewable source of cells that form the new tissues of the developing organism and supply cells for the tissue growth and renewal of the adult organism **(Krtolicaa, 2005).**

Properties of Stem Cells :

Stem cells are characterized by their capacity for ***self-renewal*** and ***differentiation***, but there are multiple cellular functions that are critical for maintaining the homeostasis of adult stem cells (ASCs) in vivo. Five functional states of stem cells **(S**elf-renewal, **M**aturation, **A**poptosis, **R**esting mode and **T**rafficking) constitute an interesting ***"SMART"*** model for maintaining stem cell homeostasis in vivo **(Fig. 1).** The lack of any of these ***"SMART"*** features would affect their use in therapeutics **(Cheng, 2008).**

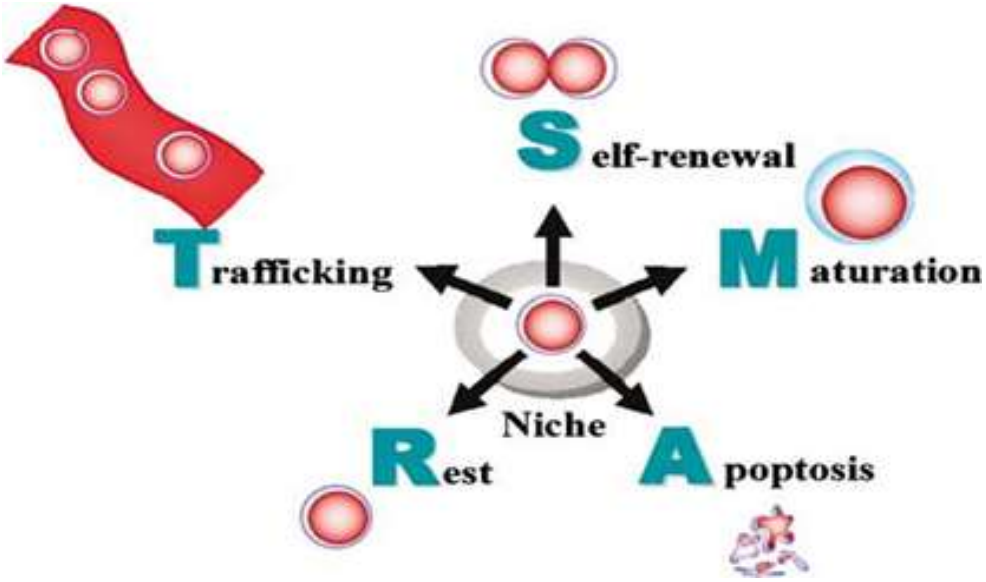

Fig. (1): The *'SMART'* physiological features of stem cells in vivo **(Cheng, 2008).**

1-Self-renewal:

When cells replicate themselves many times, it is called ***"Proliferation"***. Stem cells are capable of dividing and renewing themselves for long periods. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term ***self-renewal*** **(Watt et al, 2001).**

Following tissue damage, the replacement of lost cells often relies on the proliferation and subsequent differentiation of a population of stem cells set aside from other cells within this tissue. In order to maintain their population, stem cells

must self-renew by cell division **(Morrison and Kimble, 2006).**

Stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell **(Beckmann et al., 2007).**

2-Maturation (Differentiation):

When unspecialized cells give rise to specialized cells, the process is called ***"Maturation or Differentiation "***. Cell differentiation is triggered by certain signals inside and outside cells. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment **(Bossard and Zaret, 1998).**

One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. However, unspecialized stem

cells can give rise to specialized cells, including cardiac muscle cells, blood cells, or nerve cells, and so many others **(Levenberg et al., 2003).**

Stem cells reside in a specific microenvironment called ***"the niche"***, wherein they receive signals required to maintain their undifferentiated identity. These signals include several transcription factors. Stem cells differentiate when they leave that niche or no longer receive these signals **(Song et al., 2002).**

Potency specifies the differentiation potential of the stem cells (the potential to differentiate into different cell types). ***Totipotent*** stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent. ***Pluripotent*** stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers. ***Multipotent*** stem cells can differentiate into a number of cells, but only those of a closely related family of cells. ***Oligopotent*** stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells. ***Unipotent*** cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells **(Fig. 2) (Knoepffler et al., 2007).**