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Kursad Turksen *Editor*

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Stem Cells in Regenerative Medicine: Advances and Challenges

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Stem Cells in Regenerative Medicine: Advances and Challenges

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Preface

Much research has focused on the basic cellular and molecular biological aspects of stem cells. Much of this research has been fueled by their potential for use in regenerative medicine applications, which has in turn spurred growing numbers of translational and clinical studies. However, more work is needed if the potential is to be realized for improvement of the lives and well-being of patients with numerous diseases and conditions.

With a goal to accelerate advances by timely information exchange, I am very pleased to announce that we have initiated a series titled Cell Biology and Translational Medicine (CBTMED) as part of SpringerNature's longstanding and very successful Advances in Experimental Medicine and Biology book series. As part of the new CBTMED series, I aim to have emerging areas of regenerative medicine and translational aspects of stem cells covered in each volume. To achieve this, I have recruited outstanding researchers to highlight developments and remaining challenges in both the basic research and clinical arenas. I am pleased to say that this current volume is the first volume of a continuing series.

I would like to express my gratitude to Peter Butler, Editorial Director, who recently provided the opportunity for me to explore the CBTMED series. I thank him for his confidence in this project.

It also gives me great pleasure to acknowledge Meran Owen-Lloyd, Senior Editor, for setting the stage for the series to get off the ground.

A special thank you goes to Sara Germans-Huisman, Assistant Editor, for her efforts in getting the volume to the production stages.

Finally, I thank the contributors not only for their support of the series, but also for their efforts to capture both the advances and remaining obstacles in their areas of research. I am grateful for their efforts and trust that readers will find their contributions interesting and helpful.

Ottawa, ON, Canada — Kursad Turksen

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Embryonic Stem Cells in Development and Regenerative Medicine

Ayşegül Doğan

Abstract

After progressive improvement in embryonic stem (ES) cell field, several studies have been conducted to explore the usage of ES cells in regenerative medicine. Unlimited self renewal and pluripoteny properties, combined with encouraging preclinical trials, remark that ES cell technology might be promising for clinical practice. ES cells, which can form three germ layers *in vitro*, are potential candidates to study development at the cellular and molecular level. Understanding the cell fate decision and differentiation processes during development might enable generating functional progenitor cells for tissue restoration. Progression in gene modifications and tissue engineering technology has facilitated the derivation of desired cells for therapy. Success in differentiation protocols and identification the regulatory pathways simplify the research for clinical applications. Although there are established protocols for cell differentiation *in vitro* and promising preclinical studies *in vivo*, many challenges need to be adressed before clinical translation. In this review, ES cells are discussed as a model of development *in vitro* and as a potential candidate for regenerative medicine. This review also dissusses current challenges for ES cell based therapy.

Keywords

Embryonic stem cells · Development · Differentiation · Regenerative medicine · Tissue engineering

Abbreviations

ALS	Amyotrophic Lateral Sclerosis
ASCs	Adult Stem Cells
BDNF	Brain-Derived Neurotrophic Factor
BMP	Bone Morphogenic Protein
EB	Embryoid Body
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
ES cells	Embryonic stem cells
FACS	Fluorescence-Activated Cell Sorting
FGF	Fibroblast Growth Factor
Flt3L	Fms-like tyrosine kinase 3 ligand
FoxO1	Forkhead box O1
G-CSF	Granulocyte Colony-Stimulating Factor
GDNF	Glial-Derived Neurotrophic Factor
HSCs	Hematopoietic Stem Cells
ICM	Inner Cell Mass
IL	Interleukins
IPS	Induced Pluripotent Stem Cells

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LIF	Leukemia Inhibitory Factor
MACS	Magnetically Activated Cell Sorting
MHC	Major Histocompatibility Complex
MS	Multiple Sclerosis
MSCs	Mesenchymal Stem Cells
NGF	Nerve Growth Factor
PODXL	Podocalyxin-like protein-1
RA	Retinoic Acid
SCF	Stem Cell Factor
SCNT	Somatic Cell Nuclear Transfer
SHH	Sonic Hedgehog
TSCs	Trophoblast Stem Cells
XENCs	Extraembryonic Endoderm Cells

1 Introduction

Different types of mature human cells, residing in the specific tissues and organs, have limited capacity of proliferation which restricts tissue regeneration process (Jopling et al. 2011). However, stem cells have an unlimited lifespan and division potential with a broad range of differentiation capacity. Human stem cells are classified into two major categories based on source and differential potential: embryonic stem (ES) cells and adult stem cells (ASCs) consisting hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). ES cells are capable of differentiation into all cell lineages which makes them remarkable tools for developmental processes and cell therapy studies. ES cells derived from the inner cell mass (ICM) of blastocysts are pluripotent, providing them unrestricted differentiation potential. In defined culture conditions, ES cells could be maintained in undifferentiated state and differentiated into other cell lineages (Nishikawa et al. 2007). Pluripotent ES cells normally produces compact colonies at undifferentiated state and differentiated colonies are likely to be more flattened at the edges where colony morphology loose spherical structure (Yabut and Bernstein 2011). Pluripotent ES cells are characterized by the expression of specific markers including OCT4, cMYC, KLF44, NANOG, SOX2 (Adewumi et al. 2007) which defines the dynamics of stemness and transformation potential. In addition to the mechanisms that regulates pluripotency, self-renewal of ES cells is controlled by sustained expression of proto-oncogenes that needs to be clarified with further studies (Nishikawa et al. 2007).

Strategies to test pluripotency *in vitro* involve embryoid body (EB) formation by inducing differentiation of ES cells in feeder free non-adherent culture systems followed by triggering the transformation of specific cell populations derived from three embryonic germ layers (Itskovitz-Eldor et al. 2000). The development of teratomas as disorganized structures when ES cells are grafted into immunodeficient mice is the most well established pluripotency analysis *in vivo* (Ritner and Bernstein 2010). Because ES cells have unlimited proliferation and transformation capacity *in vitro* and *in vivo*, they have become the aim of interest of many researches in recent years as a comprehensive cell source to study development and new therapeutic approaches for regenerative medicine. Moreover, advanced genetic modification of ES cells is an important step, allowing the generation of convenient cell lineages that are desired for regenerative medicine in cell-based therapies.

In this review, the current strategies to study ES cells as a model of human development and regenerative medicine and the improvements of cell based approaches will be described in detail and the challenges for experimental research and clinical applications will be briefly discussed.

2 ES Cells in Development

Understanding the ES cells from an embryological viewpoint is required to identify ES cell biology, develop experimental model systems and establish clinically relevant protocols for therapeutic applications. In parallel, improvement of ES cell-based differentiation models could lead to overcome differences between mouse and primate embryologic development and to trace fate decision of human ES cells in *in vitro* culture conditions.

Most of the data for mammalian embryologic development are based on mouse studies. However, human and mouse embryonic development have significant differences in terms of gene expression and regulation. (Pera and Trounson 2004; Rossant 2015). Therefore, human and mouse ES cells obtained from ICM of the embryo have disparities. Both human and mouse ES cells could be kept in *in vitro* culture conditions at undifferentiated state by maintaining normal karyotype after several passages and hold great potential for regenerative medicine applications (Keller 2005). ES cells could be a suitable model for development and regenerative therapy research as they are pluripotent which allows generation of mature differentiated cells of all tissues in the adult body.

Recent advances in cell culture protocols to obtain various cell lineages could not only provide reprogramming strategies but also serve unique models for early development or even support multiple cell-based regenerative medicine approaches.

2.1 Differentiation in Culture

Under defined culture conditions that enable controlled exit from the pluripotent state, ES cells transform into differentiated cell types of the embryonic germ layers: mesoderm, endoderm, and ectoderm (Smith 2001). As developmental signals regulating the embryogenesis also contribute to the differentiation of pluripotent cells, mouse and human ES cells demonstrate disparities in culture systems. Although human ES cells could give rise to trophoectoderm derived lineages by bone morphogenic protein (BMP)-4 induction (Xu et al. 2002b), mouse ES cells do not have the ability to differentiate towards trophoblastic lineages. Cell surface glycolipids and proteoglycans including SSEA-1, -3, -4, TRA-1-60 and TRA-1-81 are differentially expressed in mouse and human ES cell lines (Pera and Trounson 2004). Phenotypic differences such as colony morphology or feeder cell and leukemia inhibitory factor (LIF) requirements are also distinct for both cell lines (Pera et al. 2000). Although mouse ES cells need LIF to maintain the pluripotency in the culture conditions as they require physiologically for mouse embryogenesis, human ES cells do not respond LIF because of differentially activated pathways including STAT3 and LIF pathways (Chen et al. 2015; Ginis et al. 2004; Sato et al. 2004). All these differences are organized by different regulatory mechanisms and precede distinct differentiation and development patterns in culture conditions.

Therefore, ES cells as attractive pluripotent cell sources represent a promising tool for development researches and cell products for therapies. Optimization of culture conditions for directed differentiation is a major challenge that should be overcome to utilize potential of ES cells to obtain functional tissue-specific cells.

2.2 Differentiated Cell Lineages from ES Cells

Embryo is a bulk of cells with the same progeny in the beginning but soon after developmental program is activated and cells switch to a differentiated state based on their position and induction signals during embryogenesis (Dvash et al. 2006). ES cell could differentiate into cell types originated from three embryonic germ layers when vital factors that keep them at undifferentiated state are removed and specific culture conditions are applied (Smith 2001).

There are three general well established approaches for ES cell differentiation: EB formation (Doetschman et al. 1985), feeder cell dependent methods (Nakano et al. 1994) and feeder free extracellular matrix protein-based techniques (Nishikawa et al. 1998). EBs as three-dimensional multicellular structures are created by ES cell aggregates in non-adherent culture conditions, and mimic embryo development and gem layer specification (Doetschman et al. 1985). Because of easy manipulation and a broad range of cell lineages generated from EB culture, the protocol is a classic method for mouse and human ES cell researches. Apart from EB culture method, stromal cell lines as LIF supplier are

used as feeder cell layers to induce cell differentiation of ES cells to hematopoietic (de Pooter et al. 2003) or mesodermal lineages (Nishikawa et al. 1998). Alternatively, ES cells are placed on extracellular proteins such as matrigel, collagen or fibronectin to induce defined cell types. These three methods and comparison are summarized in Fig. 1. Several growth factors including fibroblast growth factor- (FGF), activin A, nerve growth factor (NGF) and BMP are used to modulate ES cell differentiation and generation of cells displaying three germ layer characteristics (Dvash et al. 2006).

2.2.1 Mesodermal Cell Lineages

Mesodermal lineages including cardiomyocytes, endothelial cells and hematopoietic cells have been obtained from ES cells by using co-culture or growth factor approaches. Basically, in order to generate hematopoietic lineages such as erythroid, myeloid, and lymphoid cells (Wang et al. 2005), ES cells are induced with growth factors including stem cell factor (SCF), Fms-like tyrosine kinase 3 ligand (Flt3L), interleukins (IL-3, IL-6), BMP-4 and granulocyte colony-stimulating factor (G-CSF). Hematopoietic commitment has been well-characterized in ES cell differentiation models and expected to be useful for developing transplantable cells in therapy.

The generation of beating cardiomyocyte loci after cardiac lineage differentiation in ES cell culture has been studied in spontaneous and co-culture conditions (Mummery et al. 2003; Xu et al. 2002a). *In vitro* derived contracting cardiomyocytes were similar to early cardiac tissue (Snir et al. 2003), indicating that ES cell might give rise to physiologically functional early cardiomyocytes.

Endothelial cell differentiation and marker (CD31) expression has been detected in ES cell using EB protocol (Levenberg et al. 2002). Moreover, ES cell derived endothelial cells have demonstrated morphological features such as tube-like and vascular network-like structures.

Although early mesodermal lineage commitment and underlying mechanisms are still unknown, various mesodermal cell types have been generated from ES cells in *in vitro* cultures and seems to be promising for future therapies.

2.2.2 Endodermal Cell Lineages

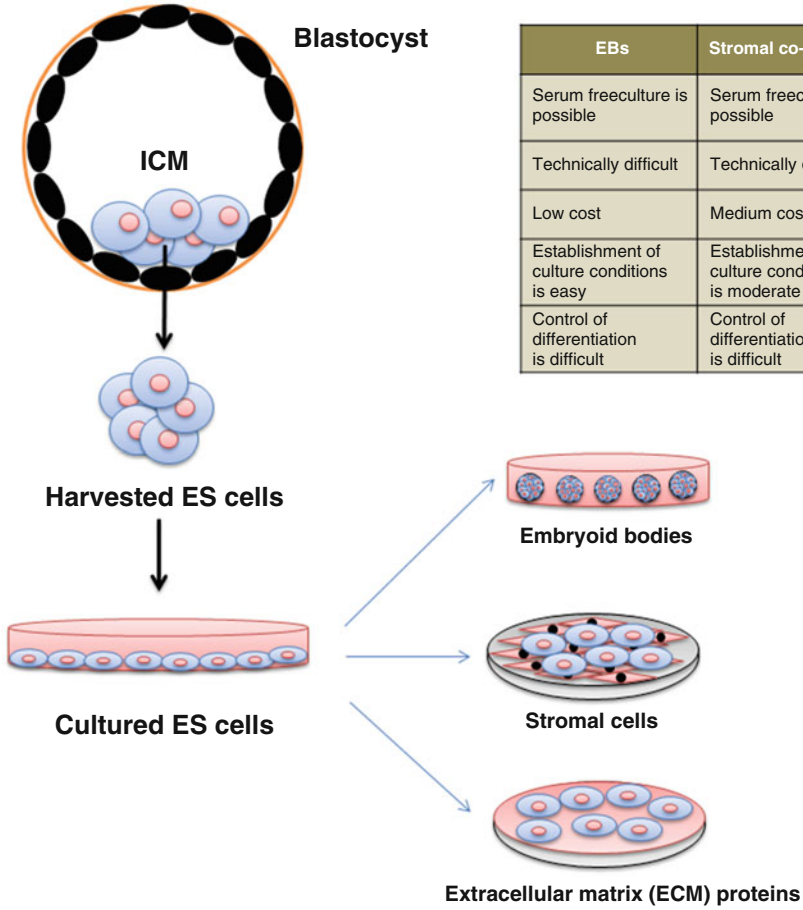
The differentiation and characterization of endodermal lineages from ES cells is crucial because they might be used as a therapeutic source for liver and pancreatic tissues. Despite the development of pancreatic β-cells and hepatocytes would be promising for the treatment of diabetes and liver disorders, derivation endoderm cell lineages is a slow process. Specific molecules to induce endoderm differentiation and identification of marker gene profiles for distinct endoderm populations should be addressed to obtain certain endodermal cells. Pancreatic and hepatic specific gene expression patterns have been observed in EB culture systems. Stimulation of human ES cells with sodium butyrate results in epithelial like cells with a hepatocyte marker profile. Similarly, insulin staining and switch to pancreatic lineage specific gene expression have been reported in EBs (Assady et al. 2001; Rambhatla et al. 2003).

However, generation of functional hepatocytes and pancreatic β-cells have to be studied in detail and improved by identification of stimulant factors and underlying mechanisms that control endodermal germ layer specification.

2.2.3 Ectodermal Cell Lineages

Ectodermal differentiation from ES cells can be generated under appropriate culture conditions including spontaneous EB differentiation, serum free monolayer culture and retinoic acid exposure. Extensive research has been focused on neural differentiation as derivation of various neural cell types is easy to obtain when treated with fully defined culture conditions and might be a solution for neurodegenerative disorders.

Neurospheres in suspension culture systems express neuroectoderm markers including nestin, N-CAM and Pax6, and these progenitor populations could give rise to neurons, astrocytes and oligodendrocyte-like cells. These ES cell derived cells might be electrophysiologically



EBs	Stromal co-culture	ECM proteins
Serum freeculture is possible	Serum freeculture is possible	Serum freeculture is possible
Technically difficult	Technically difficult	Technically easy
Low cost	Medium cost	High cost
Establishment of culture conditions is easy	Establishment of culture conditions is moderate	Establishment of culture conditions is difficult
Control of differentiation is difficult	Control of differentiation is difficult	Control of differentiation is easy

Fig. 1 Comparison of for ES-cell differentiation protocols. There are three differentiation methods available for ES cell transformation: Embryoid bodies, stromal cell sources as feeders and extracellular matrix (ECM) protein coating. Pros and cons of each method in terms of technical difficulties and efficiency is summarized

active that responded neurotransmitters and survived when injected to mice brain (Carpenter et al. 2004; Pera et al. 2004). In addition to neural cell types, ES cell could give rise to other ectodermal lineages such as keratinocyte marker expressing epithelial cells. BMP-4 promotes keratinocyte development while inhibiting neural lineage development. ES cell derived keratinocytes form mouse skin-like structures in organ culture systems (Coraux et al. 2003; Kawasaki et al. 2000). Optimization of culture conditions for ectodermal derived cell populations could be a solution for various diseases such as neural system disorders in the future.

2.2.4 Extraembryonic Tissues

The outer cell layer of the embryo known as trophectoderm arises around blastocyst and is required for embryo implantation and placenta formation. Although mouse ES cells do not form the extraembryonic tissues, human ES cell are capable of differentiating into trophoblast cells *in vitro* (Thomson et al. 1998; Xu et al. 2002b). BMP-4 administration induces trophoblast marker expression, and chorionic gonadotrophin production in human ES cell cultures (Xu et al. 2002b). Although extensive research is required to evaluate the role of BMPs in trophectodermal layer appearance in the

mammalian embryogenesis, BMP treatment in the *in vitro* human ES culture system is useful to obtain different extraembryonic structures. BMP-2 treated human ES cells have been reported to produce the extraembryonic endoderm (Pera et al. 2004). OCT4 as one of the master transcription factor has regulated the trophoblast and endoderm differentiation patterns in mouse and human ES cells distinctly (Hay et al. 2004). Wei et al used CRISPR/Cas9 and upregulated Cdx2 and Gata6 for the derivation of two extraembryonic lineages such as typical trophoblast stem cells (TSCs) and extraembryonic endoderm cells (XENCs) which were similar to their counterparts *in vivo* (Wei et al. 2016). Identification of new factors and genes regulating the extraembryonic development might help understanding the pre-implantation stage during development.

3 ES Cell Originated Cell Types in Regenerative Medicine

Although some of the vertebrate species have broad regenerative potential, humans have limited tissue regeneration capacity that restricts restoration of damaged organs. Regenerative medicine using stem cells with extensive division, proliferation and differentiation potential has been an emerging area of research in recent years. ES cells are popular in regenerative medicine due to their self-renewal and pluripotent properties. Difficulties in access to adequate numbers of adult tissue specific stem cells make ES cells promising candidates for cell based therapy. With the ability of direct differentiation protocols and genetic modification tools, ES cell based regenerative medicine could be a solution for several diseases and tissue engineering applications. The pluripotency of ES cells regulates lineage commitment during differentiation which is required for regenerative therapeutic applications. This section of the review will discuss some of the cell lineages derived from ES cells for therapy and potential regenerative applications.

3.1 Cartilage Tissue Engineering

Cartilage is an avascular supportive tissue which consists of chondrocytes and extracellular matrix (ECM) along with aggrecan and collagen fibrils. Due to its avascular nature, cartilage tissue has poor repair capacity leading to trauma induced osteoarthritis (Toh et al. 2011). Development of new cartilage tissue is challenging because of limited cell source for cell based therapies. Although some success has been achieved using MSCs and chondrocyte implantation; limited self-renawal, proliferation and differentiation abilities still remain to be solved. Therefore, understanding and controlling the ES cell differentiation to cartilage might be promising. ES cells should be cultured in defined culture conditions with appropriate cytokines, growth factors and stimulant chemicals to induce chondrocyte differentiation. Human ES cell derived EBs differentiated into chondrogenic pellets in serum free media followed by small molecules and chondrogenic medium (TGFβ3 and BMP4) treatment. (Yang et al. 2016). EB-based ES cell culture systems have led the formation of heterogeneous cartilaginous nodules and chondrocytes which de-differentiate easily to other cell types (Hegert et al. 2002; Jukes et al. 2008b). Obtaining MSCs from ES cells for subsequent chondrocyte differentiation is a suitable method as using progenitor cells instead of ES cells reduces the risk of teratoma formation and increases the success of direct differentiation to mesodermal lineages (Karlsson et al. 2009). These MSC cells derived from ES cells have been differentiated into chondrogenic cells in modified hydrogels (Hwang et al. 2006) and pellet culture systems (Nakagawa et al. 2009). Hyaline producing chondrocytes have been obtained from human ES cell derived MSCs by sequential BMP-2 and Wnt5a treatment *in vitro* and repaired rat chondral defects *in vivo* (Gibson et al. 2017).

Differentiated mouse ES cells under chondrogenic conditions have resulted in teratoma formation when injected to immunodeficient mice. However, when these differentiated cells were seeded on polycaprolactone scaffolds,

teratoma formation has not been observed (Fecek et al. 2008). Yamashita and colleagues used mouse ES cells in micromass culture system supplemented with TGF-β1 and BMP-2 to generate chondrogenic cells formed cartilage *in vivo* (Yamashita et al. 2009). In a different approach, the transformation of human ES cells into chondrocytes and cartilage-like tissue formation has been illustrated by a co-culture model and teratoma formation has not been observed following implantation (Hwang et al. 2008a). Similarly, human ES cell derived MSCs and bovine chondrocytes co-cultured and resulting cell pellets were implanted into the osteochondral defects of rat (Hwang et al. 2008b). Cartilage tissue was formed at the defect region (Takagi et al. 2007) and more hyaline containing tissue was observed when same model was used with hydrogel and growth factors such as BMP-7 and TGF-β1 (Toh et al. 2010). Consistently, human ES cells were implanted with PLLA and PEG scaffolds, and hydrogels that formed cartilaginous tissues after implantation into mice (Hwang et al. 2008a; Vats et al. 2006). Mechanical suppression induced by 3-D polydimethylsiloxane scaffolds triggered early cartilage marker gene expression and enhanced chondrogenic differentiation of ES cells (McKee et al. 2017). Cheng et al. used fibrin gel for implantation of chondrogenic cells derived from human ES cells into focal defects of nude rats and provided cartilage repair (Cheng et al. 2014). In addition to these differentiation conditions, incubation time in the culture media is also important to provide a cartilaginous structure. Chondrogenic induction of mES cells should be prolonged at least 14 days and differentiated mouse ES cells under chondrogenic condition created functional bone tissue and calcification only after 21 days (Jukes et al. 2008a). To sum up, while chondrogenic differentiation of ES cells in *in vitro* and *in vivo* culture conditions has been successful both for mouse and human ES cells, yet signaling pathways underlying the mature chondrocyte development from human ES cells, large scale production methods and appropriate delivery systems should be identified for long-term *in vivo* achievements and clinical benefits.

3.2 Neural Disease Therapies

Neurological diseases such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and spinal cord disorders are the result of severe neuron damage and loss of function in the central and peripheral nerve systems (Kim and De Vellis 2009). Because nerve system has poor regeneration ability, cell replacement therapies have been the aim of interest as potential therapeutic options for the neurological disorders. ES cells with remarkable proliferation capacity and extensive potential to differentiate any desired cell type including dopamine neurons, glial cells, astrocytes and oligodendrocytes (Joannides et al. 2007) offer the advantage of new regenerative medicine strategies. Defined culture protocols have been established including retinoic acid treatment and three dimensional neurosphere cultures supplemented with FGF-2 to generate neural progenitors from ES cells. Engineered human ES cells which overexpressed FGF-2 exerted neuroprotective role in ventral root avulsion model indicating the activity of FGF signaling in neural dierentiation (Araújo et al. 2017). Although derivation of neural cells from ES cells is simple, certain identification criteria of neural subtypes for their different physiological functions are required for clinical application.

Generation of dopaminergic neurons from ES cells is of particular interest as they are involved in the pathogenesis of motor symptoms in the Parkinson's disease. ES cell derived dopamine neurons could be used in neuron replacement therapy for neurodegenerative diseases including Parkinson's disease (Tabar and Studer 2014). Both mouse and human ES cells can give rise to dopamine neurons that actively produce tyrosine hydroxylase, secrete dopamine and form *in vitro* synapses (Murry and Keller 2008). Over the years, different protocols have been used for direct differentiation to specify dopaminergic neurons from ES cells. Although stromal cell co-culture followed by FGF8 and sonic hedgehog (SHH) administration enhanced the dopaminergic neuron induction, addition of other growth factors

including brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF) and TGF-β3 increased tyrosine hydroxylase producing neurons (Joannides et al. 2007; Murry and Keller 2008). Identification of efficient protocols that could form fully defined dopamine neurons to be implantated is required to develop a promising strategy for Parkinson's disease.

Although there is not an available human clinical trial for ES cell derived neural progenitors for the Parkinson's disease, there have been several mice and rat *in vivo* researches. Neurospheres of human ES cells formed nestin positive neurons and remained in the brain of immunosuppressed rats; however, no benefit was observed due to the absence of differentiation signals required for direct cell specification (Ben-Hur et al. 2004). Similar to this report, *in vitro* differentiated ES cells expressing tyrosine hydroxylase were not successful after transplantation (Park et al. 2005).

Another challenging cell type for neural differentiation from ES cells is engraftable glial cells such as astrocytes and oligodendrocytes which are important for neurodegenerative diseases due to myelin sheath dysfunction. The differentiation protocol involves various steps such as neurosphere formation followed by treatment with FGF2 and epidermal growth factor (EGF) containing media. Approximately, 90% of the cells in this culture system have been detected as oligodendrocytes and remaining population were noted as astrocytes and neural cells (Nistor et al. 2005). These cells myelinated the axons when transplanted into the mouse, indicating the possibility of using *in vitro* derived oligodendrocytes for the treatment. There are some research for neuron recovery and remyelination by using neural progenitors derived from mouse and human cells in mice and rat spinal cord injury models (Kimura et al. 2005; McDonald et al. 1999). Keirstead and collegues used human ES cell derived oligodendrocytes in a rat spinal cord injury model and provided remylation to axons contributing to motor function (Keirstead et al. 2005). Konig et al showed the positive effect of mouse ES cell derived neural procursors for rat spinal cord avulsion injury model (Konig et al. 2017). Spinal cord injury restoration by using human ES cells have started in recent years and seems to be promising for patient therapy in clinal applications (Shroff 2016). Abbasi et al. demonstrated the successful neural differentiation of mouse ES cells in spesifically oriented polycaprolactone scaffolds which could be used in spinal cor injury (Abbasi et al. 2016).

Moreover, derivation of neural crest cells and functional peripheral neurons from human ES cells was shown in feeder free culture system indicating the potential promising regenerative application (Zhu et al. 2017). More research and trials need to be completed to move towards from basic research to clinical applications for neural regeneration.

3.3 Pancreatic Diseases Therapies

The development of potenatial cell based therapies for type I diabetes could be managed by generation of functional pancreatic β cells. ES cells as a source of insulin producing β cells might be used for transplantation in diabetic patients. Pancreas development starts from foregut endoderm and is regulated by retinoic acid (RA) and SHH signaling. Upregulation of transcription factor Pdx1 is the first indicator of pancreatic development. Pancreas development is controlled by FGF-10 and subsequently enhanced Notch signaling which suppresses transcription factor Ngn3 and induces β cells lineage. (Murry and Keller 2008). Although *in vitro* human ES cell diffentiation protocols were not very efficient for mature β cell development, insulin production and response to glucose have been observed (Jiang et al. 2007). In two previously published studies, human ES derived Pdx1 expressing cells transplanted into kidney capsule of diabetic mice did not form teratoma after transplantation and they were insulin positive *in vivo* (Shim et al. 2007). Fetal pancreatic tissue has been used to maturate human ES cell derived Pdx1 expressing progenitors as an alternative strategy and injected into kidney capsule of mice. Resulting cell population was insulin producing pancreatic β cells (Brolen et al. 2005). Saxena et al used synthetic lineage-control network engineering method as

an interesting approach to program transcription factors that are important for pancreatic differentiation including Pdx1. This network induced the differentiation of human ES cells into insulin producing pancreatic β cells (Saxena et al. 2017). In adition to cytokine and growth factor treatment modalities in *in vitro* culture, genetic modifications in pluripotent stem cells are promising to obtain functional pancreatic cells. mir-375, a key regulator of pancreatic development, enhanced insulin producing pancreatic islet differentiation of human ES cells in culture (Lahmy et al. 2016). Silencing of Forkhead box O1 (FoxO1) which is a regulator of pancreatic β cell differentiation increased pancreatic differentiation and insulin secretion upon glucose stimuli in human ES cells. Similar effects were observed in FoxO1 inhibitor treatment indicating the potential use of FoxO1 in ES cell based therapy applications (Yu et al. 2018). Additionaly, researchers have been trying to solve immunosuppression problems of transplanted pancreatic cells in preclinical studies. Human ES cell derived pancreatic progenitors were encapsulated with alginate and transplanted into streptozotocin treated mice. Transplanted implants control glycemic regulation and had viable cells after 174 day (Vegas et al. 2016). Although cell differentiation protocols are not enough to generate fully mature pancreatic cells *in vivo*, development of insulin producing cells from ES cells via established culture conditions could promote functional cell transformation.

3.4 Heart Regeneration Therapies

Because post-natal cardiac regeneration is limited to some intrinsic cell repair processes, translation of cell based regeneration to efficient clinical trials is the focus of current research in this area. As ES cells are pluripotent, they could be able to differentiate into major heart cell types *in vitro* by using defined culture conditions and various growth factors inluding TGF- β, FGF-2 and BMP-2 (Singla 2009). The crucial point for ES cell-based cardiac regeneration is to generate mature cardiomyocytes that could achive electrical and mechanical interaction network successfuly. Mouse and human ES cell differentiation into cardiomyocytes in culture conditions could mimic the embryonic cardiac development in terms of cardiac marker gene expression. Pluripotency markers are downregulated and mesoendoderm marker Brachyury (T/Bra) and early cardiac markers such as Nkx2.5 and Tbx5 are upregulated during *in vitro* differentiation. Thereafter, late cardiac markers such as cardiac α-myosin heavy chain and cardiac β-myosin chain are observed. Although embryonic heart development is regulated by a complex series of events and gene regulatory networks, *in vitro* ES cell differentiation into cardiac lineages and marker expressions in a timely manner resembling embryogenesis is promising for fully elucidating the devolepmental process and presentation of future therapies (Zhu et al. 2009).

There are well-established protocols for both mouse and human ES cells differentiation *in vitro* including spontaneous EB and growth factor based defined culture conditons. Human ES cells maintained in monolayer culture systems in the presence of TGF-β, activin A and BMP-4 sequentially yielded at least 30% of cardiomyocytes with contracting foci and contributed to the regeneration of infarcted rat hearts (Laflamme et al. 2007). The positive contribution of human ES cell derived cardiomyocytes to myocardium regeneration has been shown in athymic nude rats. Implanted cardiomyocytes proliferated rapidly, expressed cardiac markers and produced a graft after transplantation without teratoma-like structure formation (Laflamme et al. 2005). Caspi et al. have transplanted beating human ES derived EBs to infarcted rat hearts and observed electrically active viable grafts 8 weeks after transplantation (Caspi et al. 2007). In another study, van Laake et al. have used co-culture method and transplanted human ES cell derived cardiomyocytes and endodermal cells together. Non-cardiac cells were eliminated after transplantation and grafts were viable at 12th weeks post-transplanatation (van Laake et al. 2007). Similarly, mouse ES cells derived cardiomyocytes were alive after transplantation into infarcted

animals and helped regeneration in the infarcted tissue (Kolossov et al. 2006; Ménard et al. 2005; Min et al. 2003). In another study, mouse ES cell derived cardiac progenitor cells were differentiated into cardiomyocytes when grafted into infarcted mouse hearts and did not cause teratoma. Moreover transplanted cells helped imroving heart function and differentiate into functional cells (Christoforou et al. 2010). Human ES cell derived cardiac progenitors has been used in some clinical trials for the recent years. Cardiac progenitors embedded in fibrin scaffolds were transplanted into patiend and situatons were improved without any teratoma or complication (Menasché et al. 2015). In a recent clinical trial, human ES cell derived cardiac cells were used for ischemic left ventricular dysfunction an improved for systolic function (Menasché et al. 2018). Although preclinical mouse and human ES cells studies are promising for future therapies, *in vitro* differentiation efficiency, cell purity before transplanataition, effective and stable grafting techniques should be improved and potential paracrine effets for graft integration to the host should be identified for successful clinical applications.

4 Challenges for Regenerative Therapy

There are many issues that need to be adressed for ES cell based clinical applications such as immunogenicity, tumorigenic properties, and mature and functional cell diferentiation with high purity. Potential challenges and strategies will be discussed in this part.

4.1 Teratoma Formation

Teratomas are complex tumors consisted of random tissues derived from three different germ layers and used to define pluripotency of human ES cells. Safety concerns regarding teratoma formation after transplantation is one of the major problems for therapeutic applications. In order to avoid teratoma formation, ES cells should be differentiated terminally into desired cell lineages before transplantation. Cell purification based on a visible identified phenotype, surface marker expression and genetic marker selection should be conducted carefully for most of the restoration applications. Fluorescence-activated cell sorting (FACS) and magnetically activated cell sorting (MACS) (Vodyanik et al. 2006) could be used to select cell populations based on surface marker expression profile. Although these techniques are highly efficient, lack of markers and requirements of cell expansion after sorting remain as techniqual problems. Moreover, fluorescence reporter genes could be inserted into the spesific places in the genome for selection of differentiated cell types. However; tagging these proteins in the genome and selection might cause tumorienicity problems after transplantation into host due to possible oncogenesis by chromosomal insertion. Another novel strategy to eliminate undifferentiated ES cells in culture is to use antibodies against undifferentiated cells. Choo et al have used a cytotoxic antibody against podocalyxin-like protein-1(PODXL) to remove undifferentiated cells (Choo et al. 2008). However expression of PODXL in multiple human tissues limits the usage in therapy. SSEA5, CD9, CD30, CD50 and CD200 have been used to select undifferentiated ES cells from culture as pluripotency markers. The problem with those antibodies is the unspesific expression in the differentiated tissues. SSEA5 is more spesific compared to other markers and has been utilized to remove pluripotent cells in culture (Tang et al. 2011). Similarly, targeting Claudin-6 which is a tight-junction protein and absent in differentiated cells has been proven as a succesfull strategy to sort undiferentiated cells (Ben-David et al. 2013). In addition to spesific antibodies, small molecules for certain differentiation culture systems have been developed. Targeting anti-apoptotic genes in pluripotent cells by using small molecules might block teratoma formation (Mohseni et al. 2014).

4.2 Immune Rejection

ES cells increased the expression of major histocompatibility complex (MHC) when they differentiated (English and Wood 2011). Therefore transplantation of terminally differentiated cells may cause immune rejection in tissue regeneration applications. Although immune rejection as a tough barrier for cell transplantation is adressed by several strategies including immunosupression and genetically engineered ES cells that secrete immunosuppressive cytokines, more efficient strategies should be identified. Blockage of leukocyte costimulatory molecules as a short-term immunosuppressive therapy has enabled xenogeneic engraftment of human ES cells (Pearl et al. 2011). Immune rejection might be avoided by engineering patient spesific ES cells via somatic cell nuclear transfer (SCNT) which is conducted by transfer of a somatic cell nucleus into an egg. Then ES cells could be isolated from blastocyts for further differentiaion and transplantation (Yeo and Lim 2011). This technique seems to be a solution for immune rejection; however, acquiring enough number of human eggs remains as a problem. Induced pluripotent stem cells (IPS) technology as an alternative technology is useful for eliminating immune rejection. Although IPS cells could be a sloution to immune reactions and easy in terms of ethical regulations; culture conditions, genetic manipulations for IPS formation and additional parameters that need control emerge new challenges. Moreover, expansion of IPS cells, preventing apoptosis and senesence are other obstacles for clinical research.

4.3 Patient Population and Preclinical Models

In addition, patient population and transplantation area should be selected appropriately for early clinical trials. Determination of right patient population before starting advanced clinical trials is critical. Patient populations with advanced disease stage should be selected to minimize the risk during ES cell based therapeutic applications (Murry and Keller 2008).

Identification of right preclinical models for ES cell based regenerative medicine applications is required before moving towards clinical applications. Although *in vitro* differentiation and cell purification still remain as major issues, preclinical success is another obstacle that need to be improved. Larger animal models such as non-human primates, dog or sheep could be used for preclinical studies, however; these models are expensive and diffucult to handle in practice. Murine models including the immunodefienct and genetically engineered mice are inexpensive and easy to work in the laboratory. Although small animals are usefull for preclinical studies, murine physiology is different from human and restricts the interpretation of preclinical results. Despite the limitations in ES cell research, differentiation protocols and models; ES cell based therapy still seems promising for future treatment options. Current researches and developed techniques might enable the efficient usage of ES cells for further regenerative medicine applications.

5 Future Perspectives

The main challenge for ES cell based therapy is the derivation of functional, mature and engraftable cells that are responsive to the signals provided by host tissue and differentiate into desired cell types easily. The major concern of ES cell based current clinical tirals for regenerative medicine is the unlimited differentiation capacity of ES cells (pluripotency) that likely results in teratoma formation. A strategy to bypass teratoma formation through uncontrolled ES cell proliferation and differentiaon is to establish appropriate culture conditions for *in vitro* transformation. Although several cell types have been obtained using well-established *in vitro* differentiaiton protocols, *in vivo* transplantation and disease models should be improved and optimized for successfull early stage clinical studies. Moreover, these differentiation strategies might enable scientists to understand

developmental basis of human embryologic development and tissue spesification. Identification of molecular elements that controls tissue spesification and cell fate desicion during development and cell differentiation may facilitate the derivation of various cell types that are relevant for clinical therapies.

The availability of desired cell lineages from ES cells in appropriate and efficient culture systems will provide an opportunity for the treatment of diseases in a safe and qualified way.

References

Abbasi N, Hashemi SM, Salehi M, Jahani H, Mowla SJ, Soleimani M, Hosseinkhani H (2016) Influence of oriented nanofibrous PCL scaffolds on quantitative gene expression during neural differentiation of mouse embryonic stem cells. J Biomed Mater Res A 104:155–164

Adewumi O et al (2007) Characterization of human embryonic stem cell lines by the international stem cell initiative. Nat Biotechnol 25:803–816

Araújo MR, Kyrylenko S, Spejo AB, Castro MV, Junior RSF, Barraviera B, Oliveira ALR (2017) Transgenic human embryonic stem cells overexpressing FGF2 stimulate neuroprotection following spinal cord ventral root avulsion. Exp Neurol 294:45–57

Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M (2001) Insulin production by human embryonic stem cells. Diabetes 50:1691–1697

Ben-David U, Nudel N, Benvenisty N (2013) Immunologic and chemical targeting of the tight-junction protein Claudin-6 eliminates tumorigenic human pluripotent stem cells. Nat Commun 4:1992

Ben-Hur T, Idelson M, Khaner H, Pera M, Reinhartz E, Itzik A, Reubinoff BE (2004) Transplantation of human embryonic stem cell–derived neural progenitors improves behavioral deficit in parkinsonian rats. Stem Cells 22:1246–1255

Brolen GK, Heins N, Edsbagge J, Semb H (2005) Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing β-cell–like cells. Diabetes 54:2867–2874

Carpenter MK et al (2004) Properties of four human embryonic stem cell lines maintained in a feeder-free culture system. Dev Dyn 229:243–258

Caspi O et al (2007) Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. J Am Coll Cardiol 50:1884–1893

Chen H et al (2015) Reinforcement of STAT3 activity reprogrammes human embryonic stem cells to naive-like pluripotency. Nat Commun 6:7095

Cheng A, Kapacee Z, Peng J, Lu S, Lucas RJ, Hardingham TE, Kimber SJ (2014) Cartilage repair using human embryonic stem cell-derived chondroprogenitors. Stem Cells Transl Med 3:1287–1294

Choo AB et al (2008) Selection against undifferentiated human embryonic stem cells by a cytotoxic antibody recognizing podocalyxin-like protein-1. Stem Cells 26:1454–1463

Christoforou N et al (2010) Implantation of mouse embryonic stem cell-derived cardiac progenitor cells preserves function of infarcted murine hearts. PLoS One 5:e11536

Coraux C et al (2003) Reconstituted skin from murine embryonic stem cells. Curr Biol 13:849–853

de Pooter RF, Cho SK, Carlyle JR, Zúñiga-Pflücker JC (2003) In vitro generation of T lymphocytes from embryonic stem cell–derived prehematopoietic progenitors. Blood 102:1649–1653

Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R (1985) The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. Development 87:27–45

Dvash T, Ben-Yosef D, Eiges R (2006) Human embryonic stem cells as a powerful tool for studying human embryogenesis. Pediatr Res 60:111–117

English K, Wood KJ (2011) Immunogenicity of embryonic stem cell-derived progenitors after transplantation. Curr Opin Organ Transplant 16:90–95

Fecek C et al (2008) Chondrogenic derivatives of embryonic stem cells seeded into 3D polycaprolactone scaffolds generated cartilage tissue in vivo. Tissue Eng Part A 14:1403–1413

Gibson JD, O'sullivan MB, Alaee F, Paglia DN, Yoshida R, Guzzo RM, Drissi H (2017) Regeneration of articular cartilage by human ESC-derived mesenchymal progenitors treated sequentially with BMP-2 and Wnt5a. Stem Cells Transl Med 6:40–50

Ginis I et al (2004) Differences between human and mouse embryonic stem cells. Dev Biol 269:360–380

Hay DC, Sutherland L, Clark J, Burdon T (2004) Oct-4 knockdown induces similar patterns of endoderm and trophoblast differentiation markers in human and mouse embryonic stem cells. Stem Cells 22:225–235

Hegert C et al (2002) Differentiation plasticity of chondrocytes derived from mouse embryonic stem cells. J Cell Sci 115:4617–4628

Hwang NS, Varghese S, Zhang Z, Elisseeff J (2006) Chondrogenic differentiation of human embryonic stem cell–derived cells in arginine-glycine-aspartate–modified hydrogels. Tissue Eng 12:2695–2706

Hwang NS, Varghese S, Elisseeff J (2008a) Derivation of chondrogenically-committed cells from human embryonic cells for cartilage tissue regeneration. PLoS One 3:e2498

Hwang NS et al (2008b) In vivo commitment and functional tissue regeneration using human embryonic stem cell-derived mesenchymal cells. Proc Natl Acad Sci 105:20641–20646

Itskovitz-Eldor J et al (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 6:88

Jiang J, Au M, Lu K, Eshpeter A, Korbutt G, Fisk G, Majumdar AS (2007) Generation of insulin-producing islet-like clusters from human embryonic stem cells. Stem Cells 25:1940–1953

Joannides AJ et al (2007) A scaleable and defined system for generating neural stem cells from human embryonic stem cells. Stem Cells 25:731–737

Jopling C, Boue S, Belmonte JCI (2011) Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nat Rev Mol Cell Biol 12:79–89

Jukes JM, Both SK, Leusink A, Lotus MT, Van Blitterswijk CA, De Boer J (2008a) Endochondral bone tissue engineering using embryonic stem cells. Proc Natl Acad Sci 105:6840–6845

Jukes JM, Moroni L, Van Blitterswijk CA, De Boer J (2008b) Critical steps toward a tissue-engineered cartilage implant using embryonic stem cells. Tissue Eng Part A 14:135–147

Karlsson C et al (2009) Human embryonic stem cell-derived mesenchymal progenitors—potential in regenerative medicine. Stem Cell Res 3:39–50

Kawasaki H et al (2000) Induction of midbrain dopaminergic neurons from ES cells by stromal cell–derived inducing activity. Neuron 28:31–40

Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, Steward O (2005) Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694–4705

Keller G (2005) Embryonic stem cell differentiation: emergence of a new era in biology and medicine. Genes Dev 19:1129–1155

Kim SU, De Vellis J (2009) Stem cell-based cell therapy in neurological diseases: a review. J Neurosci Res 87:2183–2200

Kimura H et al (2005) Transplantation of embryonic stem cell-derived neural stem cells for spinal cord injury in adult mice. Neurol Res 27:812–819

Kolossov E et al (2006) Engraftment of engineered ES cell–derived cardiomyocytes but not BM cells restores contractile function to the infarcted myocardium. J Exp Med 203:2315–2327

Konig N et al (2017) Murine neural crest stem cells and embryonic stem cell-derived neuron precursors survive and differentiate after transplantation in a model of dorsal root avulsion. J Tissue Eng Regen Med 11:129–137

Laflamme MA et al (2005) Formation of human myocardium in the rat heart from human embryonic stem cells. Am J Pathol 167:663–671

Laflamme MA et al (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol 25:1015–1024

Lahmy R, Soleimani M, Sanati MH, Behmanesh M, Kouhkan F, Mobarra N (2016) Pancreatic islet differentiation of human embryonic stem cells by microRNA overexpression. J Tissue Eng Regen Med 10:527–534

Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R (2002) Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci 99:4391–4396

McDonald JW et al (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 5:1410–1412

McKee C, Hong Y, Yao D, Chaudhry GR (2017) Compression induced chondrogenic differentiation of embryonic stem cells in three-dimensional polydimethylsiloxane scaffolds. Tissue Eng A 23:426–435

Ménard C et al (2005) Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. Lancet 366:1005–1012

Menasché P et al (2015) Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. Eur Heart J 36:2011–2017

Menasché P et al (2018) Transplantation of human embryonic stem cell–derived cardiovascular progenitors for severe ischemic left ventricular dysfunction. J Am Coll Cardiol 71:429–438

Min J-Y et al (2003) Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. J Thorac Cardiovasc Surg 125:361–369

Mohseni R, Hamidieh AA, Verdi J, Shoae-Hassani A (2014) Safe transplantation of pluripotent stem cell by preventing teratoma formation. Stem Cell Res Ther 4:212

Mummery C et al (2003) Differentiation of human embryonic stem cells to cardiomyocytes. Circulation 107:2733–2740

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

Nakagawa T, Lee SY, Reddi AH (2009) Induction of chondrogenesis from human embryonic stem cells without embryoid body formation by bone morphogenetic protein 7 and transforming growth factor β1. Arthritis Rheum 60:3686–3692

Nakano T, Kodama H, Honjo T (1994) Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science 265:1098–1101

Nishikawa S-I, Nishikawa S, Hirashima M, Matsuyoshi N, Kodama H (1998) Progressive lineage analysis by cell sorting and culture identifies FLK1+ VE-cadherin+ cells at a diverging point of endothelial and hemopoietic lineages. Development 125:1747–1757

Nishikawa S-I, Jakt LM, Era T (2007) Embryonic stem-cell culture as a tool for developmental cell biology. Nat Rev Mol Cell Biol 8:502–507

Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS (2005) Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 49:385–396

Park CH et al (2005) In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. J Neurochem 92:1265–1276

Pearl JI et al (2011) Short-term immunosuppression promotes engraftment of embryonic and induced pluripotent stem cells. Cell Stem Cell 8:309–317

Pera MF, Trounson AO (2004) Human embryonic stem cells: prospects for development. Development 131:5515–5525

Pera MF, Reubinoff B, Trounson A (2000) Human embryonic stem cells. J Cell Sci 113:5–10

Pera MF et al (2004) Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J Cell Sci 117:1269–1280

Rambhatla L, Chiu C-P, Kundu P, Peng Y, Carpenter MK (2003) Generation of hepatocyte-like cells from human embryonic stem cells. Cell Transplant 12:1–11

Ritner C, Bernstein HS (2010) Fate mapping of human embryonic stem cells by teratoma formation. J Vis Exp 42:2036

Rossant J (2015) Mouse and human blastocyst-derived stem cells: vive les differences. Development 142:9–12

Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med 10:55–63

Saxena P, Bojar D, Zulewski H, Fussenegger M (2017) Generation of glucose-sensitive insulin-secreting beta-like cells from human embryonic stem cells by incorporating a synthetic lineage-control network. J Biotechnol 259:39–45

Shim J, Kim S, Woo D, Kim S, Oh C, McKay R, Kim J (2007) Directed differentiation of human embryonic stem cells towards a pancreatic cell fate. Diabetologia 50:1228–1238

Shroff G (2016) Human embryonic stem cell therapy in chronic spinal cord injury: a retrospective study. Clin Transl Sci 9:168–175

Singla DK (2009) Embryonic stem cells in cardiac repair and regeneration. Antioxid Redox Signal 11:1857–1863

Smith AG (2001) Embryo-derived stem cells: of mice and men. Annu Rev Cell Dev Biol 17:435–462

Snir M, Kehat I, Gepstein A, Coleman R, Itskovitz-Eldor J, Livne E, Gepstein L (2003) Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. Am J Physiol Heart Circ Physiol 285:H2355–H2363

Tabar V, Studer L (2014) Pluripotent stem cells in regenerative medicine: challenges and recent progress. Nat Rev Genet 15:82–92

Takagi M, Umetsu Y, Fujiwara M, Wakitani S (2007) High inoculation cell density could accelerate the differentiation of human bone marrow mesenchymal stem cells to chondrocyte cells. J Biosci Bioeng 103:98–100

Tang C et al (2011) An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. Nat Biotechnol 29:829–834

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

Toh WS, Lee EH, Guo X-M, Chan JK, Yeow CH, Choo AB, Cao T (2010) Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. Biomaterials 31:6968–6980

Toh WS, Lee EH, Cao T (2011) Potential of human embryonic stem cells in cartilage tissue engineering and regenerative medicine. Stem Cell Rev 7:544–559

van Laake LW et al (2007) Human embryonic stem cell-derived cardiomyocytes survive and mature in the mouse heart and transiently improve function after myocardial infarction. Stem Cell Res 1:9–24

Vats A et al (2006) Chondrogenic differentiation of human embryonic stem cells: the effect of the microenvironment. Tissue Eng 12:1687–1697

Vegas AJ et al (2016) Long-term glycemic control using polymer-encapsulated human stem cell–derived beta cells in immune-competent mice. Nat Med 22:306

Vodyanik MA, Thomson JA, Slukvin II (2006) Leukosialin (CD43) defines hematopoietic progenitors in human embryonic stem cell differentiation cultures. Blood 108:2095–2105

Wang L, Menendez P, Cerdan C, Bhatia M (2005) Hematopoietic development from human embryonic stem cell lines. Exp Hematol 33:987–996

Wei S et al (2016) Conversion of embryonic stem cells into extraembryonic lineages by CRISPR-mediated activators. Sci Rep 6:19648

Xu C, Police S, Rao N, Carpenter MK (2002a) Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ Res 91:501–508

Xu R-H et al (2002b) BMP4 initiates human embryonic stem cell differentiation to trophoblast. Nat Biotechnol 20:1261–1264

Yabut O, Bernstein HS (2011) Human embryonic stem cells in regenerative medicine. In: Tissue engineering in regenerative medicine. Springer, New York, pp 17–38

Yamashita A, Krawetz R, Rancourt D (2009) Loss of discordant cells during micro-mass differentiation of embryonic stem cells into the chondrocyte lineage. Cell Death Differ 16:278–286

Yang D, Chen S, Gao C, Liu X, Zhou Y, Liu P, Cai J (2016) Chemically defined serum-free conditions for cartilage regeneration from human embryonic stem cells. Life Sci 164:9–14

Yeo RWY, Lim SK (2011) Embryonic stem cells for therapies–challenges and possibilities. In: Embryonic stem cells-basic biology to bioengineering. InTech, Rijeka

Yu F et al (2018) FoxO1 inhibition promotes differentiation of human embryonic stem cells into insulin producing cells. Exp Cell Res 362:227–234

Zhu W-Z, Hauch KD, Xu C, Laflamme MA (2009) Human embryonic stem cells and cardiac repair. Transplant Rev 23:53–68

Zhu Q et al (2017) Directed differentiation of human embryonic stem cells to neural crest stem cells, functional peripheral neurons, and corneal Keratocytes. Biotechnol J 12. https://doi.org/10.1002/biot.201700067

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Adult Stem Cells and Medicine

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Abstract

Stem cells can be either totipotent, pluripotent, multipotent or unipotent. Totipotent cells have the capability to produce all cell types of the developing organism, including both embryonic and extraembryonic tissues. The Hematopoietic Stem Cells (HSC) are the first defined adult stem cells (ASC) that give rise to all blood cells and immune system. Use of HSCs for treatment of hematologic malignancies, which is also called bone marrow (BM) transplantation or peripheral blood stem cells (PBSC) transplantation is the pioneer of cellular therapy and translational research. However, stem cell research field is developing so fast that, innovative approaches using HSCs for treatment of refractory diseases are growing rapidly. Hematopoietic stem cell transplantation (HSCT) has been widely used to achieve cure in different hematological diseases. Applications include the treatment of marrow failure syndromes, leukemia, lymphoma, multiple myeloma (MM), certain inherited blood disorders, autoimmune diseases and as an enzyme replacement in metabolic disorders. Innovative approaches such as haploidentical stem cell transplantation, new monoclonal antibodies and immunotherapies as well as Chimeric Antigen Receptor T-cell (CAR-T cell) therapies are on the way as promising treatment options especially for patients with refractory hematologic malignancies and even in solid tumors. However, there are still some challenges remaining before some of these therapies are translated into clinical application. In this paper, HSCs including its properties, niches, clinical usage and its contribution to modern medicine today and in the future will be discussed.

Keywords

Adult stem cells · Hematologic malignancies · Hematopoietic stem cells · Stem cell plasticity · Stem cell transplantation

Abbreviations

ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloblastic Leukemia
ASC	Adult Stem Cell
CAR T-cell	Chimeric Antigen Receptor T-cell
CY	Cyclophosphamide
DFS	Disease Free Survival
ESFT	Ewing Sarcoma Family tumors
FL	Follicular Lymphoma
GvHD	Graft versus Host Disease
HD	Hodgkin's Disease

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HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic Stem Cell Transplantation
IPS	Induced Pluripotent Cell
MCL	Mantle Cell Lymphoma
MDS	Myelodysplastic Syndrome
MM	Multiple Myeloma
NHL	Non-Hodgkin Lymphoma
NRM	Non-relapse Mortality
PBSC	Peripheral Blood Stem Cell
PNET	Primitive Neuroectodermal Tumors
RIC	Reduced Intensity Conditioning
SLE	Systemic Lupus Erythematosus
TRM	Transplant Related Mortality
UD	Unrelated Donor

1 Introduction

Stem Cells are the cardinal cells found in every organ and tissues in the body. These highly specialized cells are responsible for replacing injured tissues and cells that are lost everyday such as those in the blood, hair, skin and gut. Stem Cells have two main characteristics: I) the ability to self renew, making copies of themselves, and II) the ability to differentiate, giving rise into the mature types of cells that reside in different organs and tissues (Spangrude 2003). Every tissue has its own stem cells which are sometimes referred to as "adult" or "somatic" stem cells. The Hematopoietic Stem Cells (HSC) are the first defined adult stem cells (ASC) that give rise to all blood cells and immune system. Use of HSCs for treatment of hematologic malignancies, which is also called bone marrow (BM) transplantation or peripheral blood stem cells (PBSC) transplantation is the pioneer of cellular therapy and translational research (Ray et al. 2015). However, stem cell research field is developing so fast that, innovative approaches using HSCs for treatment of refractory diseases are growing rapidly. In fact, HSCs are lacking of critical features of stem cells and are more accurately defined as a subset of progenitor cells derived from embryonic mesoderm (Gale and Lazarus 2013). In this paper, we are going to discuss HSCs including its properties, niches, clinical usage and its contribution to modern medicine today and in the future.

2 Adult Stem Cells: Definition and Sources

Stem cells can be either totipotent, pluripotent, multipotent or unipotent. Totipotent cells have the capability to produce all cell types of the developing organism, including both embryonic and extraembryonic tissues (Spangrude 2003; Wright et al. 2001; https://stemcells.nih.gov). Pluripotent cells can only make all cells of the embryo including germ cells and cells from any of the germ layers. Multipotent cells can only make cells within a given germ layer. That is to say, multipotent stem cells from a mesodermal tissue can not make cells of a different germ layer such as ectoderm or endoderm. Unipotent cells make cells of a single cell type, such as germ cell stem cells which can become only an egg or sperm when they mature (Wright et al. 2001). It is well known that, the potency of cells is dependent on the time of embryonic development of the organism. The cells arising from the first few divisions following fertilization of the egg are generally the only cells that have totipotency (Spangrude 2003; Wright et al. 2001). On the other hand, the cells deriving from either the inner cell mass of the blastocyst or nascent germ layer cells in the embryo, approximately 7–10 days after fertilization, have pluripotency. Cell cultures or cell lines established from these structures are called embryonic stem cell or embryonic germ cells. The pluripotent cells shown to be arising from other cells called induced pluripotent stem cells (IPS) (Spangrude 2003; Wright et al. 2001).

The cells, the subject of the interest, are restricted to become either multipotent or unipotent once the primitive streak forms in the embryonic development (day 10–14 of post fertilization in the human). These cells are called

ASC or may be called "somatic stem cells"as well, if they are derived from tissue other than the germ cells (Robey 2000). ASCs are thought to be present in most but not all tissues and to persist throughout life. There is clear evidence for a resident stem cell population for some tissues such as blood, skin, intestine, muscle, brain, lung and kidney. ASCs are essential cells to provide the basis for tissue maintenance and response to injury. These cells are capable of self-renewal, but do not replicate indefinitely in culture. ASCs can differentiate to produce progenitor, precursor and mature cells but this is limited to the cells contained in the tissue of origin (Daley et al. 2009). ASCs comprise a small minority of the total tissue mass and they are quite difficult to identify and isolate (Ray et al. 2015, https://stemcells.nih.gov). ASCs have ability to differentiate from one tissue to a specialized cell type of another tissue. This is called transdifferentiation or stem cell plasticity. BM stem cells are the most popular cells in this concept, and they have been shown to differentiate into muscle, epithelium and liver (Ray et al. 2015, https://stemcells.nih.gov).

The features of ASCs can be sum up as follows: in vivo and invitro self renewal, asymmetric and symmetric cell division, potency, quiescency and niche dependency (Ferraro et al. 2010). Up until now, the most well-characterized example of ASCs is that of the hematopoietic system. Biological features of true HSCs should include: I) multipotency and asymmetrical cell divisions that can give rise to different cell types; II) persistence in a quiescent state and a slow rate of self renewal; III) ability to remain in an undifferentiated state in stem cell niches; IV) ability to restore BM function in lethally irradiated animals including rodents and primates and V) ability to differentiate into diverse hematopoietic lineages including red blood cells, myeloid and lymphoid cells and megakaryocytes (Spangrude 2003; Ray et al. 2015; Gale and Lazarus 2013; Wright et al. 2001; https://stemcells.nih.gov).

3 Stem Cell Plasticity

HSCs can constitute different types of tissues which can be referred as plasticity (Lakshmipathy and Verfaillie 2005). Fibroblast/myofibroblasts are found to be important for structural integrity of many tissues. Different types of fibroblast/myofibroblasts like glomerular mesangial cells of the kidney, brain microglial cells, perivascular cells, the adult heart valves, inner ear fibrocytes have been reported to be derived from HSCs in several studies (Ogawa et al. 2013; Ogawa et al. 2006;Hess et al. 2004; Visconti et al. 2006; Lang et al. 2006). It has been previously believed that adipocytes are derived from mesanchymal cells. Sera et al. showed the generation of adipocytes from uncommitted HSCs in mice studies (Sera et al. 2009). Also, recent data showed that support and presence of bone marrow adipocytes are important factors on HSC survival (Mattiucci et al. 2018). Mesanchymal stem cells possess potential to differentiate into osteocyte, osteoblast and chondrocyte (Heino and Hentunen 2008). In fact, a single HSC transplantation model in uninjured bone and tibia fracture model of mice demonstrated the ability of HSCs giving rise into osteo-chondrogenic cells (Mehrotra et al. 2013).

4 Hematopoietic Stem Cells

HSCs, are multipotent cells that are capable of differentiation and maintenance of the entire hematopoietic system. HSCs reside in special localizations called niches. The niche commonly refers to the pairing of hematopoietic and mesenchymal cell populations that regulate HSC self-renewal, differentiation and proliferation (Busch et al. 2015). Knowledge of the features and functions of HSC niches has markedly improved in recent years. The niche forms a regulatory unit that limits the entry of HSCs into the cell cycle, thereby protecting them from exhaustion or from errors in DNA replication, this is called "niche hypothesis". Both long term (LT) and short term (ST) progenitor populations of HSCs have been

defined. ST hematopoietic progenitor cells (ST-HPCs) can ensure the hematopoiesis for a short period of time like 6–8 weeks whereas LT-HPCs are responsible for the long term reconstitution of hematopoietic system (Sun et al. 2014; Abel Sánchez-Aguilera and Méndez-Ferrer 2017) LT-HPCs reside in the hypoxic niche of bone marrow in a quiescent state until stress factors occur like chronic infections (Suda et al. 2011). Differentiation and maintenance of HSCs have been found to be regulated by the transcriptional networks interfering with cytokines and cell-cell contacts. Recently, the nutritional metabolism was found to be related with the fate of HSCs (Hsu and Qu 2013). Also recent studies showed that lineage specific progenitors are not committed as oligopotent progenitors like previously believed but emerge directly from HSCs as unipotent progenitors (Velten et al. 2017).

5 Use of HSCs for Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) has been widely used to achieve cure in different hematological diseases (Gratwohl et al. 2004). Applications include the treatment of marrow failure syndromes, leukemia, lymphoma, multiple myeloma (MM), certain inherited blood disorders, autoimmune diseases and as an enzyme replacement in metabolic disorders. HSCT refers to the administration of hematopoietic progenitor cells from bone marrow, peripheral blood or umbilical cord and donor may be either autologous or allogeneic (Demirer et al. 1995a, 1996a, b, c, 1999; De Giorgi et al. 2005a, b; Bensinger et al. 1996). In autologous HSCT, cells are derived from the individuals inflicted with a particular disease, however in allogeneic HSCT, cells are collected from someone who has fully or partially HLA match with the patient. Worldwide, more than 40.000 HSCTs are performed each year. According to CIMBTR data PBSC grafts have been preferred more than BM or cord blood grafts. Lack of anesthesia and hospitalization for donors are the main advantages of stem cell mobilization from peripheral blood (Demirer et al. 1996b; Bensinger et al. 1996; Demirer et al. 2002; Bakanay and Demirer 2012; Demirer et al. 2001; Ataca et al. 2017a; Demirer et al. 1995b). Graft failure risk is more in patients who had bone marrow and cord blood transplantations than those who had PBSC (Tsai et al. 2016; Champlin et al. 1989). The faster neutrophil and platelet engraftment rates in PBSC transplantations can significantly decrease transplant related mortality rates (Gratwohl et al. 2004).

In recent years, the HSCT surveys include not only cell therapies with HSCs but also non-hematopoietic use of non-hematopoietic stem and progenitor cells (Passweg et al. 2016). The analysis of the EBMT survey data spanning over 20 years has shown a continued and constant increase in the annual numbers of HSCT and transplant rates (numbers of HSCT per 10 million inhabitants) for both allogeneic and autologous HSCT. Main indications are lymphoma, myeloma and leukemia. Of 11,853 patients (33% of total) including primarily acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN), 96% were allo-HSCT. Of 20,802 patients (57% of total) with lymphoid neoplasia including non-Hodgkin lymphoma (NHL), Hodgkin's disease (HD) and plasma cell disorders, only 11% were allogeneic and remaining were autologous HSCT. Of 1458 patients (4% of total) with solid tumors, 3% were allogeneic and remaining were autologous HSCT. Of 2203 patients (6% of total) with non-malignant disorders, 88% were allogeneic HSCT. As seen in previous years, the majority of HSCT for lymphoid malignancies were autologous while most transplants for leukemia were performed using stem cells from allogeneic donors (Brunvand et al. 1996; Demirer et al. 1996a, b). Autologous HSCT for non-malignant disorders predominantly include patients with autoimmune disorders (Fig. 1a and b). For the first time since 1990 45% of patients receiving

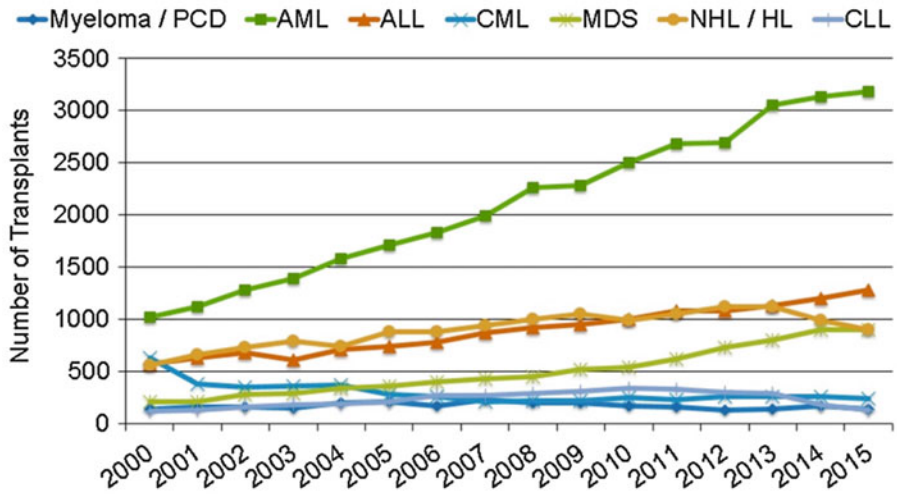

Fig. 1 Allogeneic HSCT trends in various diseases (Adapted from CIBMTR 2016 slides)

autologous HSCT had plasma cell disorders (Fig. 1b) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages). Transplants performed with alternative donors are increasing from 2003 to 2011, there was a steady increase in the number of transplants using umbilical cord blood as a result of several published studies demonstrating its benefit in both children and adults. From 2012 onward, there was an increase in the number of transplants from other relatives, which is likely due to the use of haploidentical donors with post-transplant cyclophosphamide (PTCY) strategy. In 2015, haploidentical donor transplant is the only group of donor type that is increasing associated with a decline or stability in other donorships all over the world (https://www.cibmtr.org/Reference Center/Slides Reports/Summary Slides/Pages; Passweg et al. 2017). At the beginning, older age was one of the contraindications for transplantation, however, the use of allo-HSCT with reduced intensity conditioning (RIC) the number of allogeneic transplants for treatment of malignant diseases in > 60 years continue to increase, furthermore, nonmyeloablative or RIC regimens allow allogeneic HSCT in patients aged up to 75 years (https://www.cibmtr.org/Reference Center/Slides Reports/Summary Slides/Pages; Passweg et al. 2017).

6 Stem Cell vs Marrow as a Source for HSCT

The higher content of T cells, natural killer cells and monocytes in PBSC grafts is associated with increased graft versus host disease (GvHD) rates. Although the preliminary data showed non significant GvHD rates in HLA identical HSCT, the later data was associated with higher GvHD rates in leukemia patients (Bensinger et al. 2001; Schmitz et al. 2002). EBMT Acute Leukemia Working Party analyzed the incidence of GvHD and transplant outcomes in AML patients with complete remission undergoing transplantation with myeloablative conditioning regimens from unrelated donors (UD) (Ringdén et al. 2012). In a comparison of peripheral blood with BM as stem cell source, acute GvHD rates were similar but chronic GvHD rates were significantly higher in PBSC group. No significant difference in non-relapse mortality (NRM), relapse rate and disease-free survival (DFS) was observed in the analysis of all patients in this study. But interestingly NRM was lower in advanced stage patients and DFS was significantly prolonged in PBSC group. In another study of the same group, higher incidence of acute GvHD and NRM but a lower

incidence of relapse and nonsignificant DFS difference were reported in a comparison of unrelated PBSC to BM grafts after allo-HSCT with RIC (Nagler et al. 2012).

In comparison of PBSC and BM as stem cell sources in T cell repleted haploidentical stem cell transplantations, no significant engraftment and survival differences were observed between the groups. But acute grade II-IV and chronic GvHD rates were lower with BM transplantation as expected (Bashey et al. 2017). Cord blood can be another stem cell source for either malign and non-malignant diseases. Less stringent HLA match compatibility in cord blood transplantations increases the donor availability. Infused stem cell dose should be increased in correlation with increasing mismatch number. Lower CD34+ cell dose/kg for adult patients can result in delayed immune reconstitution. To overcome this problem double unit cord blood has been introduced especially for adult patients (Ballen and Lazarus 2016).

7 Use of HSCs in the Treatment of Leukemia

Most malignant transformations in HSCs usually occur at the pluripotent stem cell level and as a result, abnormal proliferation, clonal expansion, and diminished apoptosis cause replacement of normal blood and marrow cells with blasts. Recent advances in the discovery of the genomic landscape of disease, in the development of assays for genetic testing and for detecting minimal residual disease prompted to update current treatment guidelines in acute leukemia. Based on this, European Leukemia Network reclassified the AML prognostic groups according to genetic risk stratification, that has a great impact on the choice of therapy including conventional chemotherapies, novel agents such as targeted drugs and cellular therapies, HSCT and even trial designs for drug development (Döhner et al. 2017).

7.1 HSCT Indications in Leukemia

The decision to perform allogeneic HSCT depends on the assessment of the risk-benefit ratio (i.e., NRM/morbidity vs reduction of relapse risk) based on cytogenetic and molecular genetic features as well as patient, donor and transplant factors. Indications of HSCT for leukemias have been revised by EBMT, BSBMT and ASBMT. According to ASBMT task force, HSCT indications are as follows: I) Standard of care, where indication for HSCT is well defined and supported by evidence; II) Standard of care, clinical evidence available, where large clinical trials and observational studies are not available but HSCT has been shown to be effective therapy; III) Standard of care, rare indication, for rare diseases where HSCT has demonstrated effectiveness but large clinical trials and observational studies are not feasible; IV) Developmental, for diseases where preclinical and/or early phase clinical studies show HSCT to be a promising treatment option; and V) Not generally recommended. The indications for acute leukemia, MDS and chronic leukemias are shown in the Table 1 (Majhail et al. 2015; Demirer 2017; Yuksel and Demirer 2017).

The most common allogeneic indications for HSCT in the US and Europe are acute leukemia (AML, ALL), MDS and MPNs. They are accounting for 70% of allogeneic HSCT (https://www.cibmtr.org/ReferenceCenter/Slides Reports/Summary Slides/Pages; Passweg et al. 2017; Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017; Atilla et al. 2017a). On the other side, the allogeneic transplant activity is decreasing in various indications particularly in chronic leukemias (CLL-Chronic Lymphocytic Leukemia, CML-Chronic Myeloid Leukemia) and lymphomas because of the availability of non-transplant treatment options (Fig. 1) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

Table 1 Hematopoietic stem cell transplantation indications

Indication and Disease Status	Allogeneic HCT	Autologous HCT
Acute myeloid leukemia		
CR1, low risk	N	N
CR1, intermediate	S	C
CR1, high risk	S	C
CR2	S	C
CR3+	C	C
Not in remission	C	N
Acute promyelocytic leukemia		
CR1		
CR2, molecular remission	N	N
CR2 not in molecular remission	C	S
CR3+	S	N
Not in remission	C	N
Relapse after autologous transplantation	C	N
Acute lymphoblastic leukemia		
CR1 standard risk	S	C
CR1high risk	S	N
CR2	S	C
CR3+	C	N
Not in remission	C	N
Chronic myeloid leukemia		
Chronic phase 1TKI intolerant	C	N
Chronic phase 1TKI refractory	C	N
Chronic phase 2+	S	N
Accelerated phase	S	N
Blast phase	S	N
Myelodysplastic syndromes		
Low/intermediate 1 risk	C	N
Intermediate 2/high risk	S	N
Therapy related AML/MDS		
CR1	S	N
Chronic lymphocytic leukemia		
High risk, first or greater remission	C	N
T cell prolymphocytic leukemia	R	R
B cell prolymphocytic leukemia	R	R
Transformation to high grade lymphoma	C	C

N, indicates not generally recommended; C, standard of care, clinical evi dence available; S, standard of care; R, standard of care, rare indication; CR1, first complete response; CR2, second CR; CR3, third CR; TKI indicates tyrosine kinase inhibitor; AML/MDS, acute myelogenous leukemia/myelodysplastic syndrome

7.2 Outcome of HSCTs in Leukemia and MDS

After an autologous HSCT, primary disease is the most commonly reported cause of death representing 69% of deaths. Among HLA-matched sibling transplant recipients, within the first 100 days, primary disease accounts for 29% of deaths while infection and graft versus host disease represents 25% of deaths. After 100-day, 57% of deaths are attributed to primary disease. On the other hand, after UD allogeneic HSCT within 100 days, mortality related to GvHD, infection, organ failure and graft rejection accounts for 33% of deaths. After 100 days, 46% of deaths are related to primary disease (Atilla et al. 2017b; Sahin et al. 2016a, b; Ataca et al. 2016).

The CIBMTR has data for 32,272 patients receiving an HLA-matched sibling (n = 13,118) or UD (n = 19,154) transplant for AML between 2004 and 2014. According to this data, disease status at the time of transplant and donor type are the major predictors of post transplant survival. The 3-year probabilities of overall-survival (OS) after HLA-matched sibling transplant in this cohort were 59% ± 1%, 51% ± 1%, and 27% ± 1% for patients with early, intermediate, and advanced disease, respectively. The probabilities of OS after an UD transplant were 51% ± 1%, 48% ± 1%, and 25% ± 1% for patients with early, intermediate, and advanced disease, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages) (Fig. 2a and b).

Older age at disease onset is a high-risk feature in ALL. Consequently, a larger proportion of ALL patients who are 18 years of age or older undergo allogeneic HSCT for early disease. Among 4249 patients ≥ 18 years of age receiving HLA-matched sibling HSCT for ALL between 2004–2014, the 3-year OS probabilities were 59% ± 1%, 38% ± 2%, and 27% ± 2% for patients with early, intermediate, and advanced disease, respectively. The corresponding probabilities among the 5315 recipients of UD HSCT for ALL were 57% ± 1%, 37% ± 1%, and 24% ± 2% for early, intermediate, and advanced disease, respectively (Fig. 3a and b) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SumarySlides/Pages).

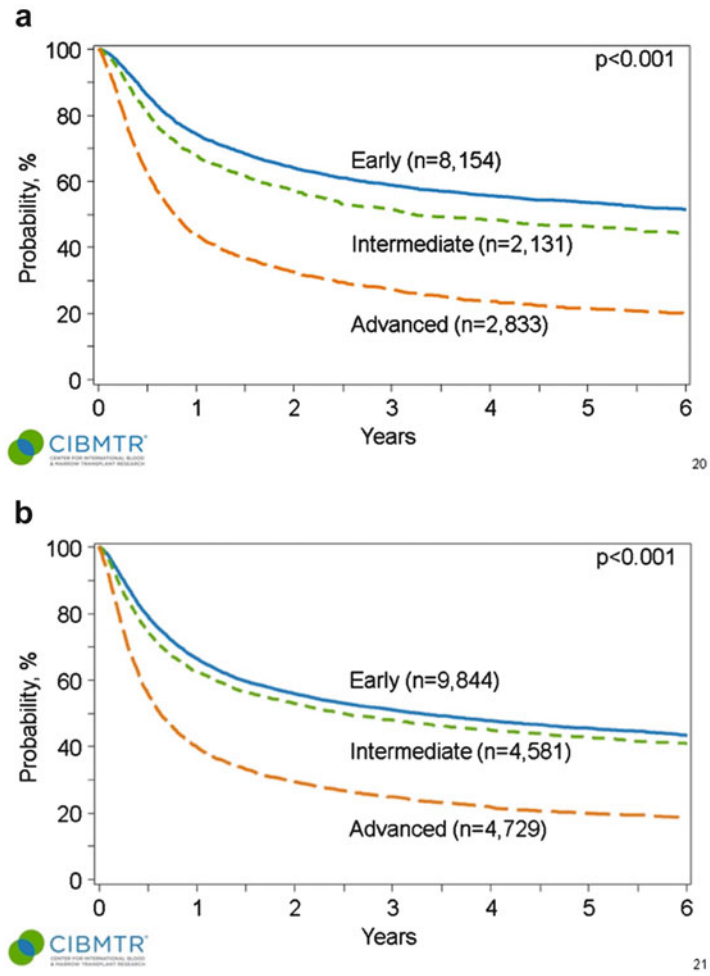

Fig. 2 (**a**) Survival after HLA matched sibling donor HSCT for AML between years 2004–2014. (**b**) Survival after unrelated donor HSCT for AML between years 2004–2014. Early phase (first complete remission [CR1]), intermediate phase (second or subsequent CR), advanced phase (primary induction failure, active disease)

Allogeneic transplant is a potentially curative treatment approach for MDS. Outcomes differ according to disease status at the time of transplant. The data on 7048 patients receiving an allotransplant for early (n = 2588) and advanced (n = 4460) MDS performed between 2004 and 2014, showed that a 3-year probabilities of OS were 53% ± 2% and 49% ± 1% for recipients of sibling and UD transplants for early MDS, respectively. Among patients with advanced MDS, corresponding probabilities were 45% ± 1% and 40% ± 1%, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

Allogeneic transplants for CML are currently reserved for patients where tyrosine kinase inhibitors have failed or are poorly tolerated. The CIBMTR has data for 2026 HLA-matched sibling donor transplants for CML from 2004 to 2014. Based on this data, 3-year probabilities of OS were 68% ± 1%, 51% ± 3% and 26% ± 4% for patients in chronic phase, in accelerated phase and blast phase, respectively. Allogeneic transplants are the main transplant modality for curative treatment of CLL patients when standard chemotherapy fails or high-risk features are present (e.g. cytogenetic abnormalities, short remission intervals, purine analog resistant disease). Among 2975 patients who underwent transplantation for CLL between 2004 and 2014, the 3-year probabilities of OS were 58% ± 2% and 51% ± 1% for HLA-matched sibling and UD transplants, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

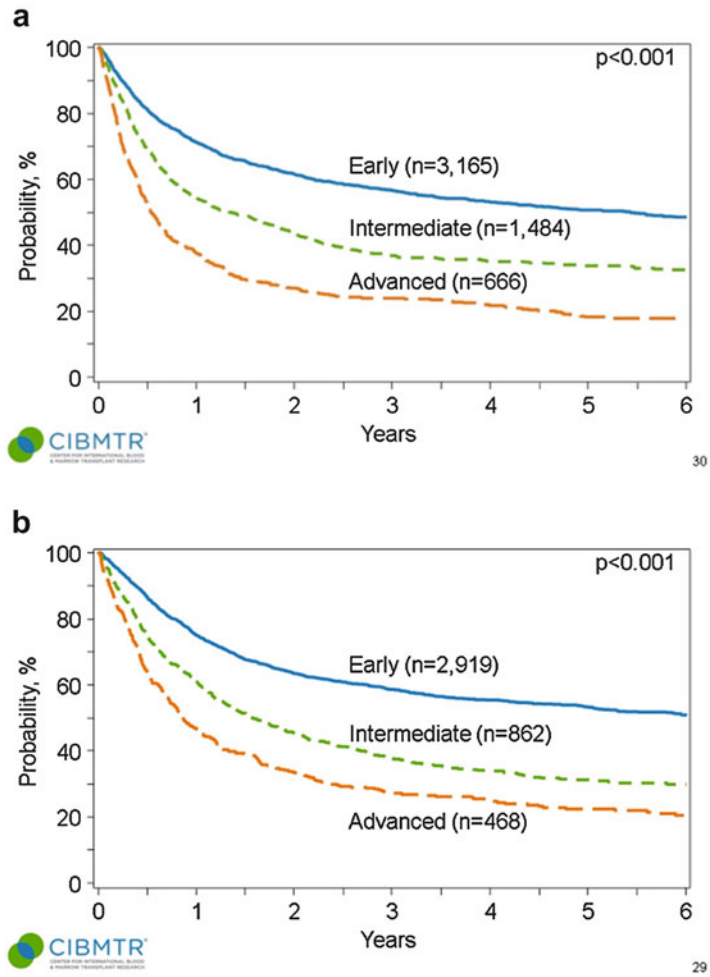

Fig. 3 (**a**) Adult ALL match sibling donor HSCT 2004–2014). (**b**) Adult ALL match sibling donor HSCT 2004–2014 Early phase (first complete remission [CR1]), intermediate phase (second or subsequent CR), advanced phase (primary induction failure, active disease)

8 Use of HSCs in the Treatment of Lymphoma

Autologous HSCT has been extensively used for treatment of various solid tumors, lymphomas and multiple myelomas since the beginning of 1990s (Brunvand et al. 1996; Holmberg et al. 1998, Berry et al. 2011). In considering that autologous HSCT related mortality rates due to regimen related toxicities and transplant related complications decreased to 1–2% in highly sophisticated transplant centers (Demirer et al. 1996a; Giorgi et al. 2004), this treatment modality now remains as an important tool for treatment of lymphomas in the settings of first line and chemosensitive relapses without marrow involvement as well as sometimes in second complete remission in appropriate patients (Robinson et al. 2015; Dreyling et al. 2005; Bernstein et al. 2010; Fenske et al. 2014).

Although HD is a curable disease in most of the patients, relapse has been observed in 10–20% of early stage and 30–40% of advanced stage disease (Gordon et al. 2003; Radford et al. 2015). Salvage chemotherapy can induce remission and help to proceed into autologous HSCT. Survival benefit of autologous HSCT in compared to conventional chemotherapy was shown in several randomized trials (Aparicio et al. 1999; Sasse et al. 2016; Baetz et al. 2003). Based on the accumulated literature data, autologous HSCT was accepted as standard approach in chemosensitive relapsed HD. According to the CIBMTR data, 3-year OS rate in HD patients with chemosensitive relapse who had autologous HSCT is reported as 82% (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages). Allogeneic HSCT with match sibling donor or UD using RIC regimens in patients with refractory HD resulted in a 5-year OS rates of 56% and 52%, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

In relapsed/refractory aggressive NHL, patients remission can be achieved in one-half after salvage chemotherapy (Cabanillas and Shah 2017). Proceeding with autologous HSCT, especially in patients without marrow involvement, can help to achieve long term remission rates (Sureda et al. 2015; Philip et al. 1995). Unfortunately, long term survival outcomes are not seen in chemorefractory patients who had autologous HSCT (Crump et al. 2017). Autologous HSCT is an accepted treatment indication for diffuse large B-cell lymphoma (DLBCL) which is often used at first chemosensitive relapse. Among the 12,161 patients who received an autologous transplant for DLBCL between 2004 and 2014, the 3-year probabilities of OS were 65% ± 1% and 45% ± 2% for patients with chemosensitive and chemoresistant disease, respectively. Allogeneic HSCT for treatment of DLBCL is performed less frequently and is generally used only in patients with aggressive disease that has been resistant to previous therapies, including autologous transplants (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages; Demirer et al. 1995c). Among 1055 patients who underwent an HLA-matched sibling HSCT for DLBCL from 2004 to 2014, the 3-year probabilities of OS were 51% ± 2% and 27% ± 3% for patients with chemosensitive and chemoresistant disease, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages) Therefore, allo HSCT with RIC regimens from match sibling donors is a frequently used approach in this group of refractory patients with NHL (Sureda et al. 2015; van Kampen et al. 2011).

In follicular lymphoma (FL) patients, randomized clinical trials did not show a survival benefit of autologous HSCT as consolidation treatment. But in relapsed/refractory patients, autologous HSCT has been widely preferred by transplanters due to low transplant related mortality. The randomized CUP trial in pre-rituximab setting, showed a statistically significant PFS advantages in autologous HSCT arm over chemotherapy arm (Schouten et al. 2000). The DFS and OS advantage remained to be significant in the later French study performed in the rituximab era (Sebban et al. 2008). Allogeneic transplants for FL are performed in patients who experience disease relapse after multiple lines of therapy or who have refractory disease and an available HLA match donor. Among 993 patients receiving

HLA-matched sibling donor transplants for FL between 2004 and 2014, the 3-year probabilities of OS were 72% ± 2% and 59% ± 4% for patients with chemosensitive and chemoresistant disease, respectively. The corresponding probabilities among 878 patients receiving UD transplants in the same period were 66% ± 2% and 43% ± 4% for chemosensitive and chemoresistant disease, respectively (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

Based on the current literature data, the use of autologous HSCT in patients with mantle cell lymphoma (MCL) with relapsed/refractory diseases is not as preferred as in the frontline. Because the outcomes of autologous HSCT in relapse setting were documented to be inferior when compared to its use in first-line consolidation (Artz et al. 2006; Lefrère et al. 2002; Freedman et al. 1998). If the chosen first line therapy is conventional, autologous HSCT may be a treatment option following first CR as a consolidation especially in high risk patients with high CRP, *B2* microglobulin, sedimentation, LDH levels and bulky disease during presentation. The use of autologous HSCT for patients with relapsed MCL has not demonstrated as promising results as with front line use. However, Fenske *et al.* suggested that even later in the disease course, autologous HSCT can offer a meaningful clinical benefit (Atilla et al. 2017c). In appropriate patients with relapsed MCL who have been successfully salvaged, autologous HSCT in second CR may be a consideration. Current data indicate that autologous HSCT may especially be a useful approach in MCL in the front line setting in patients in first complete or partial remission following induction chemotherapy (Freedman et al. 1998).

Although there are no prospective comparisons of myeloablative allo-HSCT with RIC allo-HSCT in MCL, RIC regimens have extended the utility of allo-HSCT to elderly patients and patients with co-morbidities. In high risk relapse/refractory MCL, allo-HSCT remains the only curative treatment option on the basis of the graft versus MCL effect. Current literature data suggests that Allo-HSCT with RIC regimen for chemo-sensitive relapse or refractory MCL seems to provide long term remission for some patients (Vanderberghe et al. 2003). The beneficial graft-versus-MCL effect has also been shown by the efficacy of donor lymphocyte infusions (Freedman et al. 1998; Tessoulin et al. 2016; Marks et al. 2002). As a result, the optimal timing and type of HSCT for MCL is not well defined. As with other mature B-cell lymphoproliferative disorders, autologous HSCT is the most common transplant approach. Among the 4746 patients who received an autotransplant for MCL between 2004 and 2014, the 3-year probability of OS was 79% ± 1%. Among 1591 patients who underwent an allogeneic transplantation for MCL during the same period, the 3-year probability of OS was 53% ± 1% (Fig. 4) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages).

The efficacy of autologous HSCT as a consolidation approach following induction was also shown in some studies in patients with T cell lymphoma (Iams and Reddy 2014; Corradini et al. 2006; D'Amore et al. 2012). Therefore, in peripheral T cell lymphoma subtypes except ALK positive anaplastic large cell lymphoma due to its better prognosis, autologous HSCT in first CR as a consolidation is generally recommended (Gkotzamanidou and Papadimitriou 2014).

9 Use of HSCs in the Treatment of Multiple Myeloma

Current literature data show that autologous HSCT improves survival in multiple myeloma (MM) patients (Barlogie et al. 2006; Fermand et al. 2005). Introduction of novel agents could not be able to decrease the role of autologous HSCT in the treatment of MM patients (Dhakal et al. 2018). Achievement of deeper response rates with induction treatments before transplantation can result in better outcomes (Lonial and Anderson 2014). If the patient is transplant eligible, induction therapy with triplets can be used up to six cycles.

It has been well documented that duration of post-transplant CR is highly associated with

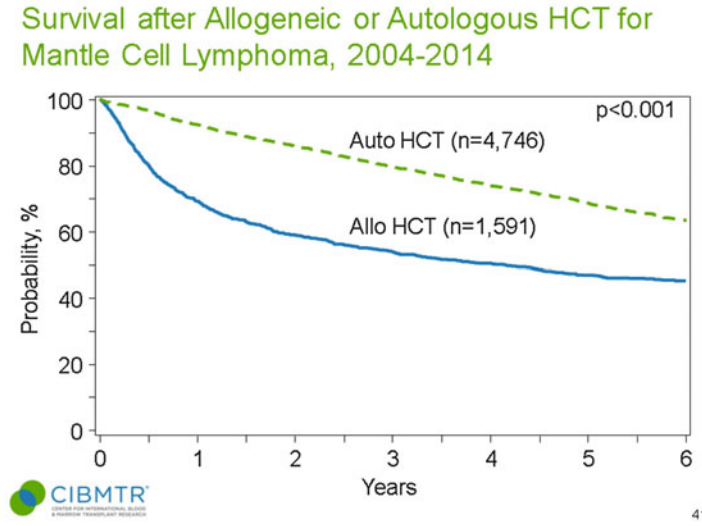

Fig. 4 Survival rates after auto and allo-HSCT in patients with MCL

prolonged PFS in MM. Based on the CIBMTR data, 3-year OS rates of MM patients receiving autologous HSCT were 70% ± 1%, 74% ± 0.4% and 78% ± 0.3% between years 2001 to 2004, 2005 to 2008 and 2009 to 2012, respectively. (Fig. 5) (https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages. In order to improve the depth of response, maintenance treatment after autologous HSCT is currently a standard of care. Lenalidomide maintenance in post-transplant setting has translated into longer PFS in 2 studies and longer OS in one of these studies (Attal et al. 2012; McCarthy et al. 2017).

There have been contradictory results of the impact of double autologous HSCT in MM patients. Double autologous HSCT has been shown to be superior to single autologous HSCT in high risk MM patients who are not achieving CR or very good PR with the first transplant (Cavo et al. 2016; Attal et al. 2003). The role of tandem autologous HSCT can be more important for patients with high risk genetic factors like del 17p and t(4,14) (Sonneveld et al. 2016).

Relapse still remains to be a challenging problem in the treatment of MM patients. Second autologous HSCT can still be an option in patients who benefited from first autologous HSCT with a long treatment free interval (at least 18–24 months) (Jimenez-Zepeda et al. 2012). One has to keep in mind that allo-HSCT with RIC regimens is still the only curative option but it is associated with a high NRM rates (Sureda et al. 2015). Allo-HSCT with RIC regimens after autologous HSCT resulted in better survival rates than double autologous HSCT in 2 studies with long term follow-up. The first study revealed a significantly longer EFS and OS in patients with HLA identical siblings compared with those without (Giaccone et al. 2011). In the second study, 96-month PFS was 49% and 22% and OS was 36% and 12% in patients who received autologous HSCT alone and auto-allo HSCT groups, respectively (Gahrton et al. 2013). But there are some studies, in which there are no significant benefit mostly due to the high NRM of allo-HSCT (Rosiñol et al. 2008).

EBMT Society recommends autologous HSCT as a standard option in all MM patients and allo-HSCT from sibling or well matched UDs as a clinical option in refractory patients (Sureda et al. 2015). Allo-HSCT from alternative donors is not recommended.

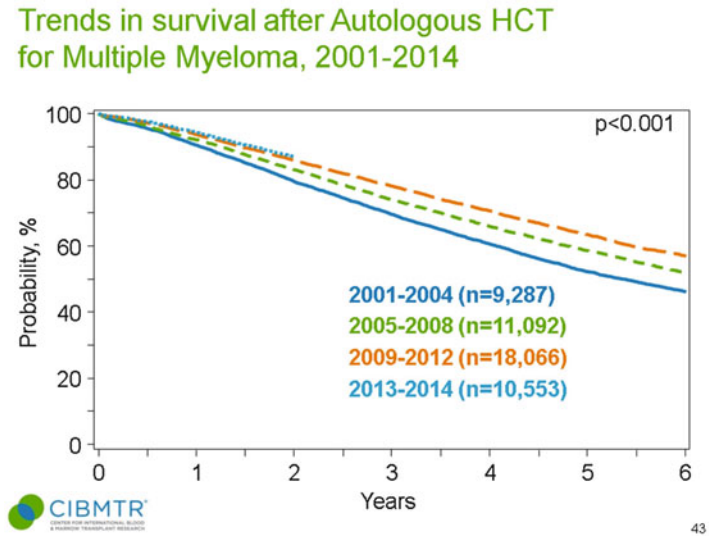

Fig. 5 verall survival rates in multiple myeloma and improvement of the outcome during last 2 decades

10 Use of HSCs in the Treatment of Solid Tumors

Autologous HSCT is a valuable strategy in young adults for indications similar to those of a pediatric population such as Ewing Sarcoma Family tumors (ESFT) and primitive neuroectodermal tumors (PNET), and those with germ cell tumors, in particular in the setting of salvage treatment, and is now not indicated for epithelial solid tumors, for which the introduction of new molecularly targeted therapies have changed the approach to therapy without,however, substantially altering the clinical course of the diseases such as triple negative breast cancer, ovarian cancer and small cell lung cancer (Demirer et al. 1996d, Gratwohl et al. 2004; Pedrazzoli et al. 2003; Pedrazzoli et al. 2006; De Giorgi et al. 2005a, b; Kroger et al. 2003; Marco 2012). In fact, allogeneic transplant has been utilised as an adoptive immunotherapy in the period of 2000–2005 for epithelial solid tumors such as renal, ovarian and colorectal cancers and the presence of graft versus solid tumor effect has been documented in the various EBMT Solid Tumors Working Party studies but this strategy has mainly remained as an experimental approach (Peccatori et al. 2005; Ueno et al. 2008; Aglietta et al. 2009; Bay et al. 2010; Secondino et al. 2007).

Autologous HSCT is reserved for patients with relapsing or refractory germ cell tumors according to recommendations of National Comprehensive Cancer Network (NCCN) guidelines (http://www.nccn.org/professionals/physician_gls/pdf/testicular.pdf). Currently, according to EBMT guidelines and recommendations, autologous HSCT in patients with relapsed chemosensitive germ cell tumors remains as a clinical option after a careful assessment of risk and benefits, whereas autologous HSCT remains as a standard approach for patients with third-line refractory disease (Karadurmuş et al. 2017).

Based on the current literature data, NCCN and EBMT guidelines, use of autologous HSCT for treatment of some subgroup of high risk patients with ESFT/PNET is remaining as a clinical option but it is an experimental treatment approach for patients with osteosarcoma. Current accumulated data show that Ewing's sarcoma patients may benefit from autologous HSCT much more than osteosarcoma patients (Marco 2012). We have to emphasize that prospective randomized clinical trials are very crucial to document the efficacy of autologous HSCT compared to conventional chemotherapy in ESFT/PNET patients, as well as to determine some

specific sub-group of high risk patients, if any, who may benefit from this treatment modality (Burdach et al. 1993; Barker et al. 2005; McTiernan et al. 2006).

11 Use of HSCs in the Treatment of Autoimmune Diseases

Autologous HSCT was introduced as a therapeutic option for autoimmune diseases in 1997 (Marmont et al. 1997). The latest data gives a promising outcome rates due to the better patient selection criteria (Alchi et al. 2013). Although treatment with CY and mycophenolate mofetil (MMF) somehow improved the outcomes in systemic lupus erythematosus (SLE) patients, high progression rates and morbidities still remain as a challenging problem. The largest registry data from EBMT revealed OS rates of over 50% for refractory SLE patients who had autologous HSCT (Alchi et al. 2013). In a recent study, Leng et al., reported an OS rate of 80% in 10 years follow up of 24 SLE patients who were treated with high dose immunosupressive treatment and autologous HSCT (Leng et al. 2017). Cao et al., showed a decrease in the levels of antibodies and normalization of complements in post-transplant 100 days (Cao et al. 2017).

Recent data revealed that long term remission rates could be achieved with autologous HSCT in diffuse cutaneous systemic sclerosis patients refractory to standard immunosupressive treatment (Sullivan et al. 2018). In a systemic review of 38 studies including 344 patients, patients who were treated with CY were compared with patients who had Autologous HSCT (Eyraud et al. 2017). Improvement in modified Rodnan skin score, pulmonary functions and also PFS were observed in the transplant group (Rhijn-Brouwer et al. 2017).

Despite these promising outcomes and low transplant related mortality (TRM) rates with autologous HSCT, secondary autoimmune diseases and high infection rates remain as problems to be solved.

12 Use of HSCs in the Treatment of Neurological Diseases

Multiple sclerosis (MS) is a relapsing neurological disease which can mostly be controlled with approved therapies. Autologous HSCT was found to have impact on disease activity in clinically definite MS patients. Autologous HSCT can reduce inflammation in MS and prevent new MRI lesions and relapses.

The only randomized trial, ASTIMS trial, showed a superior effect of autologous HSCT over best treatment option (Mancardi et al. 2015). In a review of all studies including MS patients who had autologous HSCT, 4-year PFS was 87% and 5-year OS was reported to be 70–91%. Although TRM has been decreased within recent years in relapsing–remitting patients and patients with higher baseline expanded disability status scale, TRM was found to be still high (Mancardi et al. 2018). In Stiff Person Syndrome, chronic inflammatory demyelinating polyneuropathy and myasthenia gravis, case reports and series were published (Sanders et al. 2014; Burman et al. 2018).

13 Innovative Approaches

13.1 Haploidentical Stem Cell Transplantation as an Emerging Treatment Modality

Historically, the extent of donor-recipient HLA mismatch showed an inverse relationship with HSCT outcomes. The deleterious effects of HLA-mismatch have been substantially eliminated after the advent of modern conditioning regimens and GvHD prophylaxis strategies. Recent studies using PTCY for GvHD prophylaxis have reported similar OS and DFS rates for haploidentical grafts when compared to HLA-matched sibling grafts (52–54). Therefore, haploidentical SCT, as a new treatment modality, which has almost a comparable outcome with match sibling transplants, brought hope and cure chance to many patients awaiting for donor. Use

of haplo transplants increasingly, will remarkably decrease the requirement for time consuming UD search procedures and cord blood transplants (Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017).

In 2014, the numbers of transplants using other relatives surpassed the total numbers of umbilical cord transplant performed in the US, accounting for 11% of all allogeneic transplants performed in the US. In a retrospective comparative study of haplo and UD transplant recipients, all of whom were treated with PTCY, calcineurine inhibitors and MMF, no significant difference in the incidence of acute or chronic GvHD was reported and there were no significant differences found in any outcome, with the exception of neutrophil engraftment which was faster in the UD transplantation (Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017).

While comparative studies show survival rates to be similar to unrelated donor transplants, these studies are nonrandomized and underpowered, and none to date have shown a superior survival advantage with haplo donors. But it is yet hard to interpret and compare the results of reported studies regarding used approaches and methods, since the current data about haploidentical HSCT mainly come from the results of non-randomised trials with retrospective comparison (Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017; Atilla Ataca et al. 2017b). Thus, current recommendations for haploidentical HSCT substantially depend on expert opinions. Seeing that an appropriately powered randomized trial to show a non-inferiority in DFS in haplo transplants would require approximately 3000 patients, this is a challenging task and unlikely to be feasible. Future studies should particularly focus onto head-to-head comparisons of other donor sources such as match sibling, match UD and umbilical cord with haploidentical donors, as well as conditioning regimens and strategies involving graft manipulation. For all these reasons, it is currently too early to know whether transplantation with a haplo donor will ultimately be as good or better than transplantation using a matched UD (Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017).

Further research with high quality features (i.e.; randomised, homogenous population and larger sample size) are needed before recommending haploidentical HSCT for a more extended list of indications (Demirer 2017; Yuksel and Demirer 2017; Sahin and Demirer 2017).

14 HSCs in the Future

Stem cells have remarkably altered the care of individuals with hematologic diseases during last three decades. The evolving role of stem cells in medicine is incrementally developing. In the next two decades or so, stem cells can be used as replacement therapy to regain the cell lines that have been destroyed or to modify the function of the cells, as targets of drug therapy and in vitro studying of disease models for drug development as well as in combination with gene therapy. Innovative approaches such as haploidentical stem cell transplantation, new monoclonal antibodies and immunotherapies as well as CAR-T cell therapies are on the way as promising treatment options especially for patients with ALL, CLL, refractory HD and NHL, multiple myeloma and even in solid tumors (Gauthier and Yakoub-Agha 2017; Xu et al. 2017; Yong et al. 2017). However, there are still some challenges remaining before these therapies are translated into clinical application. Last but not least, the political and ethical conflicts and considerations have to be overcome with plausible approaches and strategies.

References

Abel Sánchez-Aguilera A, Méndez-Ferrer S (2017) The hematopoietic stem-cell niche in health and leukemia. Cell Mol Life Sci 74(4):579–590

Aglietta M, Barkholt L, Carnevale Schianca F et al (2009) On behalf of the EBMT solid tumor working party. Reduced intensity allogeneic hematopoietic stem cell transplantation in metastatic colorectal cancer as a novel adoptive cell therapy approach. The European group for blood and marrow transplantation experience. Biol Blood Marrow Transplant 15(3):326–335

Alchi B, Jayne D, Labopin M, et al. EBMT Autoimmune Disease Working Party members (2013) Autologous haematopoietic stem cell transplantation for systemic lupus erythematosus: data from the European group for blood and marrow transplantation registry. Lupus 22 (3):245–253

Aparicio J, Segura A, Garcera S et al (1999) ESHAP is an active regimen for relapsing Hodgkin's disease. Ann Oncol 10:593–595

Artz AS, Pollyea DA, Kocherginsky M et al (2006) Performance status and co-morbidity predict transplant-related mortality after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 12:954–964

Ataca P, Atilla E, Toprak SK et al (2016) Non-infectious early complications of allogeneic stem cell transplantations. Int J Hematol/Oncol 26(4):239–250

Atilla Ataca P, Mine OS, Demirer T (2017a) How to manage poor mobilizers for high dose chemotherapy and autologous stem cell transplantation? Transfus Apher Sci 56(2):190–198

Atilla Ataca P, Atilla E, Bozdağ SC et al (2017b) A review of infectious complications after Haploidentical hematopoietic stem cell transplantations. Infection 45:403–411

Atilla E, Atilla Ataca P, Demirer T (2017a) A review of Myeloablative vs reduced intensity/non-Myeloablative regimens in allogeneic hematopoietic stem cell transplantations. Balkan Med J 34(1):1–9

Atilla E, Atilla Ataca P, Toprak SK et al (2017b) A review of late complications of allogeneic hematopoietic stem cell transplantations. Clin Transpl 31(10)

Atilla E, Atilla Ataca P, Demirer T (2017c) Current treatment strategies in relapsed/refractory mantle cell lymphoma: where are we now? Int J Hematol 105:257–264

Attal M, Harousseau JL, Facon T et al. InterGroupe Francophone du Myélome (2003) Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med 349(26):2495–2502

Attal M, Lauwers-Cances V, Marit G et al. IFM Investigators (2012) Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med 366(19):1782–1791

Baetz T, Belch A, Couban S et al (2003) Gemcitabine, dexamethasone and cisplatin is an active and non-toxic chemotherapy regimen in relapsed or refractory Hodgkin's disease: a phase II study by the National Cancer Institute of Canada Clinical Trials Group. Ann Oncol 14:1762–1767

Bakanay SM, Demirer T (2012) Novel agents and approaches for stem cell mobilization in normal donors and patients. Bone Marrow Transplant 47:1154–1163

Ballen KK, Lazarus H (2016) Cord blood transplant for acute myeloid leukaemia. Br J Haematol 173(1):25–36

Barker LM, Pendergrass TW, Sanders JE et al (2005) Survival after recurrence of Ewing's sarcoma family of tumors. J Clin Oncol 23:4354–4362

Barlogie B, Kyle RA, Anderson KC et al (2006) Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of phase III US Intergroup Trial S9321. J Clin Oncol 24(6):929–936

Bashey A, Zhang MJ, McCurdy SR et al (2017) Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete Haploidentical donor transplantation using post-transplant cyclophosphamide. J Clin Oncol 35 (26):3002–3009

Bay JO, Cabrespine-Faugeras A, Tabrizi R et al (2010) Allogeneic stem cell transplantation in ovarian cancer. The EBMT experience. Int J Cancer 127 (6):1446–1452

Bensinger WI, Demirer T, Buckner CD et al (1996) Syngeneic marrow transplantation in patients with multiple myeloma. Bone Marrow Transplant 18(3):527–531

Bensinger WI, Martin PJ, Storer B et al (2001) Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 344 (3):175–181

Bernstein SH, Epner E, Unger JM (2010) A phase II multicenter trial of hyperCVAD MTX/Ara-C and rituximab in patients with previously untreated mantle cell lymphoma; SWOG 0213. Br J Haematol 150:200–208

Berry DA, Ueno NT, Johnson MM et al (2011) High-dose chemotherapy with autologous hematopoietic stem-cell transplantation in metastatic breast cancer: overview of six randomized trials. J Clin Oncol 29 (24):3224–3231

Brunvand MW, Bensinger WI, Soll E et al (1996) High-dose fractionated total-body irradiation, etoposide and cyclophosphamide for treatment of malignant lymphoma: comparison of autologous bone marrow and peripheral blood stem cells. Bone Marrow Transplant 18(1):131–141

Burdach S, Jurgens H, Peters C et al (1993) Myeloablative radiochemotherapy and hematopoietic stem-cell rescue in poor-prognosis Ewing's sarcoma. J Clin Oncol 11 (8):1482

Burman J, Tolf A, Hägglund H et al (2018) Autologous haematopoietic stem cell transplantation for neurological diseases. J Neurol Neurosurg Psychiatry 89 (2):147–155

Busch K, Klapproth K, Barile M et al (2015) Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. Nature 518(7540):542–546

Cabanillas F, Shah B (2017) Advances in diagnosis and management of diffuse Large B-cell lymphoma. Clin Lymphoma Myeloma Leuk 17(12):783–796

Cao C, Wang M, Sun J et al (2017) Autologous peripheral blood haematopoietic stem cell transplantation for systemic lupus erythematosus: the observation of long-term outcomes in a Chinese centre. Clin Exp Rheumatol M35(3):500–507

Cavo M, Petrucci MT, Raimondo DF et al (2016) Upfront single versus double autologous stem cell transplantation for newly diagnosed multiple myeloma: an

intergroup, multicenter, phase III study of the European myeloma network (EMN02/HO95 MM Trial). Blood 128(22):991

Champlin RE, Horowitz M, van Bekkum DW et al (1989) Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. Blood 73:606–613

Clinical Practice Guidelines in Oncology. National Comprehensive Cancer Network, v2. 2016, 2016. at http://www.nccn.org/professionals/physician_gls/pdf/testicular.pdf

Corradini P, Tarella C, Zallio F et al (2006) Long-term follow-up of patients with peripheral T-cell lymphomas treated up-front with high-dose chemotherapy followed by autologous stem cell transplantation. Leukemia 20:1533–1538

Crump M, Neelapu SS, Farooq U et al (2017) Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood 130 (16):1800–1808

D'Amore F, Relander T, Lauritzsen G et al (2012) Up-front autologous stem cell transplantation in peripheral T-cell lymphoma : NLG-T-01. J Clin Oncol 30:3093–3099

Daley GQ, Lensch W, Jaenisch R et al (2009) Broader implications of defining standards for the pluripotency of iPSCs. Cell Stem Cell 4:200

De Giorgi U, Rosti G, Slavin S et al (2005a) Salvage high-dose chemotherapy for children with extragonadal germ-cell tumors. Group authors: European grp blood marrow transplantation. Br J Cancer 93(4):412–441

De Giorgi U, Demirer T, Wandt H et al (2005b) Second-line high-dose chemotherapy in patients with mediastinal and retroperitoneal primary non-seminomatous germ cell tumors: the EBMT experience. Ann Oncol 16(1):146–151

Demirer T Preface (eds) (2017) In Haploidentical stem cell transplantation: an emerging treatment modality. Humana Press/Springer International Publishing, Cham

Demirer T, Gooley T, Buckner CD et al (1995a) Influence of total nucleated cell dose from marrow harvests on outcome in patients with acute myelogenous leukemia undergoing autologous transplantation. Bone Marrow Transplant 15(6):907–913

Demirer T, Buckner CD, Petersen FB et al (1995b) Rapid engraftment after autologous transplantation utilizing marrow and recombinant granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells in patients with acute myelogenous leukemia. Bone Marrow Transplant 15(6):915–922

Demirer T, Weaver CH, Buckner CD et al (1995c) High-dose, carmustine, and etoposide (CBV) followed by allogeneic bone marrow transplantation cyclophosphamide in patients with lymphoid malignancies who had received dose-limiting radiation therapy. J Clin Oncol 13(3):596–602

Demirer T, Buckner CD, Appelbaum FR et al (1996a) Busulfan, cyclophosphamide and fractionated total body irradiation for autologous or syngeneic marrow transplantation for acute and chronic myelogenous leukemia: Phase I dose escalation of busulfan based on targeted plasma levels. Bone Marrow Transplant 17 (4):491–495

Demirer T, Buckner CD, Appelbaum FR et al (1996b) Busulfan, cyclophosphamide and fractionated total body irradiation for allogeneic marrow transplantation in advanced acute and chronic myelogenous leukemia: phase I dose escalation of busulfan based on targeted plasma levels. Bone Marrow Transplant 17 (3):341–346

Demirer T, Petersen FB, Bensinger WI et al (1996c) Autologous transplantation with peripheral blood stem cells collected after granulocyte colony-stimulating factor in patients with acute myelogenous leukemia. Bone Marrow Transplant 18(1):29–34

Demirer T, Buckner CD, Appelbaum FR et al (1996d) High-dose busulfan and cyclophosphamide followed by autologous transplantation in patients with advanced breast cancer. Bone Marrow Transplant 17 (5):769–774

Demirer T, Celebi H, Arat M et al (1999) Autoimmune thrombocytopenia in a patient with small cell lung cancer developing after chemotherapy and resolving following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant 24(3):335–337

Demirer T, Ilhan O, Arat M et al (2001) CD41+ and CD42 + hematopoietic progenitor cells predict platelet engraftment after allogeneic peripheral blood stem cell transplantation. J Clin Apher 16:67–73

Demirer T, İlhan O, Aylı M, Arat M et al (2002) Monitoring of peripheral blood CD34+ cell counts on the first day of apheresis is highly predictive for efficient CD34 + cell yield. Ther Apher 6(5):384–389

Dhakal B, Szabo A, Chhabra S et al (2018) Autologous transplantation for newly diagnosed multiple myeloma in the era of novel agent induction: a systematic review and meta-analysis. JAMA Oncol. https://doi.org/10.1001/jamaoncol.2017.4600

Döhner H, Estey E, Grimwade D et al (2017) Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129(4):424–447

Dreyling M, Lenz G, Hoster E et al (2005) Early consolidation by myeloablative radiochemotherapy followed by autologous stem cell transplantation in first remission significantly prolongs progression-free survival in mantle-cell lymphoma: results of a prospective randomized trial of the European MCL Network. Blood 105:2677–2684

Eyraud A, Scouppe L, Barnetche T et al. FHU ACRONIM (2017) Efficacy and safety of autologous haematopoietic stem cell transplantation in systemic sclerosis: A systematic review of literature. Br J Dermatol. https://doi.org/10.1111/bjd.15993

Fenske TS, Zhang MJ, Carreras J et al (2014) Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-

sensitive mantle-cell lymphoma: analysis of transplantation timing and modality. J Clin Oncol 32:273–281
Fermand JP, Katsahian S, Divine M, et al. Group Myelome-Autogreffe (2005) High-dose therapy and autologous blood stem-cell transplantation compared with conventional treatment in myeloma patients aged 55 to 65 years: long-term results of a randomized control trial from the Group Myelome-Autogreffe. J Clin Oncol 23(36):9227–9233
Ferraro F, Celso CL et al (2010) Adult stem cells and their niches. Adv Exp Med Biol 695:155–168
Freedman AS, Neuberg D, Gribben JG et al (1998) High-dose chemoradiotherapy and anti-B-cell monoclonal antibody-purged autologous bone marrow transplantation in mantle-cell lymphoma: no evidence for long-term remission. J Clin Oncol 16:13–18
Gahrton G, Iacobelli S, Bjorkstrand B et al (2013) Autologous/reduced-intensity allogeneic stem cell transplantation vs autologous transplantation in multiple myeloma: long-term results of the EBMT-NMAM2000 study. Blood 121:5055–5063
Gale RP, Lazarus HM (2013) A rose by any other name would smell as sweet', or would it? Bone Marrow Transplant 48:881–883
Gauthier J, Yakoub-Agha I (2017) Chimeric antigen-receptor T-cell therapy for hematological malignancies and solid tumors: clinical data to date, current limitations and perspectives. Curr Res Transl Med 65 (3):93–102
Giaccone L, Storer B, Patriarca F et al (2011) Long-term follow-up of a comparison of nonmyeloablative allografting with autografting for newly diagnosed myeloma. Blood 117(24):6721–6727
Giorgi UD, Wandt H, Lioure B et al (2004) First-line high-dose chemotherapy for patients with poor prognosis extragonadal germ cell tumors. The experience of the European Bone Marrow Transplantation (EBMT) solid tumors working party. Bone Marrow Transplant 34 (12):1033–1037
Gkotzamanidou M, Papadimitriou CA (2014) Peripheral T-cell lymphoma: the role of hematopoietic stem cell transplantation. Crit Rev Oncol Hematol 89 (2):248–261
Gordon LI, Hong F, Fisher RI et al (2003) Randomized phase III trial of ABVD versus Stanford V with or without radiation therapy in locally extensive and advanced-stage Hodgkin lymphoma: an intergroup study coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol 31:684–691
Gratwohl A, Baldomero H, Demirer T et al (2004) Hematopoietic stem cell transplantation for solid tumors in Europe. EBMT; STWP; PDWP. Ann Oncol 15(4):653–660
Heino TJ, Hentunen TA (2008) Differentiation of osteoblasts and osteocytes from mesenchymal stem cells. Curr Stem Cell Res Ther 3(2):131–145
Hess DC, Abe T, Hill WD et al (2004) Hematopoietic origin of microglial and perivascular cells in brain. Exp Neurol 186:134–144
Holmberg LA, Demirer T, Rowley S et al (1998) High-dose busulfan, melphalan and thiotepa followed by autologous peripheral blood stem cell (PBSC) rescue in patients with advanced stage III-IV ovarian cancer. Bone Marrow Transplant 22:651–659
Hsu P, Qu CK (2013) Metabolic Plasticity and Hematopoietic Stem Cell Biology. Curr Opin Hematol 20(4):289–294. https://stemcells.nih.gov/info/2001report/chapter4.htm#Holtzer (Adult Stem Cells). -https://www.cibmtr.org/ReferenceCenter/SlidesReports/SummarySlides/Pages/index.aspx (CIBMTR data)
Iams W, Reddy NM (2014) Consolidative autologous hematopoietic stem-cell transplantation in first remission for non-Hodgkin lymphoma: current indications and future perspective. Ther Adv Hematol 5 (5):153–167
Jimenez-Zepeda VH, Mikhael J et al (2012) Second autologous stem cell transplantation as salvage therapy for multiple myeloma: impact on progression-free and overall survival. Biol Blood Marrow Transplant 18:773–779
Karadurmuş N, Şahin U, Arpacı F, Demirer T (2017) An overview of high dose chemotherapy with autologous stem cell rescue for germ cell tumors in current practice. Balkan Union Oncol 22(2):306–311
Kroger N, Damon L, Zander AR et al (2003) Secondary acute leukemia following mitoxantrone-based high-dose chemotherapy for primary breast cancer patients. Bone Marrow Transplant 32(12):1153–1115
Lakshmipathy U, Verfaillie C (2005) Stem cell plasticity. Blood Rev 19(1):29–38
Lang H, Ebihara Y, Schmiedt RA et al (2006) Contribution of bone marrow hematopoietic stem cells to adult mouse inner ear: mesenchymal cells and fibrocytes. J Comp Neurol 496:187–201
Lefrère F, Delmer A, Suzan F et al (2002) Sequential chemotherapy by CHOP and DHAP regimens followed by high-dose therapy with stem cell transplantation induces a high rate of complete response and improves event free survival in mantle cell lymphoma: a prospective study. Leukemia 16(4):587–593
Leng XM, Jiang Y, Zhou DB et al (2017) Good outcome of severe lupus patients with high-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation: a 10-year follow-up study. Clin Exp Rheumatol 35(3):494–499
Lonial S, Anderson KC (2014) Association of response endpoints with survival outcomes in multiple myeloma. Leukemia 28:258–268
Majhail NS, Farnia SH, Carpenter PA et al (2015) Indications for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant 21 (11):1863–1869
Mancardi GL, Sormani MP, Gualandi F et al. ASTIMS Haemato-Neurological Collaborative Group, On behalf of the Autoimmune Disease Working Party

(ADWP) of the European Group for Blood and Marrow Transplantation (EBMT); ASTIMS Haemato-Neurological Collaborative Group On behalf of the Autoimmune Disease Working Party ADWP of the European Group for Blood and Marrow Transplantation EBMT (2015) Autologous hematopoietic stem cell transplantation in multiple sclerosis: a phase II trial. Neurology 84(10):981–988

Mancardi G, Sormani MP, Muraro PA et al (2018) Intense immunosuppression followed by autologous haematopoietic stem cell transplantation as a therapeutic strategy in aggressive forms of multiple sclerosis. Mult Scler 24(3):245–255

Marco B (2012) Solid tumours in adults. In: Apperley J, Carreras E, Gluckman E, Masszi T (eds) EBMT-ESH handbook on Haematopoietic stem cell transplantation, 6th edn. European School of Haematology pp 526–538

Marks DI, Lush R, Cavenagh J et al (2002) The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. Blood 100:3108–3114

Marmont AM, van Lint MT, Gualandi F et al (1997) Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. Lupus 6(6):545–548

Mattiucci D, Maurizi G, Izzi V et al (2018) Bone marrow adipocytes support hematopoietic stem cell survival. J Cell Physiol 233(2):1500–1511

McCarthy PL, Holstein SA, Petrucci MT et al (2017) Lenalidomide maintenance after autologous stem-cell transplantation in newly diagnosed multiple myeloma: a meta-analysis. J Clin Oncol 35(29):3279–3289

McTiernan A, Driver D, Michelagnoli MP et al (2006) High dose chemotherapy with bone marrow or peripheral stem cell rescue is an effective treatment option for patients with relapsed or progressive Ewing's sarcoma family of tumours. Ann Oncol 17:1301–1305

Mehrotra M, Williams CR, Ogawa M et al (2013) Hematopoietic stem cells give rise to osteochondrogenic cells. Blood Cells Mol Dis 50(1):41–49

Nagler A, Labopin M, Shimoni A, et al. ALWP-EBMT (2012) Mobilized peripheral blood stem cells compared with bone marrow from HLA-identical siblings for reduced-intensity conditioning transplantation in acute myeloid leukemia in complete remission: a retrospective analysis from the Acute Leukemia working party of EBMT. Eur J Haematol 89(3):206–13

Ogawa M, LaRue AC, Drake CJ (2006) Hematopoietic origin of fibroblasts/myofibroblasts: its pathophysiologic implications. Blood 108:2893–2896

Ogawa M, LaRue A, Mehrotra M (2013) Hematopoietic stem cells are pluripotent and not just "hematopoietic". Blood Cells Mol Dis 51(1):3–8

Passweg JR, Baldomero H, Bader P et al (2016) Hematopoietic stem cell transplantation in Europe 2014: more than 40.000 transplants annually. Bone Marrow Transplant 51(6):786–792

Passweg JR, Baldomero H, Bader P et al (2017) Use of haploidentical stem cell transplantation continues to increase: the 2015 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant 52(6):811–817

Peccatori J, Barkholt L, Demirer T et al. on behalf of EBMT (2005) Prognostic factors for survival in patients with advanced renal cell carcinoma undergoing nonmyeloablative allogeneic stem cell transplantation. Cancer 104(10):2099–2103

Pedrazzoli P, Ferrante P, Kulekci A et al (2003) Autologous hematopoietic stem cell transplantation for breast cancer in Europe: critical evaluation of data from the European Group for Blood and Marrow Transplantation (EBMT) Registry 1990-1999. Bone Marrow Transplant 32(5):489–494

Pedrazzoli P, Ledermann JA, Lotz J-P et al (2006) Group author(s): European grp blood marrow transplant. High dose chemotherapy with autologous hematopoietic stem cell support for solid tumors other than breast cancer in adults. Ann Oncol 17(10):1479–1488

Philip T, Guglielmi C, Hagenbeek A et al (1995) Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 333(23):1540–1545

Radford J, Illidge T, Barrington S (2015) PET-directed therapy for Hodgkin's lymphoma. N Engl J Med 373:392

Ray P, De A, Yaghoubi S, Khanna A (2015) Application of adult stem cells in medicine. Stem Cells Int. Article ID 258313, 2

van Rhijn-Brouwer FCC, Spierings J, van Laar JM (2017) Autologous hematopoietic stem cell transplantation in systemic sclerosis: a reset to tolerance? Immunol Lett Nov 16

Ringdén O, Labopin M, Beelen DW et al. Acute Leukaemia Working Party of European Group for Blood and Marrow Transplantation (EBMT) (2012) Bone marrow or peripheral blood stem cell transplantation from unrelated donors in adult patients with acute myeloid leukaemia, an Acute Leukaemia Working Party analysis in 2262 patients. J Intern Med 272(5): 472–83

Robey PG (2000) Stem cells near the century mark. J Clin Invest 105:1489–1491

Robinson S, Dreger P, Caballero D et al (2015) The EBMT/EMCL consensus project on the role of autologous and allogeneic stem cell transplantation in mantle cell lymphoma. Leukemia 29:464–473

Rosiñol L, Pérez-Simón JA, Sureda A et al. Programa para el Estudio y la Terapéutica de las Hemopatías Malignas y Grupo Español de Mieloma (PETHEMA/GEM) (2008) A prospective PETHEMA study of tandem autologous transplantation versus autograft followed by reduced-intensity conditioning allogeneic transplantation in newly diagnosed multiple myeloma. Blood 112(9):3591–3

Sahin U, Demirer T (2017) Chapter 13: Future perspectives for Haploidentical stem cell transplantation. In: Demirer T (ed) Haploidentical stem cell

transplantation: an emerging treatment modality. Humana Press/Springer, Cham, pp 189–199
Sahin U, Toprak SK, Atilla Ataca P et al (2016a) An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. J Infect Chemother 22:505–514
Sahin U, Atilla Ataca P, Atilla E et al (2016b) An overview of hematopoietic stem cell transplantation related thrombotic complications. Crit Rev Oncol Hematol 107:149–155
Sanders S, Bredeson C, Pringle CE et al (2014) Autologous stem cell transplantation for stiff person syndrome: two cases from the Ottawa blood and marrow transplant program. JAMA Neurol 71 (10):1296–1299
Sasse S, Alram M, Muller H et al (2016) Prognostic relevance of DHAP dose-density in relapsed Hodgkin lymphoma: an analysis of the German Hodgkin-study group. Leuk Lymphoma 57:1067–1073
Schmitz N, Beksac M, Hasenclever D et al. European Group for Blood and Marrow Transplantation (2002) Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood 100(3):761–767
Schouten HC, Qian W, Kvaloy S et al (2000) High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. J Clin Oncol 21:3918–3927
Sebban C, Brice P, Delarue R et al (2008) Impact of rituximab and/or high-dose therapy with autotransplant at time of relapse in patients with follicular lymphoma: a GELA study. J Clin Oncol 26:3614–3620
Secondino S, Carrabba MG, Pedrazzoli P et al (2007) Reduced intensity stem cell transplantation for advanced soft tissue sarcomas in adults: a retrospective analysis of the European group for blood and marrow transplantation. Haematologica 92:418–420
Sera Y, LaRue AC, Moussa O et al (2009) Hematopoietic stem cell origin of adipocytes. Exp Hematol 37:1108–1120
Sonneveld P, Avet-Loiseau H, Lonial S et al (2016) Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the international myeloma working group. Blood 127(24):2955–2962
Spangrude GJ (2003) Stem cells and tissue regeneration when is a stem cell really a stem cell? Bone Marrow Transplant 32:7–11
Suda T, Takubo K, Semenza GL (2011) Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell 9:298–310
Sullivan KM, Goldmuntz EA, Keyes-Elstein L et al (2018) SCOT study investigators Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med 378(1):35–47
Sun J, Ramos A, Chapman B et al (2014) Clonal dynamics of native haematopoiesis. Nature 514(7522):322
Sureda A, Bader P, Cesaro S et al (2015) Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe. Bone Marrow Transplant 50(8):1037–1056
Tessoulin B, Ceballos P, Chevaller P et al (2016) Allogeneic stem cell transplantation for patients with mantle cell lymphoma who failed autologous stem cell transplantation: a national survey of the SFGM-TC. Bone Marrow Transplant:1–7
Tsai S, Liu H, Shore T et al (2016) Frequency and risk factors associated with cord graft failure after transplant with single-unit umbilical cord cells supplemented by Haploidentical cells with reduced-intensity conditioning. Biol Blood Marrow Transplant 22(6):1065–1072
Ueno NT, Rizzo D, Demirer T et al (2008) Allogeneic hematopoietic cell transplantation for metastatic breast cancer. Bone Marrow Transplant 41:537–545
van Kampen RJ, Canals C, Schouten HC et al (2011) Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European group for blood and marrow transplantation registry. J Clin Oncol 29:1342–1348
Vanderberghe E, Elvira CR, Loveriza F et al (2003) Outcome of autologous transplantation for mantle cell lymphoma: a study by the European blood and bone marrow transplant and autologous blood and marrow transplant registries. Br J Haematol 120:793–800
Velten L, Haas SF, Raffel S et al (2017) Human haematopoietic stem cell lineage commitment is a continuous process. Nat Cell Biol 19(4):271–281
Visconti RP, Ebihara Y, LaRue AC et al (2006) An in vivo analysis of hematopoietic stem cell potential: hematopoietic origin of cardiac valve interstitial cells. Circ Res 98:690–696
Wright DE, Wagers AJ, Gulati AP (2001) Physiological migration of hematopoietic stem and progenitor cells. Science 294:1933–1936
Xu J, Tian K, Zhang H, Li L et al (2017) Chimeric antigen receptor-T cell therapy for solid tumors require new clinical regimens. Expert Rev Anticancer Ther 17 (12):1099–1106
Yong CSM, Dardalhon V, Devaud C et al (2017) CAR T-cell therapy of solid tumors. Immunol Cell Biol 95 (4):356–363
Yuksel MK, Demirer T (2017) Chapter 4: Toxicity of conditioning regimens in haploidentical stem cell transplantation. In: Demirer T (ed) Haploidentical stem cell transplantation: an emerging treatment modality. Humana Press/Springer, Cham, pp 43–56

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Stem Cells in Regenerative Cardiology

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Abstract

The common prevalence of heart failure and limitations in its treatment are leading cause of attention and interest towards the induction of cardiac regeneration with novel approaches. Recent studies provide growing evidence regarding bona fide cardiac regeneration post genetic manipulations, administration of stimulatory factors and myocardial injuries in animal models and human studies. To this end, stem cells of different sources have been tested to treat heart failure for the development of cellular therapies. Endogenous and exogenous stem cells sources used in regenerative cardiology have provided a proof of concept and applicability of cellular therapies in myocardial improvement. Recent clinical studies, especially, based on the endogenous cardiac progenitor and stem cells highlighted the possibility to regenerate lost cardiomyocytes in the myocardium. This review discusses emerging concepts in cardiac stem cell therapy, their sources and route of administration, and plausibility of de novo cardiomyocyte formation.

Keywords

Cardiac stem cells · Cardiac progenitors · Cardiovascular therapies · Clinical trials based on resident CSCs · Heart regeneration

1 Introduction

Heart failure affects more than 23 million people worldwide. Heart transplantation is still the only available treatment, provided that an appropriate donor is present (Jessup and Brozena 2003; Jessup et al. 2009; Mozaffarian et al. 2015). Several strategies and mechanisms haven been reported to be important to achieve cardiac regeneration as an alternative approach to support cardiomyocyte renewal post cardiovascular issues including but not limited to small molecule inhibitors of proteins, noncoding RNAs and stem cell administration (Garbern and Lee 2013; Boon and Dimmeler 2015; Boon et al. 2016; Turan et al. 2016). However, it is still unclear whether newly formed cardiomyocytes derived from preexisting cardiomyocytes or cardiac stem cells. In addition, in recent years, several clinical investigators and researchers have proposed the induction of cardiomyocyte proliferation as a

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plausible strategy for the development of novel cardiac regenerative approaches (Aslan et al. 2015).

2 Evidence of the Cardiac Regeneration at Baseline

Adult heart, historically, was considered as terminally differentiated and suggested as a non-regenerative organ. However, intensive studies on lower vertebrate and mammalian indicated that heart is not a post mitotic organ and there exists a constant cardiomyocyte turnover (Poss et al. 2002; Kajstura et al. 2010; Bergmann et al. 2012; Senyo et al. 2013). Cardiac regeneration studies in different organisms including zebrafish, amphibian, newt, human and neonatal mice indicated regenerative response of heart (Rumyantsev 1966, 1973; Oberpriller and Oberpriller 1974; Poss et al. 2002; Porrello et al. 2011; Witman et al. 2011). Using electron microscopy, Oberpriller et al. (1974) demonstrated that newt is capable to regenerate myocardium (Oberpriller and Oberpriller 1974). Poss et al. (2002) reported zebrafish could regenerate myocardium after amputation of 20% of ventricular apex of heart (Poss et al. 2002).

In 2000s, evidences for mammalian heart regeneration were reported in successive studies. In a landmark study, proliferative time period of neonatal mice were reported. It was shown that neonatal mice capable to regenerate myocardium until postnatal day 7 (P7) (Porrello et al. 2011). Moreover, using this model, we successfully identified Meis1 as one of the important regulator of this regenerative response (Kocabas et al. 2013). In the last decade, different modulators of cardiac regeneration were reported and suggested to be important to reactivate cardiomyocyte cell cycle.

In study regarding the administration of cardiogenic factors such as IL3, FGF10, C3orf58, Oncostatin M, TNF-related weak inducer of apoptosis (TWEAK) and periostin increased cardiomyocyte number (Kühn et al. 2007; Novoyatleva et al. 2010; Kubin et al. 2011; Beigi et al. 2013; Rochais et al. 2014; O'Meara et al. 2015). In gene manipulation studies, knockout profile of $p27^{KIP1}$, mir-133a and salvador homolog (Salv) resulted with increased cardiomyocyte proliferation determined by mitosis-specific marker phospho-histone 3 (pH 3) (Jung et al. 2005; Liu et al. 2008; Heallen et al. 2011). Moreover, overexpression studies regarding c-myc, E1A, cyclin B1-CDC2, cyclinA2, cyclin D2, Notch signaling pathway, YAP and ERBB2 induce cardiomyocyte proliferation determined by mitosis markers (pH 3, Ki67 and aurora B) (Jackson et al. 1990; Li et al. 1996; Bicknell et al. 2004; Pasumarthi et al. 2005; Cheng et al. 2007; Campa et al. 2008; D'Uva et al. 2015).

Kajstura et al. (2010) and Bergmann et al. (2009) reported that cardiomyogenesis occurs in adult heart depending on analysis of post mortem heart samples obtained from thymidine analogue iododeoxyuridine (IdU) treated cancer patients and ^{14}C labeled human subject due to nuclear weapon test during Cold War, respectively (Bergmann et al. 2009; Kajstura et al. 2010). In recent study, Canseco et al. (2015) reported adult heart cardiomyocytes proliferate response to injury due to integration of left ventricular assist device (LVAD) (Canseco et al. 2015). However, it is still unclear whether these newly formed cardiomyocytes derived from preexisting cardiomyocytes or cardiac stem cells.

3 Stem Cells in Cardiac Regeneration (Fig. 1)

After successive discovery of multiple cardiac progenitor and stem cells, cardiac clinical stem cell research area generated hope for their use in heart failure. Despite the fact that reactivating cardiomyocyte proliferation is one of the prominent approaches in cardiac regeneration, many researchers investigated embryonic stem cells (ESCs), bone marrow derived stem cells (BMCs), cardiac resident stem cells (CSCs) and skeletal myoblast cells (Murry et al. 1996, Orlic et al. 2001a, b, 2003). It was suggested that embryonic stem cells (ESCs), derived from inner cell mass of blastocysts, could differentiate

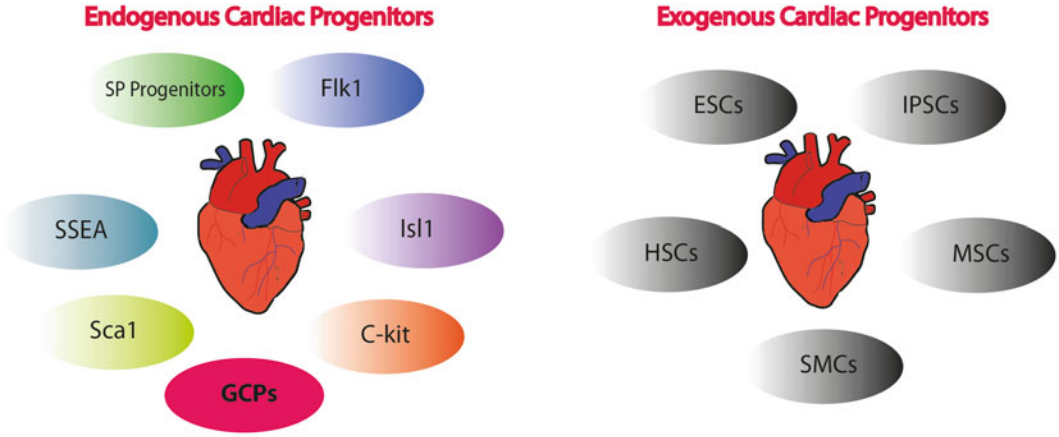

Fig. 1 Endogenous and exogenous cardiac progenitor and stem cells studied in cardiac regeneration. **SP progenitors**: Cardiac Side Population Cells, **Sca1**: Sca1+ Stem Cells, **c-Kit**: Lin-, c-Kit + Stem Cells, **Isl1**: Isl1+ Progenitor Cells, SSEA: SSEA-1+ Progenitor Cells, **Flk1**: Flk1+ Progenitor Cells, **GCPs**: Glycolytic Cardiac Progenitors, **ESC**: Embriyonic stem cells, **IPSCs**: Induced pluripotent stem cells, **HSCs**: Hematopoietic stem cells, **MSCs**: Mesenchymal stem cells, **SMCs**: Skeletal myoblast cells

into beating cardiomyocytes. Induced pluripotent stem cells (IPSCs) created a new aspect in stem cell field and made possible to establish patient specific cardiomyocyte differentiation studies (Takahashi and Yamanaka 2006).

ESCs are derived from the inner cell mass of an early stage embryo called as blastocyst (Bishop et al. 2002). ESCs are pluripotent and give rise to three different types of primary germ layers, which are ectoderm, endoderm and mesoderm. They represent a renewable progenitor cell source. The differentiation of ESCs into cardiomyocytes includes different stages: First step starts with induction and stepwise differentiation into mesoderm followed by differentiation into cardiosphere (expressing GATA4 and Nkx2.5 transcription factors). Cardiomyocyte differentiation occurs with the expression of MHC, cTNI, α-actinin and other proteins inducing contraction (Kehat et al. 2001; Beqqali et al. 2006). ESCs can differentiate into almost all cell lines. However, there have not been any clinical studies regarding use of embryonic stem cells in myocardial regeneration. Beside the ethical issues, teratoma formation and the possibility of immunologic rejection might be the major limitations to utilize ESCs (Oettgen et al. 2006).

Takahashi and Yamanaka discovered induced pluripotent stem cells (IPSCs), which are differentiated from somatic cells into embryonic like pluripotent state. IPSCs can differentiate into different type of cells including but not limited to blood cells, islet cells, neurons and muscle cells (Takahashi and Yamanaka 2006). Differentiation into IPSCs starts with retroviral introduction of the transcription factors Oct4, Sox2, cMyc and Klf4 (Zhou et al. 2009) and process takes 3–4 weeks for human cells. Several groups reprogrammed the human skin fibroblasts into iPSCs. In a study, fully functional cardiomyocytes were derived from these fibroblasts (Gai et al. 2009; Zhang et al. 2009; Zwi et al. 2009). In two other recent studies, injection of IPSCs to the infarct area resulted with in vivo differentiation into the cardiac cells (Kensah et al. 2013; Yu et al. 2013). Moreover, in vitro differentiation of IPSCs into cardiomyocytes, endothelial cells and smooth muscle cells were reported (Lee et al. 2014). However tumorigenic property and difficulties in obtaining IPSCs are two major problems complicating use of IPSC (Dixit and Katare 2015).

Skeletal myoblast cells (SMCs) are the first cell types utilized in cardiac regeneration studies. Besides their contraction ability, SMCs can resist hypoxic environment and they can endure several hours of severe ischemia without being irreversibly injured (Marelli et al. 1992; Reinecke et al. 2002b). However, injections of SMCs into patients with depressed Left Ventricular (LV) function failed to improve heart function in the MAGIC clinical study (Multicenter randomized myoblast autologous grafting in ischemic cardiomyopathy) (Menasche et al. 2008). Various factors were reported to lead the inefficient integration of injected myoblasts. It was suggested that adhesion proteins involved in myoblast integration could be an important factor (Reinecke et al. 2002b). Moreover, the intravenous injection of granulocyte colony-stimulating factor (G-CSF) for the mobilization of bone marrow stem cell to myocardium was reported. However, no significant difference was observed with the G-CSF treatment (Zimmet et al. 2012).

The bone marrow derived mononuclear cells (MNCs), hematopoietic stem cells (HSCs), and mesenchymal stem cells (MSCs) have been investigated in regenerative cardiology. MNCs are classified as single nucleated cells in bone marrow and mostly contain differentiated cells with a less number of HSCs (Garbern and Lee 2013; Aslan et al. 2015). A study conducted by Orlic et al. (2001a, b) shows that intravenous transplantation of isolated HSCs to infarcted left ventricle (LV) could increase cardiomyocyte and vasculature renewal (Orlic et al. 2001a, b). However further studies focusing on HSC transplantation shows that the regeneration capacities of HSCs are limited for a clinical benefit (Murry et al. 2004). Bone marrow MSCs are also promising for the cardiac regenerative therapies. MSCs are found in many different tissue types including adipose tissue and cord blood. Besides MSCs also can be differentiated in to vascular endothelial cells in vitro and in vivo. It has been shown that MSCs could be differentiated into cardiomyocytes to some extent (Pittenger et al. 1999; Fatkhudinov et al. 2014). However, applicability of MSCs post-acute myocardial injuries and heart failure is still investigated.

4 Endogenous Progenitor and Stem Cells in Cardiac Regeneration

4.1 Resident Cardiac Stem Cells

Resident cardiac stem cells (CSCs) are probably the most promising cell types in the context of cardiac regeneration. After the discovery of stem cell-like cell populations in heart, successive studies showing the contribution towards cardiac regeneration were reported. Beltrami et al. (2003) discovered the c-Kit+ CSCs through the analysis of c-Kit receptor expression in myocardium (Beltrami et al. 2003). Depending on the surface marker such as Sca1 (Morrison et al. 1997), Abcg-2 and Flk-1 (Iida et al. 2005; Maher et al. 2014), successive identification of different types of CSCs have been reported. Furthermore, Menasche (2004) reported the clonogenicity of resident CSCs in vivo and in vitro (Menasche 2004).

CSCs exist in supportive cell niches and their ratio in the heart tissue is reported to be approximately 1/30.000 (Beltrami et al. 2003; Bearzi et al. 2007, 2009; Hosoda et al. 2009). In addition, their important roles in regeneration of myocytes, fibroblasts, smooth muscle cells and endothelial cells were reported in back to back reports (Beltrami et al. 2003; Linke et al. 2005; Bearzi et al. 2007, 2009; Hosoda et al. 2009). After characterization of different CSCs including c-Kit+, Isl1 progenitors (Laugwitz et al. 2005), epicardial progenitors, side population (SP) progenitors, Stem cell antigen-1 (Sca1+) progenitors, cardiac mesenchymal stem cells were classified into different subgroups of CSCs (Leri et al. 2011).

In another study regarding characterization of a population of epicardium-derived multipotent cardiac progenitor cells (cCFU-Fs) were established. Moreover, it was suggested that these cells resemble MSCs and may participate

in cardiac development, homeostasis, and repair (Chong et al. 2011).

4.2 Side Population Progenitor Cells

Side population (SP) progenitors exist in a variety of organs such as brain, lungs, skin, bone marrow, liver and skeletal muscle. SP progenitors were firstly isolated from bone marrow and constitute 0.03–3.5% of mononuclear cardiac cells (Goodell et al. 1996). SP progenitor cells specifically defined as their ability to efflux the DNA binding Hoechst dye by ATP binding cassette. Moreover, SP progenitors are easily isolated by Fluorescent Activated Cell Sorting (FACS) technique (Unno et al. 2012). It was reported that bone marrow SP progenitors differentiate into endothelial cells and cardiomyocytes in vitro (Gussoni et al. 1999). In addition, heart originated cardiac SP progenitors demonstrate Sca1+, CD34-, c-Kit- markers and express MEF2c, GATA4, Nkx2-5 transcription factors (Table 1). Moreover, SP progenitors can differentiate into cells expressing sarcomeric proteins like troponin and cardiac α-actinin (Pfister et al. 2005; Liang et al. 2010).

4.3 Flk1+ Progenitor Cells

Studies in mouse cardiac development showed that the endocardium and a population of the myocardium develop from an intermediate population, which expresses Fetal liver kinase1 (Flk1) (Motoike et al. 2003; Ema et al. 2006). Yamashita et al. (2005) showed that cardiomyocytes could be generated from Flk1+ progenitors isolated from ES cell differentiation cultures and early embryos (Yamashita et al. 2005). The Flk1+ cells exit the primitive streak and migrate for the formation of cardiac crescent (Ema et al. 2006). The results support the idea that the myocardial and endothelial lineages develop from a common Flk1+ progenitor (Kattman et al. 2006). Moreover, multipotent Flk1+ progenitors can give rise to cardiomyocyte, endothelial and vascular smooth muscle lineages. A recent study revealed that CD45-, Flk1+ cells are present in adult human circulation and increase with acute myocardial infarction (Suresh et al. 2013). Flk1+ cells might be assigned for improvement of LV systolic function after myocardial infarction.

4.4 Isl1+ Progenitor Cells

First evidence related to Insulin gene enhancer protein1+ (Isl1+) progenitor cell population in the anterior heart field of mouse was identified by Kelly and Buckingham. (Kelly and Buckingham 2002). The study based on Isl1 mutants suggested that Isl1-expressing cells might contribute to the outflow tract of the heart.

Laugwitz et al. (2005) identified Isl1+ cardiac progenitor cell population in neonatal heart (Laugwitz et al. 2005). The Isl1+ progenitors isolated after pediatric surgery were cultured on cardiac mesenchymal feeder layers, which are positive for Nkx2.5 and GATA-4 markers. Although Isl1+ cardiac progenitors may not necessarily have the same molecular identity, they are able to differentiate into mature cardiomyocytes. Unfortunately, Isl1+ cells are detected only in neonates. The absence of Isl1+ progenitors in adult heart suggests that cardiac progenitors identified during later stages of life might arise *de novo* from epicardium (Wu et al. 2008). Later studies show that Isl1 is not a marker for cardiac progenitors in adult heart albeit it is a marker of the adult sinoatrial node (Weinberger et al. 2012). Moreover, another study showed that Isl1 also labels cardiac the neural crest besides the second heart field progenitors in adult murine and human hearts (Engleka et al. 2012). It has been recently shown that by Bone Morphogenic Protein 4 (BMP4) signal activation, functional cardiomyocytes can be differentiated from Isl1+ CSC population (Cagavi et al. 2014). The first clinical case study was conducted by Menasche et al. (2015) and demonstrated improvement to some extent in short term (Menasche et al. 2015).

Table 1 Classification and characteristics of resident CSCs

Cell Types	(+) Markers	(−) Markers	Species	In vivo MR	In vitro Differentiation	References
Cardiac SP cells	ABCG2, Sca1, C-kit[a], CD34[a]	CD31	Adult mouse	N/A	Co culture with cardiac cells	Hierlihy et al. (2002)
Sca1+ stem cells	Sca1	c-kit, CD34, CD45, Lin, Flk1	Adult mouse	Yes	5-Azacytidine or Oxytocine treatment	Oh et al. (2003) and Matsuura et al. (2004)
c-Kit+ Stem cells	c-kit, Nkx2.5, GATA4, GATA5	CD34, CD45, Lin	Adult rat, mouse, dog, porcine, human	Yes	Medium (MEM+ 10%, FCS+ 10 nM)	Beltrami et al. (2003)
Isl1+ progenitor cells	Isl1, GATA4	Sca1, c-kit	Neonatal mouse, rat and human	N/A	Co culture with neonatal cardiomyocytes	Laugwitz et al. (2005)
SSEA-1+ progenitor cells	Nkx2.5, GATA4, Oct 3/4	c-kit, Sca1, TnnT	Adult rat, neonatal rat, human	Yes	5-Azacytidine treatment	Ott et al. (2007)
Flk1+ progenitor cells	Flk1,	CD45	Adult human	N/A	Collagen type IV	Ishitobi et al. (2011)
Glycolytic cardiac progenitors (GCPs)	Hif-1α, Nkx2.5, GATA4, WT-1, Tbx 18, preferential cytoplasmic glycolysis	CD45, Sca1, c-kit, CD31, FSP1	Adult mouse	N/A	Co culture with neonatal cardiomyocytes, 5-Azacytidine treatment, serum withdrawal, endothelial differentiation medium	Kocabas et al. (2012)

[a]Low amount of identification, *N/A* Not available, *MR* myocardial regeneration

4.5 c-Kit+ Cardiac Stem Cells

c-Kit is a tyrosine kinase receptor factor (CD117) and c-Kit+ cells are well identified population existing in heart. c-Kit is one of the intensively studied cell surface marker and it was reported that the intracoronary delivery of isolated murine heart c-Kit+ cells to the infarcted rat hearts improved LV function at 35 d. Due to low retention and rapid disappearance of CSCs from the recipient heart, authors suggested that paracrine effects could be the underlying mechanism in this recover process (Hong et al. 2014). These cells have the mesenchymal origin and it has been shown that c-Kit+ cells migrate through the infarct zone and may give rise to cardiomyocytes in vivo (Sullivan et al. 2015). Another study reveal that the infusion of c-Kit+ CSCs reduce the rate of oxidative stress and apoptosis in both cardiomyocyte and non-cardiomyocyte cell populations (Kazakov et al. 2015).

c-Kit+ cells also express Nkx2-5, GATA4 and GATA5 transcription factors. Tillmanns et al. (2008) indicated that the myocardial injection of IGF1 and hepatocyte growth factor treated c-Kit+ cells increase the regeneration capacity of myocardium (Tillmanns et al. 2008). Moreover, Choi et al. (2013) reported that c-Kit+ CSCs proliferate in vitro provided that optimal culture and enzymatic isolation methods maintained (Choi et al. 2013).

4.6 Sca1+ Cardiac Stem Cells

Stem cell antigen1 (Sca1) expressing cardiac stem cells, which are characterized by CD45-, CD34-, Flk1- markers as well, comprise differentiation capability into myocardium, smooth muscle cells and endothelial vascular cells (Linke et al. 2005; Unno et al. 2012). Sca1+ cells can differentiate into cardiomyocytes through formation of cardiospheres. In a study conducted by Matsuura et al. (2004) Sca1+, CD45- mice cells were exposed to oxytocin. Oxytocin leads to the expression of cardiac transcription factors and contractile proteins. Thus, it leads to the contraction of Sca1+ CSCs *in-vitro* (Matsuura et al. 2004). Moreover, intramyocardial injection of these Sca1+ CSCs after the infarction increased cardiac function (Tateishi et al. 2007). Another study revealed that Sca1 knockout mice lead to myocardial contraction dysfunction (Bailey et al. 2012).

In another study, Sca1+ cells were identified in the stromal area of the myocardium (Uchida et al. 2013). The location and relationship of these Sca1+ cells are behaviorally similar to skeletal muscle stem cells, which are located in below the basal lamina and containing laminin. Moreover, studies regarding laminin showed that the Sca1+ located under the basal lamina and tightly associated with cardiomyocytes (Uchida et al. 2013). Their easily accessible nature and optimized culturing conditions makes Sca1+ CSCs an ideal candidate to study and research cardiac tissues (Jha et al. 2015).

4.7 SSEA1+ Cardiac Progenitors

Stage-specific embryonic antigen1+ (SSEA1+) cardiac stem cell population has been isolated from neonatal and adult rat hearts (Ott et al. 2007). Adult rat heart SSEA1+ cells are characterized by OCT3/4+, c-Kit-, and Sca1+ surface markers. Moreover, neonatal rat heart SSEA1+ cells express Nkx2.5, GATA4 and cardiac myosin heavy chain indicating their cardiomyogenic differentiation potential. Moreover, in the case of co-culture with primary cardiomyocytes, SSEA1+ progenitor cells differentiate and express α-sarcomeric actin or cardiac myosin heavy chain, leads to formation of beating colonies. In addition, transplantation of SSEA1+ cells into an infarcted rat heart (10^6 cells injected intromyocardially) induce myocardial regeneration and functional improvement (LVEF = 57% in treated vs LVEF = 28% in control at 3 weeks compared to 2-week baseline LVEF = 36%) (Ott et al. 2007). Ott et al. (2007) suggested that the SSEA1 identifies the most primitive cardiac stem cells present in the adult mammalian heart suggesting that c-Kit, Sca-1 and Abcg2 identify cardiac stem cells at later stages of cardiac-specific differentiation (Ott et al. 2007).

In another study, SSEA1 surface marker was used for the isolation of CPCs from human embryonic hearts isolated neonatal human SSEA-1+ cells also express Oct4, Nkx2.5, Isl1 and Tbx5 at the mRNA level. However, they did not express troponin T (TnnT) which is defined as mature cardiomyocyte marker. Moreover, human neonatal SSEA1+ cells co-express the mesenchymal stem cell markers such as CD105, CD166, CD73, CD59 and CD44 but not the hematopoietic markers (CD45, CD133 and CD34).

4.8 Glycolytic Cardiac Progenitors (GCPs)

Over the last decade, CSC niches were suggested to be more complicated than other epithelial organs owing to basal and apical morphology of heart (reviewed in (Leri et al. 2014)). Utilization of different anatomical methods on epicardial region revealed numerous different cardiac niches (Limana et al. 2007; Zhou et al. 2008; Di Meglio et al. 2010a, b; Smart et al. 2011; Kocabas et al. 2012). Moreover, it was reported that CSC niches not only existed in subepicardium, but also differentially disturbed in myocardium (Urbanek et al. 2005).

A recent study, demonstrated an epicardial/subepicardial microenvirenment with charecteristics of low oxygen tension. This was evident by low capillary density compared to

myocardium and endocardium. In addition, they have shown that epicardial/subepicardial microenvirenment houses hypoxia inducible 1 alpha (Hif-1α) positive cells. Hif-1α gene is constitutively expresses but it has been known to be stabilized at protein level only in hypoxic (<2% O_2) microenvirenments. These suggests that 3–7 cell layer of epicardial/subepicardial microenvironment could be a niche for long-term maintenance of cardiac stem and progenitors. Indeed, Kocabas et al. (2012) shows that they could metabolically profile and isolate cells from adult mouse heart with low mitochondrial potential (Low MP) using FACS.

These Low MP cells, named glycolytic cardiac progenitors (GCPs) express bone fide cardiac progenitor markers Nkx2.5, GATA4, WT1, Tbx18. GCPs could be differentiated into endothelial, smooth muscle and cardiac lineages. In addition, GCPs are clonogenic and could be maintained in cell culture upto 60 passages in ES medium. GCPs marked with HIF-1α protein stabilization even in normoxic (21% O_2) cell culture conditions. GCPs drastically resistant to anoxia and hypoxia with concomitant higher survival rates, which might be related to their localization to hypoxic cardiac epicardial/subepicardial microenvironment. GCPs demonstrate higher levels of lactate production compared to cardiac fibroblasts. In addition, GCPs poses lower ATP content and lower rates of oxygen consumption. Overall, GCPs preferentially utilize cytoplasmic glycolysis to meet their energy demands. Intriguingly, down regulation of Hif-1α in GCPs leads to induction of cardiomyocyte and vascular gene expression, which could be further explored in regenerative cardiology.

4.9 Relationship Between Different Types of Cardiac Progenitor and Stem Cells

The identification of cardiac progenitor and stem cells (CPCs) depends on expression of one or more cell surface markers. However, it is unknown that the variability in marker distribution reflects cells with distinct functional properties (Anversa et al. 2006). Moreover, more than one type of stem cell might be present in a particular tissue (Cai et al. 2004). However the main role of resident CPCs in the aspect of cellular response to scar formation and cardiac homeostasis is not clearly understood (Barile et al. 2007).

Fibro-proliferative response occurs after the myocardial infarction that causes a fibrotic scar tissue around the infarct area. Formation of fibrotic scar might be due to the limitation of recruitment signals near the infarction zone. Another possible reason is the prevention of mobilization of resident CPCs to the infarct area. Moreover, the surrounding area of the myocardium might have a negative environmental effect on stem cell viability and differentiation. The fibro-proliferative scar formation is the reason of limitation in endogenous myocardial repair capacity. Thus delivery of resident CPCs to the myocardial infarction area after the *ex vivo* expansion might be the aim of further resident CPC related studies. Moreover, surrounding area of the myocardium might have a negative environmental effect on stem cell viability and differentiation.

5 Human CSC Clinical Trials (Fig. 2)

5.1 Route of Administration

Intracoronary injection method is one of the major delivery techniques used in cardiovascular injection. Throughout the years it was modified to increase efficiency of targeting which than gave rise to method called ‘intracoronary infusion (IC)’ (Suzuki et al. 2001). Method is initiated by catheterization into coronary artery, which enhance selective delivery of stem cells into different parts of the heart trough different vessel routes. Two different step could be used trough this method. In the first one, stem cells delivered without arresting continuous coronary flow and in second one arrest-flow are mediated using balloon catheters (Keith et al. 2015).

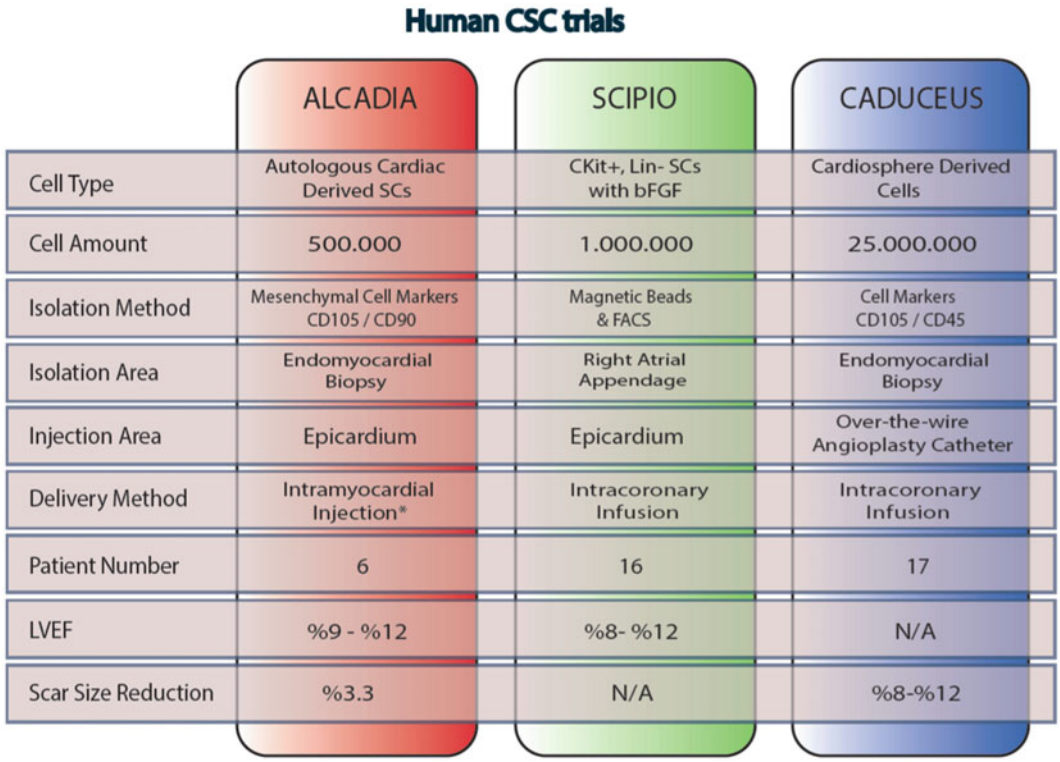

Fig. 2 Scheme of major human CSC injection experiments. ALCADIA: Autologous Human Cardiac-Derived Stem Cell to Treat Ischemic Cardiomyopathy, SCIPIO: Cardiac stem cells in patients with ischemic cardiomyopathy, and CADUCEUS: Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction

Although they differ in the action of mechanism, continuous flow delivery method is preferred and widely used (Tseliou et al. 2014). Moreover, using this method, homogenous distributions of injected cells were supported, which do not occur in injection into specific area.

Intravenous injection simply defined as injection or infusion of the stem cell population into the veins. Albeit injected cell population may migrate to undesired parts of the body, variety of homing signals including SDF-1 are used to enhance immobility of injected cells (Larrivee et al. 2006).

Intramyocardial injection is one of the widely used delivery method involving through direct injection of cells into epicardium or cardiac vein using needle to proximal area of injury site. Since cells are transferred into a particular area by intramyocardial injection, they cannot be homogenously distributed and therefore form clusters. These clusters interrupt the interaction between delivered and pre-existing cells since they are covered with stromal cells (Reinecke et al. 2002a; Leobon et al. 2003).

Transendocardial injection is defined as the subgroup of intramyocardial injection in which cardiac catheterization is utilized. It is initiated by placing special catheter into the left ventricle trough femoral artery and aortic cells valve in retrograde fashion followed by directly delivery of cells into the endocardium (reviewed in (Mushtaq et al. 2014) Transendocardial injection is generally coupled with several sensory systems to determine injured area within the myocardium, which provides more accurate delivery of stem cells. In addition, 3D MRI-electromagnetic fusion mapping, a sensory system, was recently introduced to elaborately

analyse myocardium (Hatt et al. 2013). Taking these recent advancements into consideration, transendocardial injection could be one of the promising delivery method to successfully analyse effect of injected cells on cardiac regeneration (Perin et al. 2011; Vrtovec et al. 2013).

5.2 Major Clinical Trials Based on Resident CSCs

After successful in vivo studies in animal myocardial models, human clinical trials were conducted to understand potential use of CSCs in cardiac regenerative therapies. Clinical studies known as ALCADIA (Autologous Human Cardiac-Derived Stem Cell to Treat Ischemic Cardiomyopathy), SCIPIO (Cardiac stem cells in patients with ischemic cardiomyopathy) (Bolli et al. 2011) (NCT00474461) and CADUCEUS (Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction) (Makkar et al. 2012) (NCT00893360) have got high interest (Yacoub and Terrovitis 2013). The design of both SCIPIO and CADUCEUS were based on intracoronary injection of autologous human CPCs. In CADUCEUS study cardiospheres derived from inter-ventricular septum biopsies, whereas in SCIPIO study c-Kit expressing cells were isolated from explanted atrial tissue. In both studies, injection of those cells decreased scar area. However, only in the SCIPIO trial, the improvement of left ventricular ejection fraction was reported. Although both of these studies had a small treatment group, these trials were the first two studies that showed a successful human CPC treatment for ischemic heart disease.

5.3 ALCADIA

ALCADIA study aimed cardiac regeneration in patients with ischemic cardiomyopathy and heart failure through establishing a hybrid cell therapy application with combination of autologous cardiac stem cells and controlled injection of basic Fibroblast Growth Factor (bFGF) (Yacoub and Terrovitis 2013). ALCADIA was a phase 1 non-randomized study covering six patients (55.5 ^ 10.8 years old, 5 men and one woman) with ischemic cardiomyopathy (left ventricular ejection fraction between 15–45%), symptomatic heart failure, myocardial viability and indication for coronary artery bypass surgery (Yacoub and Terrovitis 2013).

Cardiac-derived cells (CDCs) with mesenchymal cell markers (CD105+, CD90+), were isolated from endomyocardial biopsies followed by in vitro expansion. Half millions of CDCs were injected into the epicardium of patients via intramyocardial injections during coronary artery bypass graft (CABG) surgery. Upon the injection of CDCs into epicardium, the area was treated with bFGF containing biodegradable hydrogel mixture. The patients were monitored for 1 year and at the 6th month after surgery, the efficiency of left ventricular ejection fraction (LVEF), infarct volume and symptoms were measured. Further and larger, randomized studies should be carried out for successful investigation of the effect of hybrid therapy (Yacoub and Terrovitis 2013).

5.4 SCIPIO

SCIPIO was a randomized clinical trial designed to investigate improvement of patients with post infarction LV dysfunction requiring surgical revascularization. The study included 16 patients with 7 controls and c-Kit+, Lin- expressing CSCs were isolated via magnetic beads followed by intra-coronary injection into infarct area. The amount of injected CSCs differed with the infarct size of patients. Half a million cells were injected to the small posterior infarcts while one million cells were injected to the large anterior infarcts (Bolli et al. 2011). SCIPIO trial was resulted with improvement in EF by 8–12% and reduction in infarct area at one and four-year time points in the cell therapy group.

5.5 CADUCEUS

CADUCEUS is a phase 1 randomized study comprising 17 randomized cell therapy and 8 standard care patient group (Makkar et al. 2012). The study focused on to recover LV dysfunction in patients via infusion of 25 m cells into the infarct related artery. The CDCs were isolated from RV endomyocardial biopsies followed by infusion into the infarct-related artery 1,5–3 months after myocardial infarction. Trial resulted with significant reduction in the size of infarction area (7.7% at 6 months and 12.3% at 1 year) and an increase in the viable myocardium. However, there was no change in LVEF. Additionally, they have suggested that, due to small number of patient group and short period of follow up, more studies are required in the related field (Makkar et al. 2012).

6 Final Remarks

Recent involvements in cardiac regeneration field challenged that heart is not terminally differentiated organ and suggested that there exist cardiomyogenesis in adult heart (Bergmann et al. 2009; Kajstura et al. 2010). Up to know, different strategies have been utilized which have ultimate goal of reversing the heart failure trough inducing progression of cell cycle of different cells residing in the heart. Moreover, investigation of cardiomyocyte biology and identification of regulators of cardiomyocyte proliferation revealed many different mechanism including dedifferentiation of pre-existing cardiomyocytes, transdifferentiation of other cells and role of CSCs.

Although reactivation of pre-existing cardiomyocytes proliferation have been intensively researched, the impact of resident CSCs and exogenous stem cells became hot topic research areas in the context of cardiac regeneration. Utilization of exogenous stem cells including ESCs, IPSCs, SMCs and MNCs to repair cardiac injury gave promising results which led later on clinical trials in this field. However, their insufficient transdifferentation capacity into cardiomyocyte or inefficiency of integration to injury area became major obstacles to this end. Moreover, ethical and immunologic rejection issues involving in utilization of IPSCs and ESCs debated their utilization in therapeutic strategies. In the meantime, the effect of CSCs challenged the cardiac regeneration field. Due to complexity of heart organ, different population of those stem cells in different sites of heart have been recently characterized and different progenitors within heart tissue including SP progenitors, Flk1, SSEA, ISL1, Sca1, GCPs and cKit have been reported in successive studies. In different reports, the differential expression of markers led to identify those progenitors and their location in heart suggested to be important to for their function.

In the last decade, many of clinical trials have been initiated to understand the therapeutic role of either CSCs or exogenous stem cells to finalize human cardiac therapies. To this end, ALCADIA, SCIPO and CADUCEUS clinical trials utilized autologous cardiac derived SCs, ckit cells and cardiosphere derived cells, respectively. In ALCADIA clinical trial, administrated Cardiac Derived SCs decreased scar size up to %3.3 and increased LVEF %9–12. Although there was no significant in SCIPIO trial, CADUCEUS resulted with %8–12 decrease in scar size. Moreover, other ongoing clinical studies still in progress and are excitingly followed by researcher in this field.

Heart failure still remains leading cause of death worldwide and recent research in this field revealed the possibility of reversing the heart failure. To this end, the therapeutic role of exogenous stem cells and resident CSCs raised concern over. In future studies, after understanding the biology of resident CSCs and their exact function, they could be used as alternative to heart transplantation. Although many of clinical trials have been resulted with promising results, robust and well designed future studies could be more effective to finalize development of human cardiac therapies trough utilization of those cells.

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References

Anversa P, Kajstura J, Leri A, Bolli R (2006) Life and death of cardiac stem cells: a paradigm shift in cardiac biology. Circulation 113(11):1451–1463

Aslan GS, Mısır DG, Kocabas F (2015) Underlying mechanisms and prospects of heart regeneration. Turk J Biol 40:276. https://doi.org/10.3906/biy-1506-14

Bailey B, Fransioli J, Gude NA, Alvarez R Jr, Zhang X, Gustafsson AB, Sussman MA (2012) Sca-1 knockout impairs myocardial and cardiac progenitor cell function. Circ Res 111(6):750–760

Barile L, Chimenti I, Gaetani R, Forte E, Miraldi F, Frati G, Messina E, Giacomello A (2007) Cardiac stem cells: isolation, expansion and experimental use for myocardial regeneration. Nat Clin Pract Cardiovasc Med 4(Suppl 1):S9–S14

Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P (2007) Human cardiac stem cells. Proc Natl Acad Sci U S A 104(35):14068–14073

Bearzi C, Leri A, Lo Monaco F, Rota M, Gonzalez A, Hosoda T, Pepe M, Qanud K, Ojaimi C, Bardelli S, D'Amario D, D'Alessandro DA, Michler RE, Dimmeler S, Zeiher AM, Urbanek K, Hintze TH, Kajstura J, Anversa P (2009) Identification of a coronary vascular progenitor cell in the human heart. Proc Natl Acad Sci U S A 106(37):15885–15890

Beigi F, Schmeckpeper J, Pow-Anpongkul P, Payne JA, Zhang L, Zhang Z, Huang J, Mirotsou M, Dzau VJ (2013) C3orf58, a novel paracrine protein, stimulates cardiomyocyte cell-cycle progression through the PI3K-AKT-CDK7 pathway. Circ Res 113(4):372–380

Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114(6):763–776

Beqqali A, Kloots J, Ward-van Oostwaard D, Mummery C, Passier R (2006) Genome-wide transcriptional profiling of human embryonic stem cells differentiating to cardiomyocytes. Stem Cells 24 (8):1956–1967

Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisén J (2009) Evidence for cardiomyocyte renewal in humans. Science 324:98–102

Bergmann O, Zdunek S, Frisén J, Bernard S, Druid H, Jovinge S (2012) Cardiomyocyte renewal in humans. Circ Res 110:e17–e18

Bicknell KA, Coxon CH, Brooks G (2004) Forced expression of the cyclin B1–CDC2 complex induces proliferation in adult rat cardiomyocytes. Biochem J 382:411–416

Bishop AE, Buttery LD, Polak JM (2002) Embryonic stem cells. J Pathol 197(4):424–429

Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P (2011) Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 378(9806):1847–1857

Boon RA, Dimmeler S (2015) MicroRNAs in myocardial infarction. Nat Rev Cardiol 12(3):135–142

Boon RA, Jae N, Holdt L, Dimmeler S (2016) Long noncoding RNAs: from clinical genetics to therapeutic targets? J Am Coll Cardiol 67(10):1214–1226

Cagavi E, Bartulos O, Suh CY, Sun B, Yue Z, Jiang Z, Yue L, Qyang Y (2014) Functional cardiomyocytes derived from Isl1 cardiac progenitors via Bmp4 stimulation. PLoS One 9(12):e110752

Cai J, Weiss ML, Rao MS (2004) In search of "stemness". Exp Hematol 32(7):585–598

Campa VM, Gutierrez-Lanza R, Cerignoli F, Diaz-Trelles R, Nelson B, Tsuji T, Barcova M, Jiang W, Mercola M (2008) Notch activates cell cycle reentry and progression in quiescent cardiomyocytes. J Cell Biol 183(1):129–141

Canseco DC, Kimura W, Garg S, Mukherjee S, Bhattacharya S, Abdisalaam S, Das S, Asaithamby A, Mammen PP, Sadek HA (2015) Human ventricular unloading induces cardiomyocyte proliferation. J Am Coll Cardiol 65:892–900

Cheng RK, Asai T, Tang H, Dashoush NH, Kara RJ, Costa KD, Naka Y, Wu EX, Wolgemuth DJ, Chaudhry HW (2007) Cyclin A2 induces cardiac regeneration after myocardial infarction and prevents heart failure. Circ Res 100:1741–1748

Choi SH, Jung SY, Suh W, Baek SH, Kwon SM (2013) Establishment of isolation and expansion protocols for human cardiac C-kit-positive progenitor cells for stem cell therapy. Transplant Proc 45(1):420–426

Chong JJ, Chandrakanthan V, Xaymardan M, Asli NS, Li J, Ahmed I, Heffernan C, Menon MK, Scarlett CJ, Rashidianfar A, Biben C, Zoellner H, Colvin EK, Pimanda JE, Biankin AV, Zhou B, Pu WT, Prall OW, Harvey RP (2011) Adult cardiac-resident MSC-like stem cells with a proepicardial origin. Cell Stem Cell 9(6):527–540

Di Meglio F, Castaldo C, Nurzynska D, Miraglia R, Romano V, Russolillo V, Giuseppina L, Vosa C, Montagnani S (2010a) Localization and origin of cardiac CD117-positive cells: identification of a population of epicardially-derived cells in adult human heart. Ital J Anat Embryol 115(1–2):71–78

Di Meglio F, Castaldo C, Nurzynska D, Romano V, Miraglia R, Bancone C, Langella G, Vosa C, Montagnani S (2010b) Epithelial-mesenchymal transition of epicardial mesothelium is a source of cardiac CD117-positive stem cells in adult human heart. J Mol Cell Cardiol 49(5):719–727

Dixit P, Katare R (2015) Challenges in identifying the best source of stem cells for cardiac regeneration therapy. Stem Cell Res Ther 6(1):26

D'Uva G, Aharonov A, Lauriola M, Kain D, Yahalom-Ronen Y, Carvalho S, Weisinger K, Bassat E, Rajchman D, Yifa O, Lysenko M, Konfino T, Hegesh J, Brenner O, Neeman M, Yarden Y, Leor J, Sarig R, Harvey RP, Tzahor E (2015) ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. Nat Cell Biol 17(5):627–638

Ema M, Takahashi S, Rossant J (2006) Deletion of the selection cassette, but not cis-acting elements, in targeted Flk1-lacZ allele reveals Flk1 expression in multipotent mesodermal progenitors. Blood 107 (1):111–117

Engleka KA, Manderfield LJ, Brust RD, Li L, Cohen A, Dymecki SM, Epstein JA (2012) Islet1 derivatives in the heart are of both neural crest and second heart field origin. Circ Res 110(7):922–926

Fatkhudinov T, Bol'shakova GB, Goldshtein DV, Sukhikh GT (2014) Mechanisms of therapeutic activity of multipotent cells in heart diseases. Bull Exp Biol Med 156(4):535–543

Gai H, Leung EL, Costantino PD, Aguila JR, Nguyen DM, Fink LM, Ward DC, Ma Y (2009) Generation and characterization of functional cardiomyocytes using induced pluripotent stem cells derived from human fibroblasts. Cell Biol Int 33(11):1184–1193

Garbern JC, Lee RT (2013) Cardiac stem cell therapy and the promise of heart regeneration. Cell Stem Cell 12 (6):689–698

Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183(4):1797–1806

Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401(6751):390–394

Hatt CR, Jain AK, Parthasarathy V, Lang A, Raval AN (2013) MRI-3D ultrasound-X-ray image fusion with electromagnetic tracking for transendocardial therapeutic injections: in-vitro validation and in-vivo feasibility. Comput Med Imaging Graph 37(2):162–173

Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, Martin JF (2011) Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science 332:458–461

Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA (2002) The post-natal heart contains a myocardial stem cell population. FEBS Lett 530(1–3):239–243

Hong KU, Guo Y, Li QH, Cao P, Al-Maqtari T, Vajravelu BN, Du J, Book MJ, Zhu X, Nong Y, Bhatnagar A, Bolli R (2014) c-kit+ Cardiac stem cells alleviate post-myocardial infarction left ventricular dysfunction despite poor engraftment and negligible retention in the recipient heart. PLoS One 9(5):e96725

Hosoda T, D'Amario D, Cabral-Da-Silva MC, Zheng H, Padin-Iruegas ME, Ogorek B, Ferreira-Martins J, Yasuzawa-Amano S, Amano K, Ide-Iwata N, Cheng W, Rota M, Urbanek K, Kajstura J, Anversa P, Leri A (2009) Clonality of mouse and human cardiomyogenesis in vivo. Proc Natl Acad Sci U S A 106(40):17169–17174

Iida M, Heike T, Yoshimoto M, Baba S, Doi H, Nakahata T (2005) Identification of cardiac stem cells with FLK1, CD31, and VE-cadherin expression during embryonic stem cell differentiation. FASEB J 19 (3):371–378

Ishitobi H, Wakamatsu A, Liu F, Azami T, Hamada M, Matsumoto K, Kataoka H, Kobayashi M, Choi K, Nishikawa S, Takahashi S, Ema M (2011) Molecular basis for Flk1 expression in hemato-cardiovascular progenitors in the mouse. Development 138 (24):5357–5368

Jackson T, Allard MF, Sreenan CM (1990) The c-myc proto-oncogene regulates cardiac development in transgenic mice. Mol Cell Biol 10(7):3709–3716

Jessup M, Brozena S (2003) Heart failure. N Engl J Med 348(20):2007–2018

Jessup M, Abraham WT, Casey DE, Feldman AM, Francis GS, Ganiats TG, Konstam MA, Mancini DM, Rahko PS, Silver MA, Stevenson LW, Yancy CW (2009) 2009 focused update: ACCF/AHA guidelines for the diagnosis and Management of Heart Failure in adults: a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circ Res 119 (14):1977–1916

Jha AK, Tharp KM, Ye J, Santiago-Ortiz JL, Jackson WM, Stahl A, Schaffer DV, Yeghiazarians Y, Healy KE (2015) Enhanced survival and engraftment of transplanted stem cells using growth factor sequestering hydrogels. Biomaterials 47:1–12

Jung J, Kim TG, Lyons GE, Kim HR, Lee Y (2005) Jumonji regulates cardiomyocyte proliferation via interaction with retinoblastoma protein. J Biol Chem 280(35):30916–30923

Kajstura J, Urbanek K, Perl S, Hosoda T, Zheng H, Ogórek B, Ferreira-Martins J, Goichberg P, Rondon-Clavo C, Sanada F, D'Amario D, Rota M, Del Monte F, Orlic D, Tisdale J, Leri A, Anversa P (2010) Cardiomyogenesis in the adult human heart. Circ Res 107:305–315

Kattman SJ, Huber TL, Keller GM (2006) Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. Dev Cell 11(5):723–732

Kazakov A, Meier T, Werner C, Hall R, Klemmer B, Korbel C, Lammert F, Maack C, Bohm M, Laufs U (2015) C-kit(+) resident cardiac stem cells improve left ventricular fibrosis in pressure overload. Stem Cell Res 15(3):700–711

Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J, Gepstein L (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 108 (3):407–414

Keith MC, Tang XL, Tokita Y, Li QH, Ghafghazi S, Moore Iv J, Hong KU, Elmore B, Amraotkar A, Ganzel BL, Grubb KJ, Flaherty MP, Hunt G, Vajravelu B, Wysoczynski M, Bolli R (2015) Safety of intracoronary infusion of 20 million C-kit positive human cardiac stem cells in pigs. PLoS One 10(4): e0124227

Kelly RG, Buckingham ME (2002) The anterior heart-forming field: voyage to the arterial pole of the heart. Trends Genet 18(4):210–216

Kensah G, Roa Lara A, Dahlmann J, Zweigerdt R, Schwanke K, Hegermann J, Skvorc D, Gawol A, Azizian A, Wagner S, Maier LS, Krause A, Drager G, Ochs M, Haverich A, Gruh I, Martin U (2013) Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. Eur Heart J 34(15):1134–1146

Kocabas F, Mahmoud AI, Sosic D, Porrello ER, Chen R, Garcia JA, DeBerardinis RJ, Sadek HA (2012) The hypoxic epicardial and subepicardial microenvironment. J Cardiovasc Transl Res 5(5):654–665

Kocabas F, Mahmoud AI, Muralidhar SA, Kimura W, Koura AS, Thet S, Porrello ER, Sadek HA (2013) Meis1 regulates postnatal cardiomyocyte cell cycle arrest. Nature 497(7448):249–253

Kubin T, Pöling J, Kostin S, Gajawada P, Hein S, Rees W, Wietelmann A, Tanaka M, Lörchner H, Schimanski S, Szibor M, Warnecke H, Braun T (2011) Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling. Cell Stem Cell 9:420

Kühn B, Monte F d, Hajjar RJ, Chang Y-S, Lebeche D, Arab S, Keating MT (2007) Periostin induces proliferation of differentiated cardiomyocytes and promotes cardiac repair. Nat Med 13:962–969

Larrivee B, Olive PL, Karsan A (2006) Tissue distribution of endothelial cells in vivo following intravenous injection. Exp Hematol 34(12):1741–1745

Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, Chien KR (2005) Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 433(7026):647–653

Lee JH, Lee JB, Shapovalova Z, Fiebig-Comyn A, Mitchell RR, Laronde S, Szabo E, Benoit YD, Bhatia M (2014) Somatic transcriptome priming gates lineage-specific differentiation potential of human-induced pluripotent stem cell states. Nat Commun 5:5605

Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S (2003) Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. Proc Natl Acad Sci U S A 100 (13):7808–7811

Leri A, Kajstura J, Anversa P (2011) Role of cardiac stem cells in cardiac pathophysiology: a paradigm shift in human myocardial biology. Circ Res 109(8):941–961

Leri A, Rota M, Hosoda T, Goichberg P, Anversa P (2014) Cardiac stem cell niches. Stem Cell Res 13 (3 Pt B):631–646

Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, Mohabeer MK, Rao V, Ivanov J (1996) Cardiomyocyte transplantation improves heart function. Ann Thorac Surg 62(3):654–660. discussion 660-651

Liang SX, Tan TY, Gaudry L, Chong B (2010) Differentiation and migration of Sca1+/CD31- cardiac side population cells in a murine myocardial ischemic model. Int J Cardiol 138(1):40–49

Limana F, Zacheo A, Mocini D, Mangoni A, Borsellino G, Diamantini A, De Mori R, Battistini L, Vigna E, Santini M, Loiaconi V, Pompilio G, Germani A, Capogrossi MC (2007) Identification of myocardial and vascular precursor cells in human and mouse epicardium. Circ Res 101(12):1255–1265

Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Bohm M, Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, Anversa P (2005) Stem cells in the dog heart are self-

renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc Natl Acad Sci U S A 102(25):8966–8971

Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, Olson EN (2008) microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 22(23):3242–3254

Maher TJ, Ren Y, Li Q, Braunlin E, Garry MG, Sorrentino BP, Martin CM (2014) ATP-binding cassette transporter Abcg2 lineage contributes to the cardiac vasculature after oxidative stress. Am J Physiol Heart Circ Physiol 306(12):H1610–H1618

Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E (2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 379 (9819):895–904

Marelli D, Desrosiers C, el-Alfy M, Kao RL, Chiu RC (1992) Cell transplantation for myocardial repair: an experimental approach. Cell Transplant 1(6):383–390

Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, Komuro I (2004) Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. J Biol Chem 279(12):11384–11391

Menasche P (2004) Embryonic stem cells pace the heart. Nat Biotechnol 22(10):1237–1238

Menasche P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, Vilquin JT, Marolleau JP, Seymour B, Larghero J, Lake S, Chatellier G, Solomon S, Desnos M, Hagege AA (2008) The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 117 (9):1189–1200

Menasche P, Vanneaux V, Hagege A, Bel A, Cholley B, Cacciapuoti I, Parouchev A, Benhamouda N, Tachdjian G, Tosca L, Trouvin JH, Fabreguettes JR, Bellamy V, Guillemain R, Suberbielle Boissel C, Tartour E, Desnos M, Larghero J (2015) Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. Eur Heart J 36(30):2011–2017

Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL (1997) Identification of a lineage of multipotent hematopoietic progenitors. Development 124(10):1929–1939

Motoike T, Markham DW, Rossant J, Sato TN (2003) Evidence for novel fate of Flk1+ progenitor: contribution to muscle lineage. Genesis 35(3):153–159

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB, American Heart Association Statistics Committee and Stroke Statistics Subcommittee (2015) Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation 131(4):e29–322

Murry CE, Wiseman RW, Schwartz SM, Hauschka SD (1996) Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest 98:2512–2523

Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ (2004) Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428(6983):664–668

Mushtaq M, DiFede DL, Golpanian S, Khan A, Gomes SA, Mendizabal A, Heldman AW, Hare JM (2014) Rationale and design of the percutaneous stem cell injection delivery effects on Neomyogenesis in dilated cardiomyopathy (the POSEIDON-DCM study): a phase I/II, randomized pilot study of the comparative safety and efficacy of transendocardial injection of autologous mesenchymal stem cell vs. allogeneic mesenchymal stem cells in patients with non-ischemic dilated cardiomyopathy. J Cardiovasc Transl Res 7(9):769–780

Novoyatleva T, Diehl F, Amerongen MJ v, Patra C, Ferrazzi F, Bellazzi R, Engel FB (2010) TWEAK is a positive regulator of cardiomyocyte proliferation. Cardiovasc Res 85:681

Oberpriller JO, Oberpriller JC (1974) Response of the adult newt ventricle to injury. J Exp Zool A Ecol Genet Physiol 187(2):249–259

Oettgen P, Boyle AJ, Schulman SP, Hare JM (2006) Cardiac stem cell therapy. Need for optimization of efficacy and safety monitoring. Circulation 114 (4):353–358

Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A 100 (21):12313–12318

O'Meara CC, Wamstad JA, Gladstone RA, Fomovsky GM, Butty VL, Shrikumar A, Gannon JB, Boyer LA, Lee RT (2015) Transcriptional reversion of cardiac myocyte fate during mammalian cardiac regeneration. Circ Res 116(5):804–815

Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P (2001a) Transplanted adult bone marrow cells repair myocardial infarcts in mice. Ann N Y Acad Sci 938:221–230

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001b) Bone marrow

cells regenerate infarcted myocardium. Nature 410 (6829):701–705

Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P (2003) Bone marrow stem cells regenerate infarcted myocardium. Pediatr Transplant 7(Suppl 3):86–88

Ott HC, Matthiesen TS, Brechtken J, Grindle S, Goh SK, Nelson W, Taylor DA (2007) The adult human heart as a source for stem cells: repair strategies with embryonic-like progenitor cells. Nat Clin Pract Cardiovasc Med 4(Suppl 1):S27–S39

Pasumarthi KBS, Nakajima H, Nakajima HO, Soonpaa MH, Field LJ (2005) Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. Circ Res 96:110–118

Perin EC, Silva GV, Henry TD, Cabreira-Hansen MG, Moore WH, Coulter SA, Herlihy JP, Fernandes MR, Cheong BY, Flamm SD, Traverse JH, Zheng Y, Smith D, Shaw S, Westbrook L, Olson R, Patel D, Gahremanpour A, Canales J, Vaughn WK, Willerson JT (2011) A randomized study of transendocardial injection of autologous bone marrow mononuclear cells and cell function analysis in ischemic heart failure (FOCUS-HF). Am Heart J 161(6):1078–1087. e1073

Pfister O, Mouquet F, Jain M, Summer R, Helmes M, Fine A, Colucci WS, Liao R (2005) CD31- but not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circ Res 97(1):52–61

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147

Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA (2011) Transient regenerative potential of the neonatal mouse heart. Science 331:1078–1080

Poss KD, Wilson LG, Keating MT (2002) Heart regeneration in zebrafish. Science 298:2188–2190

Reinecke H, Bogdanski J, Woltering A, Breithardt G, Assmann G, Kerber S, Eckardstein A v (2002a) Relation of serum levels of sex hormone binding globulin to coronary heart disease in postmenopausal women. Am J Cardiol 90(4):364–368

Reinecke H, Poppa V, Murry CE (2002b) Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. J Mol Cell Cardiol 34(2):241–249

Rochais F, Sturny R, Chao CM (2014) FGF10 promotes regional foetal cardiomyocyte proliferation and adult cardiomyocyte cell-cycle re-entry. Cardiovasc Res 104:432–442

Rumyantsev PP (1966) Autoradiographic study on the synthesis of DNA, RNA, and proteins in normal cardiac muscle cells and those changed by experimental injury. Folia Histochem Cytochem 4:397–424

Rumyantsev PP (1973) Post-injury DNA synthesis, mitosis and ultrastructural reorganization of adult frog cardiac myocytes. An electron microscopic-autoradiographic study. Z Zellforsch Mik Ana 139:431–450

Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK (2013) Mammalian heart renewal by pre-existing cardiomyocytes. Nature 493(7432):433–436

Smart N, Bollini S, Dube KN, Vieira JM, Zhou B, Davidson S, Yellon D, Riegler J, Price AN, Lythgoe MF, Pu WT, Riley PR (2011) De novo cardiomyocytes from within the activated adult heart after injury. Nature 474(7353):640–644

Sullivan KE, Burns LJ, Black LD 3rd (2015) An in vitro model for the assessment of stem cell fate following implantation within the infarct microenvironment identifies ISL-1 expression as the strongest predictor of c-kit(+) cardiac progenitor cells' therapeutic potential. J Mol Cell Cardiol 88:91–100

Suresh R, Chiriac A, Goel K, Villarraga HR, Lopez-Jimenez F, Thomas RJ, Terzic A, Nelson TJ, Perez-Terzic C (2013) CXCR4+ and FLK-1+ identify circulating cells associated with improved cardiac function in patients following myocardial infarction. J Cardiovasc Transl Res 6(5):787–797

Suzuki K, Murtuza B, Suzuki N, Smolenski RT, Yacoub MH (2001) Intracoronary infusion of skeletal myoblasts improves cardiac function in doxorubicin-induced heart failure. Circulation 104(12 Suppl 1): I213–I217

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676

Tateishi K, Ashihara E, Takehara N, Nomura T, Honsho S, Nakagami T, Morikawa S, Takahashi T, Ueyama T, Matsubara H, Oh H (2007) Clonally amplified cardiac stem cells are regulated by Sca-1 signaling for efficient cardiovascular regeneration. J Cell Sci 120(Pt 10):1791–1800

Tillmanns J, Rota M, Hosoda T, Misao Y, Esposito G, Gonzalez A, Vitale S, Parolin C, Yasuzawa-Amano S, Muraski J, De Angelis A, Lecapitaine N, Siggins RW, Loredo M, Bearzi C, Bolli R, Urbanek K, Leri A, Kajstura J, Anversa P (2008) Formation of large coronary arteries by cardiac progenitor cells. Proc Natl Acad Sci U S A 105(5):1668–1673

Tseliou E, de Couto G, Terrovitis J, Sun B, Weixin L, Marban L, Marban E (2014) Angiogenesis, cardiomyocyte proliferation and anti-fibrotic effects underlie structural preservation post-infarction by intramyocardially-injected cardiospheres. PLoS One 9(2):e88590

Turan RD, Aslan GS, Yucel D, Doger R, Kocabas F (2016) Evolving approaches to heart regeneration by therapeutic stimulation of resident cardiomyocyte cell cycle. Anatol J Cardiol 16(11):881–886

Uchida S, De Gaspari P, Kostin S, Jenniches K, Kilic A, Izumiya Y, Shiojima I, Grosse Kreymborg K, Renz H, Walsh K, Braun T (2013) Sca1-derived cells are a source of myocardial renewal in the murine adult heart. Stem Cell Reports 1(5):397–410

Unno K, Jain M, Liao R (2012) Cardiac side population cells: moving toward the center stage in cardiac regeneration. Circ Res 110(10):1355–1363

Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, Anversa P (2005) Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. Circ Res 97(7):663–673

Vrtovec B, Poglajen G, Lezaic L, Sever M, Socan A, Domanovic D, Cernelc P, Torre-Amione G, Haddad F, Wu JC (2013) Comparison of transendocardial and intracoronary CD34+ cell transplantation in patients with nonischemic dilated cardiomyopathy. Circulation 128(11 Suppl 1):S42–S49

Weinberger F, Mehrkens D, Friedrich FW, Stubbendorff M, Hua X, Muller JC, Schrepfer S, Evans SM, Carrier L, Eschenhagen T (2012) Localization of Islet-1-positive cells in the healthy and infarcted adult murine heart. Circ Res 110 (10):1303–1310

Witman N, Murtuza B, Davis B, Arner A, Morrison JI (2011) Recapitulation of developmental cardiogenesis governs the morphological and functional regeneration of adult newt hearts following injury. Dev Biol 354:67–76

Wu SM, Chien KR, Mummery C (2008) Origins and fates of cardiovascular progenitor cells. Cell 132 (4):537–543

Yacoub MH, Terrovitis J (2013) CADUCEUS, SCIPIO, ALCADIA: cell therapy trials using cardiac-derived cells for patients with post myocardial infarction LV dysfunction, still evolving. Glob Cardiol Sci Pract 2013(1):5–8

Yamashita JK, Takano M, Hiraoka-Kanie M, Shimazu C, Peishi Y, Yanagi K, Nakano A, Inoue E, Kita F, Nishikawa S (2005) Prospective identification of cardiac progenitors by a novel single cell-based cardiomyocyte induction. FASEB J 19(11):1534–1536

Yu SP, Wei Z, Wei L (2013) Preconditioning strategy in stem cell transplantation therapy. Transl Stroke Res 4 (1):76–88

Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ (2009) Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 104(4):e30–e41

Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J, Jiang D, Gise A v, Ikeda S, Chien KR, Pu WT (2008) Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature 454(7200):109–113

Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Scholer HR, Duan L, Ding S (2009) Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4(5):381–384

Zimmet H, Porapakkham P, Porapakkham P, Sata Y, Haas SJ, Itescu S, Forbes A, Krum H (2012) Short- and long-term outcomes of intracoronary and endogenously mobilized bone marrow stem cells in the treatment of ST-segment elevation myocardial infarction: a meta-analysis of randomized control trials. Eur J Heart Fail 14(1):91–105

Zwi L, Caspi O, Arbel G, Huber I, Gepstein A, Park IH, Gepstein L (2009) Cardiomyocyte differentiation of human induced pluripotent stem cells. Circulation 120 (15):1513–1523

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The Potency of Induced Pluripotent Stem Cells in Cartilage Regeneration and Osteoarthritis Treatment

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Abstract

Osteoarthritis (OA) is the most common chronic disabling condition effecting the elderly, significantly impacting an individual patient's quality of life. Current treatment options for OA are focused on pain management and slowing degradation of cartilage. Some modern surgical techniques aimed at encouraging regeneration at defect sites have met with limited long-term success. Mesenchymal stem cells (MSCs) have been viewed recently as a potential tool in OA repair due to their chondrogenic capacity. Several studies have shown success with regards to reducing patient's OA-related pain and discomfort but have been less successful in inducing chondrocyte regeneration. The heterogeneity of MSCs and their limited proliferation capacity also raises issues when developing an off-the-shelf treatment for OA. Induced pluripotent stem cell (iPSC) technology, which allows for the easy production of cells capable of prolonged self-renewal and producing any somatic cell type, may overcome those limitations. Patient derived iPSCs can also be used to gain new insight into heredity-related OA. Efforts to generate chondrocytes from iPSCs through embryoid bodies or mesenchymal intermediate stages have struggled to produce with optimal functional characteristics. However, iPSCs potential to produce cells for future OA therapies has been supported by iPSC-derived teratomas, which have shown an ability to produce functional, stable articular cartilage. Other iPSCs-chondrogenic protocols are also improving by incorporating tissue engineering techniques to better mimic developmental conditions.

Keywords

Osteoarthritis · Articular cartilage · Regenerative medicine · Stem cells · iPSC · Differentiation · Chondrocytes

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Abbreviations

AC	articular cartilage
ACT	autologous cartilage transplantation
BMP	Bone Morphogenetic Protein
ECM	extracellular matrix
ESC	Embryonic Stem Cell
FOCD	Familial osteochondritis dissecans
HALPN1	Hyaluronan and proteoglycan link protein 1
ICM	Inner Cell Mass
IGF-1	Insulin-Like Growth Factor 1
iMPC	intermediate Mesenchymal Progenitor Cell
iPSC	induced Pluripotent Stem Cell
KLF4	gut-enriched Krüppel-like factor
MSC	Mesenchymal Stem Cell
OA	Osteoarthritis
Oct-4	octamer-binding transcription factor 4
SOX	Sry-related HMG box
SRY	Sex-Determining Region Y-Box
TGF-β	Transforming Growth Factor-beta
WHO	Word Health Organisation
WNT4	WNT Family Member 4

1 Introduction

Articular cartilage is essential for the pain-free and easy movement of diarthrodial joints, as it provides shock absorbance and lubrication. Unfortunately, once formed, articular cartilage has a very low capacity for self-repair in part due to its lack of vascularisation. Damage to the cartilage from excess mechanical stress can begin a progressive degradation that can lead into a condition known as osteoarthritis (OA), the most common chronic disabling condition effecting adults in later life (Loeser 2011). This condition begins with a sense of stiffness (crepitus) in the affected joints those progresses on to pain and a reduction in function and significantly impact a sufferer's quality of life. The areas most commonly affected are the highly-used joints in the hand and load bearing joints of the hips and knees (Neogi 2013). While there can be some genetic predisposition for OA, obesity and old age (Lawrence et al. 2007) can play a large role in the development of the condition due to extra strain being but on the major joints and changes in the aging cartilage matrix respectively. A Dutch population study show signs of OA in over 60% of those over 60 and found similar rates when they compared them to other populations worldwide (Saase et al. 1989). The impact of OA is set to rise further wit increasingly aging populations and rising obesity levels across the developed world. Projections in the US estimate that by 2030 over 20% of the population will have arthritis with OA being by far the most prevalent form (Murphy and Helmick 2012).

The WHO has placed OA as one of its top ten most disabling diseases in developed countries (Neogi 2013) with loss of work days and productivity due to OA valued at 10 billion dollars a year in the US (Muchmore et al. 2003) as well as placing heavy economic burdens on health care systems. Currently treatment option for OA are limited and focused on pain management, slowing degradation and reducing inflammation. Artificial joint replacement surgery for knees and hips make up much of the medical costs linked to OA. These implants are effective at returning mobility to a patient with severe OA and have very low failure rates up to 10 years after implantation (Herberts and Malchau 2000). However, this condition, so predominantly associated with older adults, has been occurring at increasing rates in people below the age of 65, providing challenge for the current strategies, where patients may be faced with managing their painful condition for decades and progressive bone loss around the site of the prosthesis is a significant concern for long-term cases. For these reasons, new therapies focused on joint repair and preservation will be important to maintain pain free mobility in into old age for the many adults. Interest has been growing in stem cell cell-based regenerative techniques as a potential source of these innovative therapies.

2 The Articular Cartilage

2.1 Overview

Diarthrodial joints are formed from a highly specialized connective tissue, the articular cartilage (AC). The principal function of AC is to supply a lubricated and smooth surface for articulation and to facilitate the transmission of loads with a low frictional coefficient. It is created by a specialised cell type, the chondrocytes, which are able to produce its exceptional collagenous extracellular matrix (ECM) composed mainly from proteoglycans, collagen, water and non-collagenous proteins and glycoproteins. AC is a hyaline-type cartilage, which differs from the other two cartilage types, the elastic and fibrocartilage in relative amounts of collagen and proteoglycan. One specialty of the AC tissue is that it does not contain blood vessels or nerves, therefore, the nutrition happens through diffusion which is powered by a fluid flow (synovial fluid) generated by the joint movements (compression or flexion). This is one reason behind the very slow turnover of its extracellular matrix and the fact that AC does not able to repair. To understand the chronic conditions that affects AC and their potential treatment possibilities, first, we have to review its development and maintenance.

2.2 Articular Cartilage Development

Chondrocyte formation begins following the condensations with the dynamic expression of cartilage specific genes such as collagen type II, type IX and type XI and Aggrecan under the regulation of SOX transcription factors, SOX9 (SRY-box 9) being the so-called master regulator of chondrogenesis (Wehrli et al. 2003), while the cells proliferate and secrete a cartilage matrix (Iwamoto et al. 2007). Early in endochondral bone development the cells in the cartilage template at the site of a future synovial joint are directly connected to each other. The separation of the long bones at the joint site begin when the chondrocytes at the site become more densely packed and form an area known as an interzone. During interzone formation the chondrocytes show a downregulation in SOX9 and collagen type II (Ito and Kida 2000). The interzone is made up of three layers: two chondrogenic, perichondrium-like layers and one intermediate layer of densely packed cells. Shortly after the formation of the interzone an apoptosis induced cavitation will occur at the site of the future synovial joint within the dense intermediate interzone, with some cells from this region going on to form the synovial tissues of the joint (Caldwell and Wang 2015). The two outer interzone layer are incorporated into the epiphysis of the cartilage growth plate to contribute to the postnatal growth of the long bone through proliferation and hypertrophy. The remaining cells form the intermediate interzone layer and are not included in this process and will separate from the epiphyseal growth plate cartilage to form a layer of chondrocytes expressing collagen type X and assembling matrices of vesicles and proteoglycans to promote the formation of the permanent articular cartilage found in the mature joint. Biochemical signals from transcription factors like WNT family member 4 (WNT4), Catenin B1 (CTNNB1) and transforming growth factor beta (TGF-β) promote the development and maintenance of articular cartilage (Hill et al. 2005). TGF-β is of particular importance to keep articular cartilage in its proper state, as seen in transgenic mice with defective TGF-β receptors where articular cartilage is replaced by hypertrophic cartilage and bone (Spagnoli et al. 2007). However, theses biochemical signals are not the only ones to have an impact on the development of articular cartilage as there is also evidence to suggest that mechanical stimulation during development may play an important role in the development of the future joint.

As the pre-chondrogenic cells are differentiating at the sites of the future bones, progenitors of muscles and tendons are also being defined (Rodríguez et al. 1988). This forming muscle mass begins contracting at the same time as the cartilaginous template is taking shape. This connection is hinted at by the severe bone and cartilage malformations seen in children born with the

congenital neuro-muscular disorders (Amthor et al. 1998). Experiments with chemically paralysed chick embryos (Nowlan et al. 2010) and mutant mice with muscle-less limbs have shown that a lack of mechanical stimulation can result in serious failures in interzone development resulting in fused joints with no synovial cavity and a lack of articular cartilage.

2.3 Articular Cartilage Maintenance

Many of these factors that play important roles in the development of articular cartilage, such as Bone Morphogenic Protein (BMP) and TGF-β, are also essential for maintaining it's healthy permanent state in adulthood. The homeostasis required for this maintenance can be disrupted by excessive damage to the cartilage and can result in the over expression of catabolic factors beginning the tissue degradation seen in OA (Fukui et al. 2001). Chondrocytes from osteoarthritic articular cartilage have also been seen to express early and late stage differentiation markers (Pfander et al. 2001) suggesting it has taken on a transient form that could differentiate into undesired forms of cartilage or calcify resulting in greater wear on the joint. Genetic variations in the strength of receptor signalling for genes related to the development and maintenance of articular cartilage are thought to be an important risk factor for the development of OA. These complex processes that develop and maintain articular cartilage also contribute to the difficulties and limitations faced by current treatments aiming to regenerate the cartilage damaged in OA.

3 Current Regeneration Based Treatments and Their Limitations

3.1 Overview

Non-surgical treatment possibilities are considered in the early stages of OA, however, their effect on the restoration of the normal tissue function has not been demonstrated convincingly (Browne and Branch 2000). Surgical methods such as arthroscopy, subchondral drilling, abrasion arthroplasty, microfracture, autologous chondrocyte implantation (ACI) or its second generation version the matrix-assisted autologous chondrocyte implantation (MACI) aim to restore the damaged cartilage itself. However, these technologies have limitations as well, mainly the formation of fibrocartilage, which is not as effective as hyaline cartilage in AC to respond frictional, compressive, shear and tensile loading. Below, we will concentrate on microfracture and ACI techniques and review their applications and major limitations.

3.2 Microfracture

Microfracture surgery arose from investigations into surgical bone marrow stimulation in the late 80s and early 90s (Freitag et al. 2016). The technique involves the drilling of small holes into the subchondral bone plate at the site where the cartilage has diminished. The aim is to allow blood and bone marrow to seep out of these fractures as with the hopes that the mesenchymal stem cells also known as mesenchymal stromal cells (MSCs) (Dominici et al. 2006) contained within the bone marrow will differentiate and form new healthy cartilage. The procedure itself is quite short and the recovery time is much less than that of joint replacement surgery, as such it has become a very popular treatment option in the world of sports medicine. However, the cartilage formed by these released MSCs will most often take the form of fibrocartilage which has a different biochemical make up from articular, also called hyaline, cartilage (Freitag et al. 2016). Fibrocartilage contains both collagen I and collagen II and form white fibrous tissues unlike articular cartilage which contains only collagen II and has a smooth, glass like appearance (Pearle et al. 2005). This difference in composition means that the biomechanical properties of fibrocartilage are less suited to the mechanical forces placed on cartilage in the joints and the new cartilage is effectively mechanically

inferior. In addition, the microfractures in the subchondral bone can result in the formation of lesions. Efforts have been made to refine the procedure and reduce the fracture size, but long-term studies (Freitag et al. 2016) have found that the relief the procedure provides is reversed 5 years after surgery regardless of fracture size. The changes to the subchondral bone surface also increase the failure rate of a more recently developed regenerative therapy, autologous cartilage transplantation (ACT) up to seven-times if applied after microfracture.

3.3 Autologous Cartilage Transplantation

The ACT procedure involves taking a biopsy of a patient's own cartilage from a non-loadbearing site on the joint. These cells are then cultured *in vitro* to expand a population of a patient's own chondrocytes with the aim to implant these healthy cells into the damaged area of the joint, where they are covered with a membrane and sutured in place. Unlike microfracture, pre-clinical and clinical trial of ACT show the formation of new hyaline-like cartilage in the joints and a study following 61 patients found the clinical outcomes rate good to excellent for 83% of the group after 5 years (Browne et al. 2005). Unfortunately, ACT is not without drawbacks of its own. Two separate operations are required with time between needed for the expansion of the chondrocytes, increasing a patient's recovery time, and the harvesting of the cartilage is an invasive and painful procedure and can cause damage to the donor site. The low cell number in native cartilage tissue and the limited amount of suitable non-loadbearing donor tissue restricts the number of cells that can be produced for implantation. The most reported cause of failure for ACTs is the hypertrophy of the membrane or periosteal flap used to secure the implanted cells (Peterson et al. 2010). A number of artificial and porcine based (Makris et al. 2015) membranes have been tried to correct this but they can cause an immune response negating the key advantage of using autologous cells in the first place. Studies have also shown that up to 40% of ACTs show signs of cartilage "dedifferentiation", with the autologous cartilage turning into fibrocartilage (Caplan and Kader 2013). This could be due to changes the cells undergo when they are being cultured *ex vivo* or the failure of these cells to properly integrate into the normal extracellular matrix of cartilage.

Both microfracture surgery and ACT are effective treatments that have been shown temporarily restore normal function to patients with cartilage damage and delay the need for drastic joint replacement surgery. However, both procedures are more suited for treating isolated defects in cartilage and not the more generalized degradation seen in OA. Nevertheless, recent efforts in generating functional chondrocytes from pluripotent and multipotent stem cells may bypass some of the short-comings of current regenerative treatment and shed new insight into the pathology of OA.

4 Using Stem Cells in Cartilage Replacement

4.1 Overview

Interest has been growing in the use of stem cell technologies to both offer new methods for studying the mechanisms of OA and new treatments offering more effective and longer-term solutions than the options currently available to patients.

4.2 Stem Cell Types Available for Cartilage Replacement

The chondrogenic capabilities of adult MSCs have been extensively investigated for the last decade as a possible source of replacement cartilage. MSCs can be easily harvested in large numbers from several sources including a patient's bone marrow and adipose tissue (Kern et al. 2006), avoiding the potential damage that can be done to the patient's existing cartilage inherent in current cell therapies.

Another cell source would be pluripotent stem cells. Embryonic stem cells (ESCs) derived from the Inner Cell Mass (ICM) of the blastocyst are

pluripotent, having the ability to form tissues from any of the three germ layers, and have also been used to produce hyaline cartilage *in vitro* (Diekman et al. 2012). Unlike MSCs, ESCs have the ability to self-renew, making them a potentially unlimited replacement cartilage (Koch et al. 2009). However, the ethical issues related to the derivation of ESCs from preimplantation embryos limits their clinical applications. Induced pluripotent stem cells (iPSCs) derived from adult somatic cells using a combination of reprogramming factors offer an alternative source of self-renewing pluripotent cells that avoid these ethical issues. Since its development by Yamanka (Takahashi et al. 2007) iPSC technology has garnered massive attention in the field of regenerative medicine with ambitions to develop new therapies with a patient patent specific pluripotent cells. While the reprogramming factors used to produce the first iPSCs, Oct3/4, Klf4, Sox2 and c-Myc (also called as 'OSKM factors') caused some concern for future clinical use due to the oncogenic nature of c-Myc and Klf4 but more recently it has been found that they can be replaced with Nanog and Lin28 (Shi et al. 2016). New methods for introducing these reprogramming factors to the cells including non-integrating viral vectors, such as a Sendai and the development of several non-viral methods using microRNA, synthetic messenger RNA and proteins have also increased the safety of iPSC derived cells. Early animal studies have been promising with iPSCs derived cardiomyocytes, able to repair cardiac defects in a porcine model (Shiba et al. 2012). Safety trials of iPSC derived cells in humans are already underway (Trounson and DeWitt 2016).

In addition to the potential for iPSCs to form replacement tissues, the ability to generate pluripotent cell from a patient's own tissue has opened up new avenues in personalised medicine and the modelling of genetic diseases. Two studies published in 2017 have found altered expressions of genes associated with some forms of OA and phenotypic differences in MSCs and osteoblast generated from iPSCs derived from patients with disorder related to bone growth. Esseltine et al. (2017) generated iPSCs from a patient with the developmental disorder oculodentodigital dysplasia, linked to a Connexin-mutation that commonly results in malformations of the facial bones. Connexin is a gap junction protein and has been shown to both be upregulated in cells at the joints during OA and to enhance the expression of several other OA-related genes (Gupta et al. 2014). This study found that connexion had a reduced expression in the patient-derived iPSCs when compared to healthy control iPSCs. The patient-derived iPSCs also showed delayed osteogenic differentiation. The osteoblast generated showed reduced levels of connexin which could negatively impact their future maturation and mineralization. Layh-Schmitt et al. (2016) produced iPSCs from patients with axial spondyloarthritis, a genetic disease that results in abhorrent bone formation at the joints and spine. They found that MSCs derived from these iPSCs shown elevated expression of number of genes related to bone formation. Mutations in one of these genes, HAPLN1 has been associated with spinal osteophyte formation in OA. In both studies iPSC derived from patient cells gave fresh insights into mechanisms of their rare conditions while also showing how mutations in genes related to OA can be successfully modelled by iPSCs. Similar studies that established *in vitro* disease models from patient derived iPSCs, such as a recent study (Cao et al. 2016) of patients with inherited erythromelalgia, have been able to use these models to test an array of drug compounds for their effectiveness in correcting or reducing the phenotypic expression of the disease mutation in these cells. Some small trials have gone on to show drugs that had been found effective on these patient-derived iPSC disease models *in vitro*, to in turn be effective at alleviating the related diseases symptoms when given those patients (Cao et al. 2016). This potential to provide models for diseases which currently lack representative animal models and creating an easily expandable population of cells that show a diseases phenotype to test the efficacy of arrays of drug compounds represents major advantages iPSCs have over MSCs and other stem cells. However, in order to realise this potential for

OA researcher must show that iPSCs can be differentiated into functional chondrocytes.

4.3 Producing Cartilage from Mesenchymal Stem Cells

Several *in vitro* techniques have been investigated to induce the differentiation of chondrocytes from MSCs with TGF-β1 and Insulin-Like Growth Factor 1 (IGF-1) commonly used together to stimulate chondrogenesis (Longobardi et al. 2005). Some other compounds including dexamethasone and BMP-7 have been found to assist in directing the cells down a chondrogenic lineage.

A number of therapies for OA based on introducing MSCs to sites of damaged cartilage have gone through preclinical and clinical trials in recent years. Some trials used a technique similar to ACT, transplanting a cellular scaffold containing MSCs instead of ACs to the site of damage (Grigolo et al. 2009). While this technique has shown some success in repairing cartilage defects in both the preclinical models and human patients, a direct comparison study showed that there was no significant difference in clinical outcome between MSC scaffold transplantation and ACT (Nejadnik et al. 2010) including the risk of non-hyaline cartilage formation. Another MSC based therapy for OA currently under investigation is the injection of MSCs into the inter-articulated region. One advantage of this approach is its potential to affect the entire joint, rather than just the site of a specific defect, making it better suited for treating OA which causes a diffuse degradation of cartilage across the joint. There are currently a number of active and recruiting phase I/II clinical trials testing the safety and efficacy of MSC-based therapies for knee OA.

The sources of MSCs most commonly used in these therapies are autologous adipose (https://clinicaltrials.gov/ct2/ MSC) MSCs or allogenic MSCs obtained from umbilical cord blood (https://clinicaltrials.gov/ct2/ UCB). In most of these cases the MSCs are applied trough an intra-articular injection either in a single dose or in 2–3 doses over a 6-months period. This single or repeated dose strategy may impact the products safety outcomes depending on the source of MSCs used as a 2017 equine model study (Joswig et al. 2017) suggested that repeated intra-articular injection of allogeneic MSCs causes an adverse response compared to autologous MSCs. Recently two phase I/II trial reports have been published for therapies using autologous MSCs and a product using allogenic cell intra-articularly to treat OA. Soler et al. (2016), expanded autologous bone marrow derived MSCs *ex vivo* and infused them in a single dose for 15 patients. They found a few patients experienced some discomfort which diminished 8 days after injection. Twelve month follow ups showed improvements in bodily pain and function and magnetic resonance T2 mapping indicated signs of cartilage regeneration. Stempeucel®, an allogenic, pooled MSC product, was administered to 60 OA patients (Gupta et al. 2016) in a single dose. While no adverse effect was observed over a 12-months period, reports of pain reduction by patients was not statically significant when compared to the placebo group.

A number of other clinical trials of MSC injections for OA have shown that a majority of patients get some pain relief following the injections (Centeno et al. 2011). However, the evidence for disease modification or cartilage regeneration resulting from this technique are inconsistent and unclear (Freitag et al. 2016). Many of the trials are unblinded and have small numbers of patients with some concerns being raised about potential bias in a number of trials. Additionally, other trials for a number of conditions have shown paracrine secretions of MSCs to have immunomodulatory and anti-inflammatory properties (Aggarwal and Pittenger 2005) and these may be responsible for the pain relief the OA patients received.

While these trials show promise in terms of slowing OA related degradation and improving patients' quality of life, MSCs have a number of drawbacks that limit their capacity as a source of

cartilage for regeneration or repair. While MSCs can be obtained from many source tissues, there is a lot of heterogeneity in the differentiation capabilities of these various stem cell populations. Bone marrow derived MSCs are commonly used to produce chondrocytes however, chondrogenic differentiation in these cells normally follows an endochondral pathway, producing transient cartilage not suitable to replace articular cartilage (Pelttari et al. 2006). Additionally, primary autologous MSCs obtained from any adult tissue have a limited proliferation capacity, limiting the amount of replacement chondrocytes that they can produce. The heterogeneity even within a population of MSCs from the same tissue means that not all of the primary cells will be capable of chondrocyte differentiation (Russell et al. 2010). This heterogeneity among adult MSC populations, may contribute to variable clinical outcomes when using autologous MSCs for cartilage repair. These limitations have caused some to look to induced pluripotent stem cells (iPSCs) made from patient derived tissue to possible provide both large numbers of autologous cells with powerful chondrogenic capabilities and provide new ways to study the molecular and genetic aspects of OA.

4.4 Chondrogenesis from iPSCs

While there is currently no generally accepted efficient protocol for differentiation chondrocytes from iPSC (Lietman 2016) (Fig. 1), the methods that have been commonly tried produce some to the conditions of cartilage development with most using one of three main approaches, (i) the induction of MSC-like iPSCs and the differentiation of these cells into chondrocytes using the growth factors used in normal bone marrow MSC *in vitro* chondrogenesis (Nejadnik et al. 2015), (ii) the co-culture of iPSCs derived MSCs with primary chondrocytes or other feeder layer cells (Qu et al. 2013) or (iii) the culturing of embryoid bodies (EB) from iPSCs, followed by the differentiation of the mesodermal cells in the EBs into chondrocytes by treatment with growth factors (Nakagawa et al. 2009). All of these techniques have had some success producing chondrocyte cells but have had some limitations, often producing very heterogeneous populations of cells, very few of which were able to generate healthy hyaline cartilage.

To discuss the issues involved in these methods we must first look at how comparable MSCs derived from iPSCs are to bone marrow derived MSCs. Diederichs and Tuan (2014) performed side-by-side genomic and functional comparisons of adult bone marrow derived MSCs and MSCs generated from human iPSCs. The iPSCs themselves were also derived from bone marrow MSCs from the same donors and the MSCs were differentiated from them were generated using several different methods including MSC growth factors, EBs and co-culture with primary MSCs. The comparative analyses showed distinct transcriptomic and functional differences between bone marrow and iPSC derived MSCs. The iPSC derived MSCs were generally found to be less responsive to chondrogenic differentiation protocols commonly used on MSCs. Diederich and colleagues recently investigated further (Diederichs et al. 2016) the chondrogenic discrepancies between iPSCs and bone marrow derived MSCs, focusing on the regulation of SOX9 in the cell, due to this protein's essential nature in cartilage development. When intermediate mesenchymal progenitor cells (iMPCs) were generated from the iPSCs SOX9 was induced and reached varying protein levels compared to bone marrow MSCs cultured under the same conditions. The iMPCs also produced less robust cartilage compared to the MSCs, though iMPCs with high levels of SOX9 produced better cartilage than those with low levels. SOX9 levels in the iMPCs were actually downregulated by the standard TGF-β based protocol for MSC chondrogenesis though this effect could be mitigated somewhat by a co-treatment of BMP-4. These results seem to indicate that there are some underlying differences between MSCs and iPSC-derived iMPCs, maybe an epigenetic memory retained from the iPSCs tissue of origin that impacts their chondrogenic potential. It is also known that differences exist

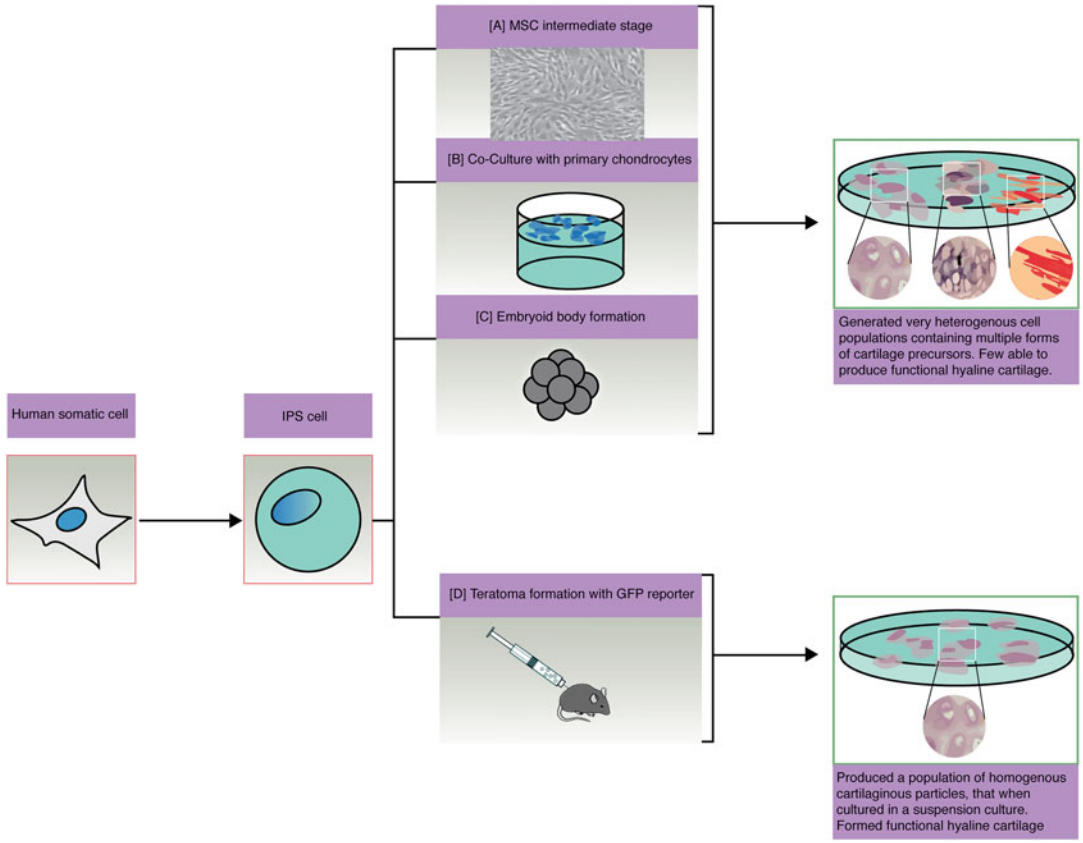

Fig. 1 A Summary of recent attempts to generate cartilage from IPSCs. Induced pluripotent stem cells (iPSCs) can be generated from any somatic cell via induction with the Yamanaka factors. The great potential of iPSCs lies in the capacity to differentiate into any cell type if they are subjected to the right conditions A number of methods have recently been attempted to generate stable homogenous cartilage from iPSC: (A) the induction of an MSC-like intermediate stage and the differentiation of these cells into chondrocytes using the growth factors used in normal bone marrow MSC in vitro chondrogenesis, (B) Co-culturing of iPSC-derived MSCs with primary chondrocytes or other feeder layer cells to promote chondrogenic differentiation or (C) culturing aggregates of iPSCs to form embryoid bodies (EB), encouraging a spontaneous differentiation toward the three germ layers followed by the differentiation of the mesodermal cells in the EBs into chondrocytes by treatment with growth factors. While all of these techniques have had some success producing chondrocyte cells but have had some limitations, often producing very heterogeneous populations of cells, very few of which were able to generate healthy hyaline cartilage. However, stable homogenous hyaline cartilage has been produced, (D) by forming in teratomas in immunodeficient mice using a line of hiPSC that expressed GFP in cartilage. This expression was used to purify a population of homogenous cartilaginous particles from the teratoma tissues, that formed hyaline cartilage tissue when cultured in a scaffold-free suspension (Figure was created by Roxana Mobasheri based on the author's original concept)

between iPSC lines from various donors, which can affect the outcome of differentiation experiments.

However, these limitations in current differentiation strategies do not mean that iPSCs are incapable of producing functional chondrocytes from patient derived cells. A key piece of evidence for early iPSCs' pluripotent capability was their ability to form teratomas with tissues from all three germ layers when implanted *in vivo* (Shi et al. 2016). Yamashita et al. (2015) produced scaffold-less hyaline cartilaginous tissue from human iPSCs, by generating a line of hiPSC that expressed GFP in cartilage

when it formed in teratomas in immunodeficient mice. They then used this expression to purify a population of homogenous cartilaginous particles from the teratoma tissues, culturing them in a scaffold-free suspension culture. These cartilaginous particles formed hyaline cartilage when implanted subcutaneously in immunodeficient mice and integrated with the native cartilage transplanted to the site of joint defects in mice. A similar method using teratoma formation to derive cartilage tissue has also been used to model genetic cartilage conditions. Xu et al. (2016) produced iPSCs from skin fibroblasts taken from patients with the inherited skeletal defect, familial osteochondritis dissecans (FOCD) which is characterised by the development of large cartilage lesions in multiple joints and early onset of severe OA. Xu injected these patient-derived iPSCs subcutaneously in immunodeficient mice and harvested teratoma tissue after 2–3 months, using Safranin-O staining to identify cartilage tissues. This teratoma derived cartilage tissue displayed irregularities that could helped explain why these patients are so susceptible to cartilage damage. The ECM around the cells was largely depleted and cells were densely packed indicating poor matrix formation. Finally, large amounts of aggrecan accumulated within the endoplasmic reticulum of the differentiated chondrocytes together with a marked absence of aggrecan in the ECM, a site it would normally be found in abundance and play a crucial role in the ECM's structural integrity.

Teratoma formation in an immunodeficient animals is not a suitable method for producing large number of cells to be used for replacement and regenerative therapies for OA patients due to several issues such as risks related to transplant animal grown or transgenic tissues into patients, the long timeframes needed to produce the final cartilage product, and the expenses and ethical issues involved with raising large numbers of animals in which to generate the teratoma. However, these experiments have shown that human iPSCs can fulfil their potential of growing hyaline cartilage that can integrate with a joint and modelling genetic diseases that can contribute to OA development.

5 Concluding Remarks

While current efforts to produce iPSC-derived cartilage that can be used to benefit OA patients *in vitro* have some ways to go, the functional cartilage produced from human iPSCs in teratomas suggests that success lies in the right combination of environmental factors. As our understanding of the developmental process necessary for chondrogenesis and the development of specialised articular cartilage grows we can develop new strategies to better replicate those processes *in vitro*. Efforts are made to replicate the mechanical stimulation that play such an important role in the cartilage and interzone development in cultured cells culture. Mechanical micro-bioreactors have recently developed to exert compressive pressure and shear stress on MSC during chondrogenesis and have produced stable cartilage with good biomechanical properties (Halvaei et al. 2016). The frequency and intensity of the mechanical stimulation applied by these bioreactors can easily be modified to test chondrogenesis in MSC and iPSC derived cells using a range of conditions best matching the natural development of specialised types of cartilage. And while iPSC still have a long way to go before they can be used for therapy, step are being made with the lessons learned from the previous attempts to produce iPSC-derived chondrocytes and surgically repair cartilage. Diederichs et al. (2016) suggested screening iPSC colonies for SOX9 expression to start the refinement of the chondrogenic process and recent papers have already been doing this to produce osteochondrogenic-progenitor from iPSCs (Wang et al. 2017). Nguyen et al. (2017) have co-cultured iPSCs into a 3D–bioprinted scaffold alongside irradiated primary chondrocytes and produced some cartilaginous-like tissue in a system that can be easily place at a joint defect site much like ACT. Finally, in an interesting twist, a very recent paper

has suggested another role iMSCs can play in the treatment of OA. As discussed earlier in this review the paracrine secretions of MSCs to have immunomodulatory and anti-inflammatory properties and may provide effective relief from the pain and discomfort caused by OA. Zhu et al. (2017) have found that exosomes taken from the paracrine secretions of iMSCs had a superior therapeutic effect on a mouse OA model when injected intra-articularly than those taken from adult MSCs derived from the synovial membrane.

In summary, while more work still needs to be done to establish the standard, reliable, reproducible method of chondrocyte production from iPSCs for therapeutic applications, the success of deriving hyaline-cartilage by the iPSC-teratoma method and continuously improving iPSC-chondrogenic protocols show the attainable promise of this technology and its potential to meet the needs of the growing numbers of OA patients around the world.

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References

Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105(4):1815–1822. https://doi.org/10.1182/blood-2004-04-1559

Amthor H, Christ B, Weil M, Patel K (1998) The importance of timing differentiation during limb muscle development. Curr Biol 8(11):642–652. https://doi.org/10.1016/s0960-9822(98)70251-9

Browne JE, Branch TP (2000) Surgical alternatives for treatment of articular cartilage lesions. J Am Acad Orthop Surg 8(3):180–189. https://doi.org/10.5435/00124635-200005000-00005

Browne JE, Anderson AF, Arciero R, Mandelbaum B, Moseley JB, Micheli LJ, Lyle J, Fu F, Erggelet C (2005) Clinical outcome of autologous chondrocyte implantation at 5 years in US subjects. Clin Orthop Relat Res 436:237–245

Caldwell K, Wang J (2015) Cell-based articular cartilage repair: the link between development and regeneration. Osteoarthr Cartil 23(3):351–362. https://doi.org/10.1016/j.joca.2014.11.004

Cao L, McDonnell A, Nitzsche A, Alexandrou A, Saintot PP, Loucif AJC, Brown AR, Young G, Mis M, Randall A, Waxman SG, Stanley P, Kirby S, Tarabar S, Gutteridge A, Butt R, McKernan RM, Whiting P, Ali Z, Bilsland J, Stevens EB (2016)

Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. Sci Transl Med 8(335). https://doi.org/10.1126/scitranslmed.aad76533

Caplan N, Kader DF (2013) Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. In: Classic papers in orthopaedics. Springer, London, pp 165–168. https://doi.org/10.1007/978-1-4471-5451-8_40

Centeno CJ, Schultz JR, Cheever M, Freeman M, Faulkner S, Robinson B, Hanson R (2011) Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr Stem Cell Res Ther 6(4):368–378. https://doi.org/10.2174/157488811797904371

Clinical Study of Umbilical Cord Tissue Mesenchymal Stem Cells (UC-MSC) for Treatment of Osteoarthritis – Full Text View – ClinicalTrials.gov. (n.d.) https://clinicaltrials.gov/ct2/show/NCT02237846?term=Mesenchymal+Stem+Cell+osteoarthritis&recr=Active%2C+not+recruiting&rank=1. Accessed 10 May 2017

Diederichs S, Tuan RS (2014) Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor. Stem Cells Dev 23 (14):1594–1610. https://doi.org/10.1089/scd.2013.0477

Diederichs S, Gabler J, Autenrieth J, Kynast KL, Merle C, Walles H, Utikal J, Richter W (2016) Differential regulation of SOX9 protein during Chondrogenesis of induced pluripotent stem cells versus mesenchymal stromal cells: a shortcoming for cartilage formation. Stem Cells Dev 25(8):598–609. https://doi.org/10.1089/scd.2015.0312

Diekman BO, Christoforou N, Willard VP, Sun H, Sanchez-Adams J, Leong KW, Guilak F (2012) Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. Proc Natl Acad Sci 109(47):19172–19177. https://doi.org/10.1073/pnas.1210422109

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317. https://doi.org/10.1080/14653240600855905

Esseltine JL, Shao Q, Brooks C, Sampson J, Betts DH, Séguin CA, Laird DW (2017) Connexin43 mutant patient-derived induced pluripotent stem cells exhibit altered differentiation potential. J Bone Miner Res. https://doi.org/10.1002/jbmr.3098

Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, Tenen A (2016) Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy – a review. BMC Musculoskelet Disord 17(1). https://doi.org/10.1186/s12891-016-1085-9

Fukui N, Purple CR, Sandell LJ (2001) Cell biology of osteoarthritis: the chondrocyte's response to injury. Curr Rheumatol Rep 3(6):496–505. https://doi.org/10.1007/s11926-001-0064-8

Grigolo B, Lisignoli G, Desando G, Cavallo C, Marconi E, Tschon M, Tschon M, Giavaresi G, Fini M, Giardino R, Facchini A (2009) Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. Tissue Eng Part C Methods 15(4):647–658. https://doi.org/10.1089/ten.TEC.2008.0569

Gupta A, Niger C, Buo AM, Eidelman ER, Chen RJ, Stains JP (2014) Connexin43 enhances the expression of osteoarthritis-associated genes in synovial fibroblasts in culture. BMC Musculoskelet Disord 15 (1). https://doi.org/10.1186/1471-2474-15-425

Gupta PK, Chullikana A, Rengasamy M, Shetty N, Pandey V, Agarwal V, Majumdar AS (2016) Efficacy and safety of adult human bone marrow-derived, cultured, pooled, allogeneic mesenchymal stromal cells (Stempeucel®): preclinical and clinical trial in osteoarthritis of the knee joint. Arthritis Res Ther 18 (1). https://doi.org/10.1186/s13075-016-1195-7

Halvaei M, Abolfathi N, Shokrgozar M, Eskandari M, Haghighipour N, Navaee F (2016) A new mechanical micro-bioreactor for cartilage tissue. Tissue Eng 22 (Suppl 1):94–95. doi:EPFL-CONF-224956

Herberts P, Malchau H (2000) Long-term registration has improved the quality of hip replacement: a review of the Swedish THR register comparing 160,000 cases. Acta Orthop Scand 71(2):111–121. https://doi.org/10.1080/0001

Hill TP, Später D, Taketo MM, Birchmeier W, Hartmann C (2005) Canonical Wnt/β-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8(5):727–738. https://doi.org/10.1016/j.devcel.2005.02.013

Ito MM, Kida MY (2000) Morphological and biochemical re-evaluation of the process of cavitation in the rat knee joint: cellular and cell strata alterations in the interzone. J Anat 197(4):659–679. https://doi.org/10.1046/j.1469-7580.2000.19740659

Iwamoto M, Tamamura Y, Koyama E, Komori T, Takeshita N, Williams JA, Pacifici M (2007) Transcription factor ERG and joint and articular cartilage formation during mouse limb and spine skeletogenesis. Dev Biol 305(1):40–51. https://doi.org/10.1016/j.ydbio.2007.01.037

Joswig A, Mitchell A, Cummings KJ, Levine GJ, Gregory CA, Smith R, Watts AE (2017) Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. Stem Cell Res Ther 8 (1). https://doi.org/10.1186/s13287-017-0503-8

Kern S, Eichler H, Stoeve J, Klüter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24(5):1294–1301. https://doi.org/10.1634/stemcells.2005-0342

Koch P, Opitz T, Steinbeck JA, Ladewig J, Brustle O (2009) A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc Natl Acad Sci 106(9):3225–3230. https://doi.org/10.1073/pnas.0808387106

Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA (2007) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: part II. Arthritis Rheum 58(1):26–35. https://doi.org/10.1002/art.23176

Layh-Schmitt G, Lu S, Navid F, Brooks SR, Lazowick E, Davis K, Montag C, Gadina M, Colbert RA (2016) Generation and differentiation of induced pluripotent stem cells reveal ankylosing spondylitis risk gene expression in bone progenitors. Clin Rheumatol 36 (1):143–154. https://doi.org/10.1007/s10067-016-3469-5

Lietman SA (2016) Induced pluripotent stem cells in cartilage repair. World J Orthop 7(3):149. https://doi.org/10.5312/wjo.v7.i3.149

Loeser RF (2011) Aging and osteoarthritis. Curr Opin Rheumatol 23(5):492–496. https://doi.org/10.1097/BOR.0b013e3283494005

Longobardi L, O'Rear L, Aakula S, Johnstone B, Shimer K, Chytil A, Horton WA, Moses HL, Spagnoli A (2005) Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-β signaling. J Bone Miner Res 21 (4):626–636. https://doi.org/10.1359/jbmr.051213

Makris EA, Gomoll AH, Malizos KN, JC H, Athanasiou KA (2015) Repair and tissue engineering techniques for articular cartilage. Nat Rev Rheumatol 11 (1):21–34. https://doi.org/10.1038/nrrheum.2014.157

Muchmore L, Lynch WD, Gardner HH, Williamson T, Burke T (2003) Prevalence of arthritis and associated joint disorders in an employed population and the associated healthcare, sick leave, disability, and workers' compensation benefits cost and productivity loss for employers. J Occup Environ Med 45 (4):369–378. https://doi.org/10.1097/01.jom.0000063621.37065.26

Murphy L, Helmick CG (2012) The impact of osteoarthritis in the United States. Orthop Nurs 31(2):85–91. https://doi.org/10.1097/nor.0b013e31824fcd42

Nakagawa T, Lee SY, Reddi AH (2009) Induction of chondrogenesis from human embryonic stem cells without embryoid body formation by bone morphogenetic protein 7 and transforming growth factor β1. Arthritis Rheum 60(12):3686–3692. https://doi.org/10.1002/art.27229

Nejadnik H, Hui JH, Choong EP, Tai B, Lee EH (2010) Autologous bone marrow–derived mesenchymal stem cells versus autologous chondrocyte implantation. Am J Sports Med 38(6):1110–1116. https://doi.org/10.1177/0363546509359067

Nejadnik H, Diecke S, Lenkov OD, Chapelin F, Donig J, Tong X, Derugin N, Chan RC, Gaur A, Yang F, JC W, Daldrup-Link HE (2015) Improved approach for chondrogenic differentiation of human induced pluripotent stem cells. Stem Cell Rev Rep 11(2):242–253. https://doi.org/10.1007/s12015-014-9581-5

Neogi T (2013) The epidemiology and impact of pain in osteoarthritis. Osteoarthr Cartil 21(9):1145–1153. https://doi.org/10.1016/j.joca.2013.03.018

Nguyen D, Hägg DA, Forsman A, Ekholm J, Nimkingratana P, Brantsing C, Kalogeropoulos T, Zaunz S, Concaro S, Brittberg M, Lindahl A, Gatenholm P, Enejder A, Simonsson S (2017) Cartilage tissue engineering by the 3D bioprinting of iPS cells in a nanocellulose/alginate bioink. Sci Rep 7(1). https://doi.org/10.1038/s41598-017-00690-y

Nowlan NC, Bourdon C, Dumas G, Tajbakhsh S, Prendergast PJ, Murphy P (2010) Developing bones are differentially affected by compromised skeletal muscle formation. Bone 46(5):1275–1285. https://doi.org/10.1016/j.bone.2009.11.026

Pearle AD, Warren RF, Rodeo SA (2005) Basic science of articular cartilage and osteoarthritis. Clin Sports Med 24(1):1–12. https://doi.org/10.1016/j.csm.2004.08.007

Pelttari K, Winter A, Steck E, Goetzke K, Hennig T, Ochs BG, Ainger T, Richter W (2006) Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. Arthritis Rheum 54 (10):3254–3266. https://doi.org/10.1002/art.22136

Peterson L, Vasiliadis HS, Brittberg M, Lindahl A (2010) Autologous chondrocyte implantation a long-term follow-up. Am J Sports Med 38(6):1117–1124. https://doi.org/10.1177/0363546509357915

Pfander D, Swoboda B, Kirsch T (2001) Expression of early and late differentiation markers (proliferating cell nuclear antigen, syndecan-3, annexin VI, and alkaline phosphatase) by human osteoarthritic chondrocytes. Am J Pathol 159(5):1777–1783. https://doi.org/10.1016/s0002-9440(10)63024-6

Qu C, Puttonen KA, Lindeberg H, Ruponen M, Hovatta O, Koistinaho J, Lammi MJ (2013) Chondrogenic differentiation of human pluripotent stem cells in chondrocyte co-culture. Int J Biochem Cell Biol 45(8):1802–1812. https://doi.org/10.1016/j.biocel.2013.05.029

Rodríguez JI, Palacios J, García-Alix A, Pastor I, Paniagua R (1988) Effects of immobilization on fetal bone development. A morphometric study in newborns with congenital neuromuscular diseases with intrauterine onset. Calcif Tissue Int 43 (6):335–339. https://doi.org/10.1007/bf02553275

Russell KC, Phinney DG, Lacey MR, Barrilleaux BL, Meyertholen KE, Oconnor KC (2010) In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells 28(4):788–798. https://doi.org/10.1002/stem.312

Saase JL, Romunde LK, Cats A, Vandenbroucke JP, Valkenburg HA (1989) Epidemiology of osteoarthritis: Zoetermeer survey. Comparison of radiological osteoarthritis in a Dutch population with that in 10 other populations. Ann Rheum Dis 48 (4):271–280. https://doi.org/10.1136/ard.48.4.271

Shi Y, Inoue H, Wu JC, Yamanaka S (2016) Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov 16(2):115–130. https://doi.org/10.1038/nrd.2016.245

Shiba Y, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H, Van Biber B, Dardas T, Mignone JL, Izawa A, Hanna R, Viswanathan M, Gold JD, Kotlikoff MI, Sarvazyan N, Kay MW, Murry CE, Laflamme MA (2012) Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature 489(7415):322–325. https://doi.org/10.1038/nature11317

Soler R, Orozco L, Munar A, Huguet M, López R, Vives J, Garcia-Lopez J (2016) Final results of a phase I–II trial using ex vivo expanded autologous mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. Knee 23(4):647–654. https://doi.org/10.1016/j.knee.2015.08.013

Spagnoli A, O'Rear L, Chandler RL, Granero-Molto F, Mortlock DP, Gorska AE, Weis JA, Longobardi L, Chytil A, Shimer K, Moses HL (2007) TGF-β signaling is essential for joint morphogenesis. J Cell Biol 177(6):1105–1117. https://doi.org/10.1083/jcb.200611031

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872. https://doi.org/10.1016/j.cell.2007.11.019

Trounson A, DeWitt ND (2016) Pluripotent stem cells progressing to the clinic. Nat Rev Mol Cell Biol 17 (3):194–200. https://doi.org/10.1038/nrm.2016.10

Use of Adipose Tissue Derived Mesenchymal Stem Cells for Knee Osteoarthrosis – Full Text View-ClinicalTrials.gov. (n.d.) https://clinicaltrials.gov/ct2/show/NCT02966951term=Mesenchymal+Stem+Cell+osteoarthritis recr=Open rank=2. Accessed 10 May 2017

Wang Y, Wu M, Cheung MP, Sham MH, Akiyama H, Chan D, Cheah KS, Cheung M (2017) Reprogramming of dermal fibroblasts into osteochondrogenic cells with elevated osteogenic potency by defined transcription factors. Stem Cell Rep 8 (6):1587–1599. https://doi.org/10.1016/j.stemcr.2017.04.018

Wehrli BM, Huang W, De Crombrugghe B, Ayala AG, Czerniak B (2003) Sox9, a master regulator of chondrogenesis, distinguishes mesenchymal chondrosarcoma from other small blue round cell tumors. Hum Pathol, 34(3):263–269. http://dx.doi.org/10.1053/hupa.2003.41

Xu M, Stattin E, Shaw G, Heinegård D, Sullivan G, Wilmut I, Colman A, Önnerfjord P, Khabut A, Aspberg A, Dockery P, Hardingham T, Murphy M, Barry F (2016) Chondrocytes derived from mesenchymal stromal cells and induced pluripotent cells of patients with familial osteochondritis dissecans exhibit an endoplasmic reticulum stress response and defective matrix assembly. Stem Cells Transl Med 5 (9):1171–1181. https://doi.org/10.5966/sctm.2015-0384

Yamashita A, Morioka M, Yahara Y, Okada M, Kobayashi T, Kuriyama S, Matsuda S, Tsumaki N (2015) Generation of scaffoldless hyaline cartilaginous tissue from human iPSCs. Stem Cell Rep 4 (3):404–418. https://doi.org/10.1016/j.stemcr.2015.01.016

Zhu Y, Wang Y, Zhao B, Niu X, Hu B, Li Q, Zhang J, Ding J, Chen Y, Wang Y (2017) Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. Stem Cell Res Ther 8(1). https://doi.org/10.1186/s13287-017-0510-9

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Pericytes: The Role of Multipotent Stem Cells in Vascular Maintenance and Regenerative Medicine

Toka A. Ahmed and Nagwa El-Badri

Abstract

Blood vessels consist of an inner endothelial cell layer lining the vessel wall and perivascular pericytes, also known as mural cells, which envelop the vascular tube surface. Pericytes have recently been recognized for their central role in blood vessel formation. Pericytes are multipotent cells that are heterogeneous in their origin, function, morphology and surface markers. Similar to other types of stem cells, pericytes act as a repair system in response to injury by maintaining the structural integrity of blood vessels. Several studies have shown that blood vessels lacking pericytes become hyperdilated and haemorrhagic, leading to vascular complications ranging from diabetic retinopathy to embryonic death. The role of pericytes is not restricted to the formation and development of the vasculature: they have been shown to possess stem cell-like characteristics and may differentiate into cell types from different lineages. Recent discoveries regarding the contribution of pericytes to tumour metastasis and the maintenance of tumour vascular supply and angiogenesis have led researchers to propose targeting pericytes with anti-angiogenic therapies. In this review, we will examine the different physiological roles of pericytes, their differentiation potential, and how they interact with surrounding cells to ensure the integrity of blood vessel formation and maintenance.

Keywords

Blood vessel integrity · Endothelial cells · MSCs · Pericytes · Vascular mural cells

1 Introduction

Pericytes were described more than 100 years ago as perivascular cells that wrap around blood capillaries. Pericytes are also known as mural cells because of their location within the blood vessel and as Rouget cells after their discoverer, Charles Rouget (Hirschi and D'Amore 1996). Furthermore, pericytes are named for the organ in which they reside. In the liver, they are known as Ito cells or hepatic stellate cells (HSCs); in the kidney, they are known as mesangial cells; and in the bone marrow, they are called adventitial reticular cells (Hirschi and D'Amore 1996; da Silva Meirelles et al. 2008). The periendothelial location of pericytes frequently overlaps with the periendothelial locations of vascular smooth muscle cells (vSMCs), macrophages, fibroblasts and epithelial cells. Although it is well known that pericytes belong to the same lineage as

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vSMCs, no specific molecular markers are currently able to distinguish pericytes from vSMCs or other mesenchymal cells. Commonly expressed markers are also neither stable nor specific in their expression (Armulik et al. 2005). It is difficult to determine the relationship between pericytes and the microvascular basement membrane (BM) at the embryonic level and during angiogenesis. Under pathological conditions and during formation or turnover of the BM, it is particularly problematic to distinguish between pericytes and other perivascular mesenchymal cells.

The BM separates the majority of the pericyte-endothelial interface, although both cell types come in contact at certain points via micro-holes in the BM. The size and number of pericyte-endothelial contacts vary between tissues, but approximately 1000 contacts have been identified for a single endothelial cell. The cells may make contact via peg-socket junctions, in which pericyte cytoplasmic projections (pegs) are inserted into endothelial invaginations (pockets). Another contact mechanism is adhesion plaques, which occur between microfilament bundles attached at the pericyte plasma membrane and electron-dense material in the corresponding endothelial cytoplasm (Gerhardt et al. 2000; Gerhardt and Betsholtz 2003). Adhesion plaques, as the name suggests, function to facilitate pericyte adherence to endothelial cells, while peg-and-socket contacts allow the diffusion of molecules and ions between the cytoplasm of the two cell types (Gerhardt and Betsholtz 2003). Adhesion plaque contacts include fibronectin deposits, while peg-and-socket contacts are secured via tight, gap and adherence junctions containing N-cadherin and β-catenin (Armulik et al. 2005).

2 Pericyte Morphology and Ultrastructure

Pericytes are fibroblast-like cells with distinguishable nuclei, low cytoplasmic content, and several long processes surrounding the endothelial wall. Mature pericytes are embedded within the BM of microvessels, which are formed by pericytes and endothelial cells (EC). Pericytes located on the outer surface of blood capillaries interact with underlying endothelial cells and are covered in the same BM (Mandarino et al. 1993). Pericyte processes are usually connected with more than one endothelial cell via adhesion plaques in addition to peg-and-socket contacts, which permit direct contact between the two cell types (Sims 1991; Larson et al. 1987; Rucker et al. 2000; Bergers and Song 2005). This feature, which was first identified by transmission electron microscopy, differentiates primary and secondary pericyte processes (Mandarino et al. 1993; Sims 1986).

Based on their location in the blood vessels, pericytes are characterized as pre-capillary, mid/true-capillary and post-capillary pericytes (Nehls and Drenckhahn 1991). Mid-capillary pericytes are distinguished from the other two types by a lack of α-smooth muscle actin within the cell and by their elongated and spindle-like shape. Pre- and post-capillary pericytes are shorter, more stellate in shape and have varying amounts of α-smooth muscle actin (Shepro and Morel 1993).

The ratio of ECs to pericytes varies according to the tissue of derivation. The vasculatures of the central nervous system (CNS) and the retina have the highest ratio of ECs to pericytes: 1:1 and 3:1, respectively. A lower ratio of 1:10 has been reported in the skin and lungs, and the ratio drops significantly to as low as 1:100 in striated muscles (Armulik et al. 2011). This variation in number is believed to be related to the function of the tissue in which the vessels reside. For example, as the blood pressure within the vessel increases, the number of pericytes also increases. This may explain the presence of more pericytes in larger central vessels and fewer pericytes in smaller peripheral vessels (Sims 2000; Risau et al. 1990; Dıaz-Flores et al. 2009)

3 The Origin of Pericytes

During embryogenesis, blood vessel formation and development occurs via the following two

mechanisms: vasculogenesis, which is *in vivo* vessel formation, and angiogenesis, which is the formation of new blood vessels from pre-existing vessels. After birth, vascularization is determined and maintained by angiogenesis. During vasculogenesis, pericytes originate either from mesenchymal stem cells (MSCs) residing in the abluminal side of the endothelial tube (mesodermal origin) or from the neural crest (Bergwerff et al. 1998; Creazzo et al. 1998; Yamashita et al. 2000; Etchevers et al. 2002; Ema et al. 2003; Korn et al. 2002). The vascular mural cells in coelomic organs (such as liver, gut and lungs) originate from the mesothelium (Wilm et al. 2005; Asahina et al. 2011; Que et al. 2008; Cai et al. 2008; Dettman et al. 1998; Mikawa and Gourdie 1996; Wessels and Perez-Pomares 2004; Zhou et al. 2008). A concept map determined that mesothelial cells undergo EMT (epithelial to mesenchymal transition) and migrate to the organs to produce different mesenchymal components, such as vSMCs and pericytes. Some studies have proposed that pericytes may also arise directly from endothelial cells and the bone marrow (DeRuiter et al. 1997; Rajantie et al. 2004).

Reports on common progenitors during vasculogenesis show that mesodermal $FLK1^{+}$ angioblasts may give rise to ECs or pericytes depending on stimulation with either vascular endothelial growth factor (VEGF) or platelet derived growth factor-β (PDGF-β), respectively (Yamashita et al. 2000; Carmeliet et al. 2001). In contrast, lineage tracking studies have reported that vSMCs do not share a common developmental origin with pericytes, although they express the same markers and have a similar morphology throughout the vasculature (Majesky 2007; Majesky et al. 2011). Despite myriad studies demonstrating the embryonic origin and development of pericytes in different organs, very little is currently known about how pericytes grow and distribute along the vasculature.

4 Regulation of Pericyte Development and Interaction with Endothelial Cells

Analysis of the anatomic relationship between pericytes and endothelial cells shows that they interact closely via juxtacrine or paracrine signalling (Gaengel et al. 2009). Possible pathways for endothelial-pericyte signalling and the role of different intercellular signalling pathways in vascular stability and development include the pathways described below.

5 PDGF-β/PDGFR-β

PDGF-β is a dimeric protein that exists in three isoforms, PDGF-AA, PDGF-BB and PDGFAB, and has alpha and beta receptor subunits (Hart et al. 1988). The subunits are expressed by angiogenic endothelial cells and recruit developing pericytes by binding to PDGFR-β (Fig. 1). The

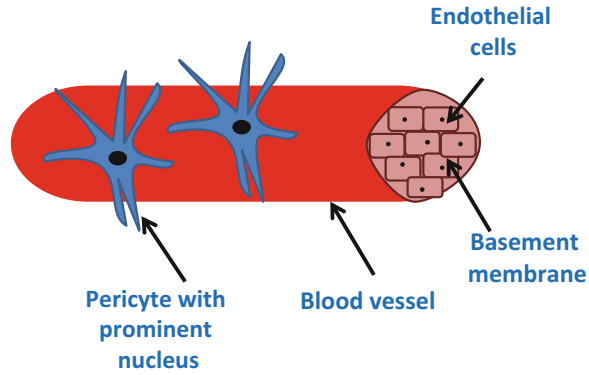

Fig. 1 Diagram showing longitudinal section of blood vessel wrapped with pericytes

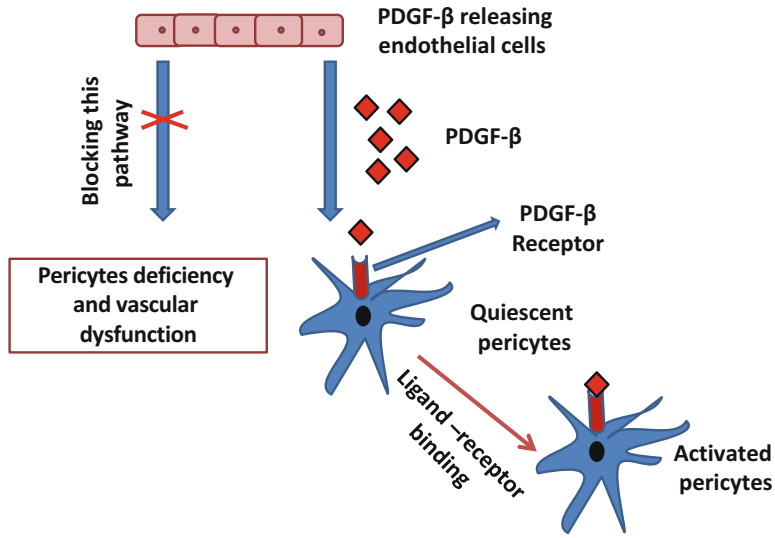

Fig. 2 Diagram showing the role of PDGFR- β/PDGF-β signaling pathway in pericyte-endothelial interaction

recruitment of pericytes to their periendothelial location is controlled via different signalling pathways. Although PDGF-β/PDGFR-β (platelet derived growth factor receptor-ß) is one of the most important signalling pathways that control pericyte development, it is not present in all organs. For example, pericyte development in the gut, heart and lungs is regulated by the PDGF-β/PDGFR-β pathway, whereas the development of liver pericytes (HSCs) is PDGF-β/PDGFR-β-independent (Hellstrom et al. 1999). Likewise, the development of pericytes derived from the neural crest is regulated by the PDGF-β/PDGFRβ pathway in the CNS (Lindahl et al. 1997) but not in the thymus (Foster et al. 2008) (Fig. 2).

PDGFRβ is also expressed by vSMCs in venous and arterial vessels and plays an important role in their development and differentiation. Blocking the *pdgfb* or *pdgfrb* genes in mice causes mural cell deficiency, resulting in vascular dysfunction and perinatal lethality (Leveen et al. 1994; Soriano 1994). The extent of pericyte deficiency is highly variable according to the organ in which they reside, suggesting that mural cell recruitment may be enhanced by other signalling pathways and have a role similar to that of PDGFβ/PDGFRβ. For example, while knocking out the *pdgfb* or *pdgfrb* genes in mouse embryos results in a lack of pericytes in the brain, lungs, kidneys, heart, and skin, hepatic pericytes (stellate cells) are unaffected (Hellstrom et al. 1999; Lindahl et al. 1997) (Fig. 3).

PDGFβ mRNA is more highly expressed in developing arteries than in corresponding veins that run parallel to the thickness of the mural cell coat. Although most studies have demonstrated PDGFβ/PDGFRβ signalling during embryonic development, there is also evidence that the PDGF-β/PDGFRβ signalling pathway has a postnatal role. Activation of PDGFRβ via targeted mutation insertion in the *pdgfrb* locus in mice enhances mural cell proliferation but inhibits differentiation (Olson and Soriano 2011), leading to postnatal phenotypes. The role of PDGFβ is not restricted to mural cell recruitment but extends to blood vessel maturation under pathological conditions, such as in patients with hereditary haemorrhagic telangiectasia as well as in mouse models of this disease (Lebrin et al. 2010).

6 TGFβ Pathway

The TGFβ (transforming growth factor beta) signalling pathway plays an important role in the induction of undifferentiated progenitor cells into PDGFR-ß-positive pericyte precursors, which are

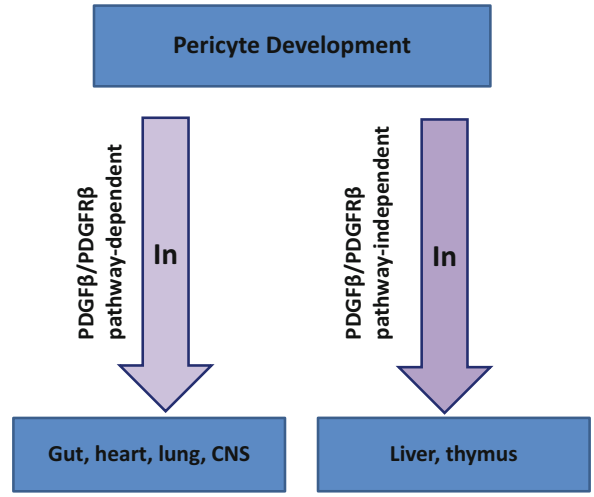

Fig. 3 Signaling pathways of pericyte development in various organs, pericyte development is PDGF-β dependent in the gut, heart, lung and central nervous system, and PDGF-β independent in the liver and thymus

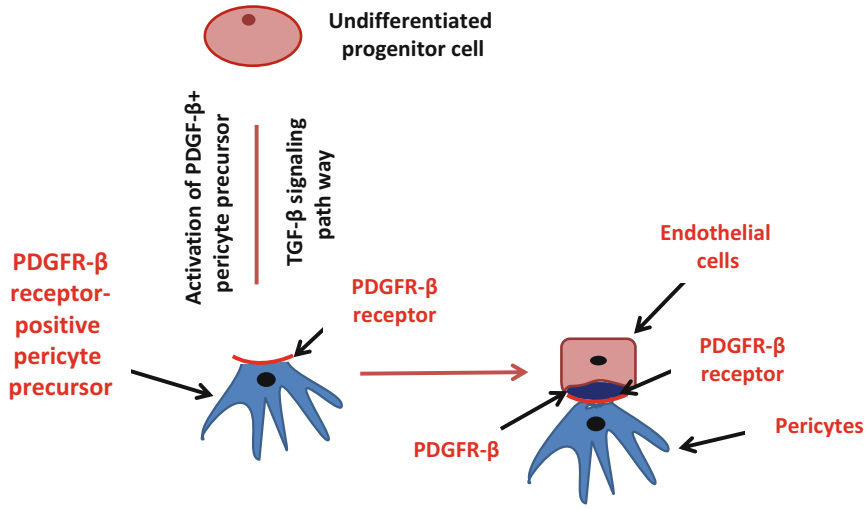

Fig. 4 Diagram showing the role of TGF-β signaling pathway in the regulation of pericyte-endothelial cell interaction

then attracted by PDGF-ß-expressing endothelial cells (Fig. 4) (Creazzo et al. 1998; Hellstrom et al. 1999). **TGFβ** regulates the proliferation and differentiation of both mural cells and endothelial cells. However, its activation requires interaction between the two cell types (Sato and Rifkin 1989). Determining the role of TGFβ signalling in the regulation of maintenance, development and function of the vascular system is challenging given that TGFβ receptors are expressed on both pericytes and ECs. Two forms of type I TGFβ receptors are expressed on both mural and endothelial cells. Both activin receptor-like kinase (Alk)-1 and Alk-5 trigger different cellular effects (Goumans et al. 2002; Oh et al. 2000).

Alk-5 activation in mesenchymal cells causes the phosphorylation of Smad2/3, which promotes mitosis and differentiation into SMC. On the other hand, Alk-1 activation phosphorylates Smad1/5 and enhances target genes, promoting cell migration and proliferation and countering SMC differentiation (Chen et al. 2003; Goumans et al. 2002; Ota et al. 2002). In endothelial cells, Alk-1 signalling interplays with the Alk-5 signalling pathway, whereas Alk-1 inhibits Alk-5, the latter of which is simultaneously required for Alk-1

signalling. While Alk-5 induces vessel maturation, Alk-1 counteracts this effect. The overall effect of TGFβ depends on the ratio of Alk-1/5 expression and TGFβ signal strength and duration. The process of TGFβ stimulation initially involves Alk-1 signalling, which leads to cell proliferation and migration, followed by Alk-5 signalling, which leads to cell differentiation and extracellular matrix production. Knockout of different TGFβ signalling pathway genes in mice, e.g., *tgfb1* (Dickson et al. 1995), *alk1* (Urness et al. 2000), *alk5* (Larsson et al. 2001), *tgfbr2* (Oshima et al. 1996), *smad4* (Lan et al. 2007), *smad5* (Chang et al. 1999; Yang et al. 1999), and *endoglin* (Li et al. 1999), leads to embryonic lethality with severe vascular abnormalities, including defects in the yolk sac vasculature, decreases in mural cell formation and, in some cases, defective haematopoiesis.

7 Pericytes Regulate Blood Flow and Maintain Blood Vessel Integrity

One of the most important functions of pericytes is their role in blood flow regulation and participation in the stability, remoulding, maintenance and permeability of blood vessels. Similar to vascular smooth muscle cells, pericytes regulate vasodilation and vasoconstriction within the blood capillaries to control vessel diameter and blood flow. This important physiological function has been confirmed by the identification of contractile proteins such as tropomyosin, myosin and α-SMA in pericytes. Pericytes have both adrenergic and cholinergic (β-2 and α-2) receptors. The adrenergic response causes relaxation, while the α-2 response promotes cell contraction. Other pericyte binding substances that have a vascular regulatory role are angiotensin II and endothelin-1. The latter is endothelial cell-dependent and produces nitric oxide, which induces blood vessel relaxation via a cGMP-dependent mechanism. These molecules exert a paracrine effect that is believed to play a central role in regulating blood flow via the contraction and relaxation of pericytes and the interaction between pericytes and endothelial cells (Rucker et al. 2000). Pericyte contraction is controlled by oxygen levels: hypoxia stimulates contraction, while increased carbon dioxide induces pericyte relaxation. Similarly, it has been postulated that vessels dilate when oxygen is required and contract when sufficient oxygen is available (Hirschi and D'Amore 1996; Tilton et al. 1979).

Mid-capillary pericytes play a limited role in blood flow regulation, as they do not express α-SMA. Rather, these pericytes are located at junctions with endothelial cells and are believed to permit the fusion of cells and transfer of fluids and proteins through the walls of blood capillaries (Hirschi and D'Amore 1996; Shepro and Morel 1993). In sites between pericytes, there are decreased amounts of proteins in the vascular BM, including collagen IV, laminin-10 and nidogen-2 (Wang et al. 2006). Furthermore, these areas are invaded by neutrophils to allow their escape into the perivascular space (Wang et al. 2012). The presence of such important areas allows local wound healing and the extravasation of blood vessels without causing any damage to membrane integrity. Pericytes interact with astrocytes to maintain the functionality, integrity and stability of the blood-brain barrier (Fig. 5) (Krueger and Bechmann 2010; Cuevas

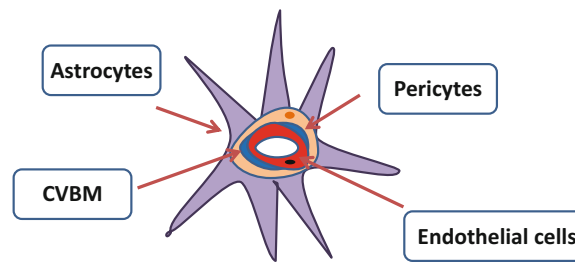

Fig. 5 Cross section in blood capillary showing pericyte-astrocyte interaction. CVBM, central vascular basement membrane

et al. 1984; Dohgu et al. 2005; Nakagawa et al. 2007; Nakamura et al. 2008; Al Ahmad et al. 2011; Shimizu et al. 2008; Armulik et al. 2010; Bell et al. 2010; Kamouchi et al. 2011; Daneman et al. 2010).

Pericytes also affect blood coagulation (Kim et al. 2006; Fisher 2009; Bouchard et al. 1997) and regulate immune function through their role in lymphocyte activation (Balabanov et al. 1999; Tu et al. 2011; Verbeek et al. 1995; Fabry et al. 1993). In adults, when an injury damages a pre-existing blood vessel and new vessel generation is required, pericytes detach from the vessel, and degradation of the vascular BM occurs, which halts the inhibitory effect of pericytes on the proliferation of endothelial cells and permits endothelial cell division and migration towards the surrounding matrix for new vessel formation (Bergers and Benjamin 2003; Yancopoulos et al. 2000). Once primitive vessels are reformed, PDGF-β, expressed by endothelial cells, triggers pericytes to resume their position on the blood vessel wall; this allows these pericytes to induce endothelial cell differentiation and inhibit their proliferation to maintain vessel stability (Tonnesen et al. 2000).

Pericytes in different organs demonstrate differing densities corresponding to their specified functions. For example, they are distributed in large capillaries and blood vessels at a higher density than in small arterioles and venules (Allt and Lawrenson 2001). Although pericytes are systematically and not randomly distributed on blood vessels, there is insufficient knowledge of the mechanism by which pericytes reside in the vessel. The number of pericytes on the vessel wall may depend on blood pressure. For example, in humans, pericytes were found to be highly distributed down the legs, where elevated blood pressure levels are required to pump blood upward. Similar to other vascular cells, pericytes acquire different specialized characteristics to perform different functions in different organs, such as the brain, kidney and liver, thus gaining different names as mentioned above.

8 Pericytes in the Brain

Neural blood vessels in the brain and retina contain the highest density of pericytes. These pericytes interact with endothelial cells to form the blood-brain barrier (BBB), which is essential to protect brain cells from toxic factors derived from the blood (Ballabh et al. 2004; Cleaver and Melton 2003). Pericytes play a very critical role in maintaining the integrity of vessels and the BBB (Verbeek et al. 1997). *In vitro* studies have demonstrated that pericytes protect against disruption of the BBB induced by hypoxia (Hayashi et al. 2004). Vascular mural cells in the brain provide immunological defence via macrophage-like effects, leading to the hypothesis that pericytes are macrophage precursors in the brain. Evidence for this hypothesis is provided by studies showing that small and soluble molecules suspended in the extracellular fluid are ingested by pericytes via pinocytosis, which is similar to macrophage functionality (Thomas 1999). The phagocytic activity of pericytes was further confirmed when researchers observed that protein traces injected in immature mice accumulated in brain and spinal cord pericytes (Kristensson and Olsson 1973). Furthermore, pericytes have scavenger receptors, which possess a broad ligand binding capacity and express Fc receptors, both of which are essential features for antigen–antibody complex formation to enhance antibody-mediated phagocytosis (Thomas 1999; Balabanov et al. 1996).

9 Pericytes in the Kidneys

Mesangial cells are renal pericytes, representing approximately 30% of the glomerular cell population. The interaction of mesangial cells with glomerular cells to form invading branches increases the capillary surface area for blood filtration. Mesangial cell development is dependent on the PDGF-β signalling pathway: PDGF-β- or PDGFR-β-deficient mice lack kidney pericytes and have abnormal kidney glomeruli (Betsholtz 2004). The kidney produces

approximately 90% of all erythropoietin (EPO) in the body, with some contribution from the liver. Many studies have postulated that mesangial cells are renal erythropoietin-producing cells (Obara et al. 2008). The mesenchymal origin of renal EPO-producing cells supports this hypothesis. For further confirmation, Obara et al. (2008) induced anaemia in a group of mice expressing erythropoietin-green fluorescent protein (EPO-GFP) by bleeding and examined renal GFP-expressing cells. EPO-GFP + cells were present at the cortical border of the medulla. These cells were stellate in morphology and expressed CD73, which is characteristic of pericytes and MSCs. Furthermore, the EPO-GFP $^{+}$ cells were in close proximity to CD31^{+} endothelial cells but did not express this endothelial marker (Maxwell et al. 1993; Bachmann et al. 1993). During anaemia or hypoxia, EPO synthesis increased via gene up-regulation. Patients with chronic kidney disease (CDK) and late stage renal disorders suffer from a reduction in renal erythropoietin production, which causes normochromic, normocytic anaemia (Babitt and Lin 2012). This reduction in EPO synthesis may be caused by the dysfunction of EPO-producing cells in the kidney. One postulated scenario to explain this process is that mesangial cells are activated, detach from the vessel wall, extend and differentiate into myofibroblasts during CDK progression. Upon initiation of this process, the cells lose their ability to produce EPO in response to the elevated levels of hypoxia-inducible factor 2.

10 Pericytes in the Liver

HSCs or Ito cells, named after their discoverer, Toshio Ito, are hepatic pericytes with highly specialized functions (Suematsu and Aiso 2001). HSCs are highly fenestrated, allowing them to line the hepatic sinusoids and regulate metabolite exchange between the portal blood, hepatocytes and Kupffer cells (Abbott 2002). HSCs play an important role in maintaining functionality and stability of the ECM by forming both ECM elements and matrix metalloproteinases. HSCs also contain a high percentage of vitamin A, estimated at 80% of the total body content (Sato et al. 2003). Similar to pericytes in other organs, HSCs recruit inflammatory cells after any hepatic injury as a repair mechanism and in response to liver fibrosis (Knittel et al. 1999). In healthy livers, HSCs are quiescent cells that play a vital role in vitamin A storage and maintaining matrix functionality. In contrast, in the fibrotic liver, several studies have indicated that HSCs become activated, highly proliferative, lose vitamin A and form a fibrotic matrix enriched with collagen type I. The mitogenic activity of PDGF in HSCs requires the expression of its receptor, which depends HSC activation (Friedman and Arthur 1989). Quiescent HSCs express the PDGF alpha receptor but do not express the beta receptor. In response to HSC activation, there is an increase in PDGFR-β synthesis without any changes in PDGFR-α synthesis (Wong et al. 1994). TGFβ is thought to up-regulate beta receptor expression and increase the mitogenic activity of PDGF-BB but not the mitogenic activity of PDGF-AA or PDGFAB. Finally, *in vitro* studies have shown that cultured HSCs express mRNA for the A and B genes and release PDGF in its active form into the culture media, which suggests the presence of an autocrine loop to maintain the proliferative ability of HSCs (Marra et al. 1994).

In 2008, Caplan reported that all MSCs are pericytes, not only because all MSCs express pericyte markers (Caplan 2008) but also because CD146^{+}, CD34^{-}, CD45^{-}, and CD56^{-} sorted pericytes demonstrate multipotent potential and differentiate into adipocytes, chondrocytes, myocytes and osteocytes (Caplan 2007). Pe´ault and colleagues reported that vascular mural cells do not act as precursors for neural or haematopoietic cells in adults. However, the perivascular locations of neural and haematopoietic stem cells in foetal tissue indicate that other stem cells accommodate this perivascular environment (Kiel et al. 2005; Hirschi and D'Amore 1996), supporting the hypothesis that not all pericytes are MSCs.

Recent studies have shown that pericytes differentiate into chondrocytes and express chondrogenic markers, such as sox-9, collagen

type II and aggrecan, when cultured in chondrogenic medium (Farrington-Rock et al. 2004). Kirton and collaborators have shown that TGF-β initiates signalling pathways that enhance the differentiation of pericytes into chondrogenic lineages (Kirton et al. 2007). Blocking this pathway causes the inhibition of chondrogenesis and decreases sox-9 and collagen type II expression (Farrington-Rock et al. 2004).

11 Pericytes and MSCs

In 2008, Caplan reported that all MSCs are pericytes, not only because all MSCs express pericyte markers (Caplan 2008), but also because $CD146^+$, $CD34^-$, $CD45^-$, and $CD56^-$ sorted pericytes demonstrate multipotent potential and differentiate into adipocytes (Chen et al. 2015), chondrocytes (Konig et al. 2016), myocytes and osteocytes (Caplan 2007). This differentiation potential of pericytes is highly associated with their lineage and the microenvironment (Herrmann et al. 2016). Pe´ault and colleagues reported that vascular mural cells do not act as precursors for neural or haematopoietic cells in adults. However, the perivascular locations of neural and haematopoietic stem cells in foetal tissue indicate that other stem cells accommodate this perivascular environment (Hirschi and D'Amore 1996; Kiel et al. 2005) supporting the hypothesis that not all pericytes are MSCs. Earlier studies have shown that pericytes can differentiate into chondrocytes, and express chondrogenic markers such as sox-9, collagen type II and aggrecan, when cultured in chondrogenic medium. Kirton and colleagues have shown that TGF-β initiates signalling pathway that enhances the differentiation of pericytes into chondrogenic lineages (Kirton et al. 2007). Blocking this pathway causes the inhibition of chondrogensis and decreases sox-9 and collagen type II expression.

12 Pericytes Contribution in Wound Healing

Wound healing is a complex multistep process that include inflammation (Chen et al. 2013; Miller et al. 1992; Proebstl et al. 2012), re-epithelialization (Paquet-Fifield et al. 2009), angiogenesis (Antonelli-Orlidge et al. 1989; Gaengel et al. 2009), matrix formation (Rajkumar et al. 2006) and tissue regeneration (Mills et al. 2013). In addition to their various physiological roles, pericytes play an important role during the wound healing process as follows:

13 Inflammation

Inflammation is important initial response that drives the wound healing process. Inflammation is enhanced within 30–40 min of the tissue injury by an influx of neutrophils from blood vessels to the wound site, to engulf cellular debris and pathogens. Earlier studies showed that pericytes cover gaps between ECs by forming umbrella-like structures over these gaps. IL-2 treatment opposes this effect by realigning of pericytes at EC junctions, resulting in microvessel leakage (Miller et al. 1992; Sims et al. 1994). T lymphocyte activity is paradoxically influenced by pericyte, based on the organ. For example in the brain, pericytes present antigens to T cells to induce lymphocyte activation (Balabanov and Dore-Duffy 1998), while retinal pericytes were reported to inhibit T cells (Tu et al. 2011). These differences result from either differences in the local population of pericytes, or from undefined microenvironmental cues. Brain pericytes can respond to different inflammatory signals as lipopolysaccharides (LPS) leadng to expression of Interferon gamma-induced protein 10 (IP-10) and Monocyte Chemoattractant Protein-1 (MCP-1) (Jansson et al. 2014). These findings lead Hung et al. to postulate that the role of pericytes is not restricted to immune cells

recruitment during inflammation, but they also recruit proinflamatory molecules. In lung injury for example, fewer pericytes were correlated with inflammatory response, leading to labeling pericytes as "interstitial immune sentinel cells" (Hung et al. 2017).

14 Re-epithelization

Another important event in the wound healing process is the reformation of epithelial barrier after wounding. This can help protect the wound from infection and return to the skin some of its vital activities, including regulation of temperature and preventing excess water loss. In their studies on the contribution of pericytes to re-epithelization, Paquet-Fifield and colleagues isolated pericytes from skin, and sorted them using human dermis-1 (HD-1) antibody. Organotypic cultures (OCs) containing fibroblasts and keratinocytes, were then prepared, with or without pericytes. The authors demonstrated that OCs which contain pericytes showed significant improvement in the epidermal layer formation compared to the OCs without PCs (Paquet-Fifield et al. 2009).

15 Angiogenesis

As previously mentioned, pericytes play an important role to support the pre-existing vessels and to regulate the proliferation and migration of EC to form new blood vessels and maintain their stabilization (Antonelli-Orlidge et al. 1989; Kutcher and Herman 2009). Pericytes were reported to respond to PDGF-β and TGF-β, which are released by platelets after an injury (Mills et al. 2013). This chemotactic response to PDGF-β leads to the migration of pericytes to the outer layer of the blood vessel, and to the wound area. This migration permits the endothelial cells proliferation at the wound site in response to VEGF (vascular endothelial growth factor) secreted upon platelet activation (Mohle et al. 1997). This process is further aided by the production of vitronctin, fibronectin, and laminins which also support the migration of endothelial cells and formation of new blood capillaries (Li et al. 2003; Tonnesen et al. 2000). These extracellular matrix (ECM) components contribute to the remodeling during healing with the help of macrophage-secreted proteases (Arroyo and Iruela-Arispe 2010). In addition, pericytes act to stabilize the newly formed capillaries via expression of TGF-β (Antonelli-Orlidge et al. 1989), which inhibits the proliferation of endothelial cells (Kutcher and Herman 2009). PDGF-β seems to also play an important role in wound remodeling as PDGFR-β knockout mice presented with endothelial hyperplasia with characteristic absence of pericytes in their blood vessels (Hellstrom et al. 2001). PDGF-β expression is controlled via Tie2 and Ang1/Ang2 interactions, whereas Tie2/Ang1 interaction causes PDGF-β expression and recruitment of pericytes; while Tie2/Ang2 interaction has an opposite effect (Maisonpierre et al. 1997).

16 Matrix Deposition/Fibrosis

Matrix deposition is crucial to speedy effective wound healing (Lin et al. 2008; Popescu et al. 2011). Under normal conditions, deposition of matrix starts as soon as the wound is cleared from infection and cellular debris. Initially, fibroblasts deposit collagen type III and fibronectin, but later these proteins are replaced by elastin and collagen type I. Fibroblasts are attracted to the wound site via PDGF-β, expressed by platelets and resident cells (Rajkumar et al. 2006). At the wound site, fibroblasts are activated to differentiate into myofibroblasts and express α-SMA to contract the wound (Mohle et al. 1997). Although some studies reported that pericytes are the collagen-producing cells (Popescu et al. 2011; Sundberg et al. 1993), others showed that the majority of collagen-producing cells were positive for α-SMA, and thus were myofibroblasts. These cells however were also positive for PDGF-β and NG-2 and thus were postulated to be pericytes (Dulauroy et al. 2012). The role of

pericytes in fibrosis remains however inconclusive as recent studies reported that pericyte deletion did not alter the recruitment of myofibroblast or the fibrosis process, leading to the hypothesis that resident MSCs, play a central role in fibrosis. Pericytes' role in fibrosis was further defined based on their location and phenotype. Based on Nestin expression; pericytes were classified into type-I which is $Nestin^{-}NG2^{+}$ and type-2 which is $Nestin^{+}NG2^{+}$. Type-1 pericytes are located near the fibrosis sites, and participate in fibrosis by the help of other factors, while type-2 have an important angiogenic role (Birbrair et al. 2014a; Birbrair et al. 2014b). Based on these findings, the role of pericyte in wound healing seems to be multifactorial based on the type, location, and local inflammatory response.

17 Pericytes Are Promising Therapeutic Targets for Regenerative Medicine

In addition to their different physiological roles, pericytes were shown to contribute to tissue regeneration and repair in various experimental settings. (Birbrair et al. 2014b; Eliasberg et al. 2017; He et al. 2010; Katare et al. 2011; Lauvrud et al. 2017; Park et al. 2011). In a study by Chen and colleagues, the therapeutic potential of perivascular cells in ischaemic heart repair was investigated. Transplantation of pericytes derived from human muscle into hearts of NOD/SCID mice with acute myocardiac infarction markedly improved cardiac functions compared to the uninjected control group (Chen et al. 2009). Furthermore administration of pericytes promoted angiogenesis, reduced fibrosis and scar formation, and ameliorated ongoing chronic inflammation by secretion of various trophic factors. Interestingly, these findings conflicted with previous data confirming the significant contribution of pericytes to fibrosis (Kida and Duffield 2011).

More recently, Dar et al. described the pericytes production from spontaneous differentiation of embryoid bodies derived from human pluripotent stem cells (hPSCs). Upon transplantation into immunodeficient mice with limb ischaemia induced by femoral artery ligation, hPSC-derived pericytes enhanced vascular regeneration and increased muscle repair (Dar et al. 2012). Enhancement of the contribution of pericytes to tissue regeneration has been achieved by switching from 2-D flat culture conditions into 3-D scaffolds. Human muscle derived pericytes were grown in ES-TIPS PEUU cylindrical synthetic scaffolds before transplantation into a sectioned rat aorta. The pericyte-seeded tissue engineered vascular grafts (TEVGs) supported better structure and function, and normal development of the blood vessel compared to the unseeded groups (He et al. 2010). *In vivo,* pericytes have also shown promising regenerative capacities in immunodeficient mouse models with skeletal muscle injury. Human skeletal muscle-derived pericytes were purified by flow cytometry, and injected into the muscles of the hind limb of cardiotoxin-injured NOD/SCID (SCID-non-obese diabetic) mice (Crisan et al. 2008). Both long-term cultured and freshly sorted pericytes generated human myofibres, and this regeneration was surprisingly more efficient than $CD56^{+}$ human skeletal myoblasts. When pericytes from adipose tissue, pancreas and placenta were administered into the muscles of mdx/SCID or cardiotoxin-treated NOD/SCID, they promoted angiogenesis and regeneration of human spectrin or dystrophin-positive myofibres (Park et al. 2011).

In addition to muscle, pericytes were also shown to promote osteogenic regeneration (Crisan et al. 2008; Konig et al. 2016). Pericytes cultured in osteogenic medium expressed alkaline phosphatase and exhibited mineral deposition. *In vivo*, pericytes developed into bony nodules when seeded on Gelfoam scaffolds, and implanted into the pockets of skeletal muscle in immunodeficient mice (Crisan et al. 2008). Osteogenic differentiation was also achieved when pericytes were cultures in media supplemented with Nell-1osteoinductive growth factor, using human cancellous bone chip (hCBC) scaffold. Pericytes seeded hCBC achieved superior bone formation than hCBC scaffold alone (Zhang et al. 2011).

In addition to skeletal differentiation, pericytes were shown to differentiate into neural cells, suggesting their potential valuable contribution to brain regeneration. The brain is a highly vascular tissue, and is rich in pericytes (Mirzadeh et al. 2008). Neurogenic cells are found close to blood vessels, wrapping them via multiple processes (Mirzadeh et al. 2008; Shen et al. 2008). Pericytes are found in a very high density in the brain compared to other organs (Diaz-Flores et al. 2009). They release neutrophins which contribute to neuroprotection during hypoxia (Ishitsuka et al. 2012). Hypoxic conditions in monkey hippocampus were found to induce pericytes into neural differentiation (Yamashima et al. 2004). In rodents, pericytes generated neurospheres after co-culture with endothelial cells, suggesting that endothelial-pericyte interaction may contribute to neural regeneration (Dore-Duffy 2008; Dore-Duffy et al. 2006), (Tavazoie et al. 2008). Furthermore, direct programming of human pericytes derived from cerebral cortex into functional neurons was recently achieved (Karow et al. 2012). Interestingly, human pericytes from non-brain tissue could also be differentiated into neural cells. Pericytes isolated from fat (Jung et al. 2011) or the aorta (Montiel-Eulefi et al. 2012) could be differentiated into cells of neural lineage.

Retinal degeneration is one of the most formidable complications of diabetic retinopathy. Loss of pericytes in the diabetic retina causes collapse of the vasculature finally leading to blindness. The role of human adipose tissue-derived pericytes which express α-SMA, PDGF-β and NG-2 in protection from diabetic retinopathy was demonstrated in mouse models. While implanting BM-MSCs alone could not ameliorate diabetic retinopathy, co-transplantation with pericytes caused revascularization of the retina (Mendel et al. 2013). This regenerative effect was enhanced after treatment with TGF-β. These results suggest a direct role for pericytes in regenerative therapies of intractable vascular disorders.

18 Conclusion

Pericytes hold reliable adeptness for vascular regenerative therapies, due to their physiological contribution to angiogenesis and maintenance of the blood vessel integrity. Pericytes are abundant throughout the body, and highly concentrated in vital organs such as the brain and the cardiovascular system. They are relatively easy to access and isolate with minimally invasive procedures. Their potential capacity to differentiate into cells of different lineages, in addition to cells of vascular tissue suggests a potentially valuable contribution to a diverse array of diseases, and especially in vascular regenerative medicine.

References

Abbott NJ (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat 200 (6):629–638

Al Ahmad A, Taboada CB, Gassmann M, Ogunshola OO (2011) Astrocytes and pericytes differentially modulate blood-brain barrier characteristics during development and hypoxic insult. J Cereb Blood Flow Metab 31(2):693–705

Allt G, Lawrenson JG (2001) Pericytes: cell biology and pathology. Cells Tissues Organs 169(1):1–11

Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA (1989) An activated form of transforming growth factor beta is produced by cocultures of endothelial cells and pericytes. Proc Natl Acad Sci U S A 86 (12):4544–4548

Armulik A, Abramsson A, Betsholtz C (2005) Endothelial/pericyte interactions. Circ Res 97(6):512–523

Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K et al (2010) Pericytes regulate the blood-brain barrier. Nature 468(7323):557–561

Armulik A, Genove G, Betsholtz C (2011) Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21 (2):193–215

Arroyo AG, Iruela-Arispe ML (2010) Extracellular matrix, inflammation, and the angiogenic response. Cardiovasc Res 86(2):226–235

Asahina K, Zhou B, Pu WT, Tsukamoto H (2011) Septum transversum-derived mesothelium gives rise to hepatic stellate cells and perivascular mesenchymal cells in developing mouse liver. Hepatology 53(3):983–995

Babitt JL, Lin HY (2012) Mechanisms of anemia in CKD. J Am Soc Nephrol 23(10):1631–1634

Bachmann S, Le Hir M, Eckardt KU (1993) Co-localization of erythropoietin mRNA and ecto-5-′-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. J Histochem Cytochem 41(3):335–341

Balabanov R, Dore-Duffy P (1998) Role of the CNS microvascular pericyte in the blood-brain barrier. J Neurosci Res 53(6):637–644

Balabanov R, Washington R, Wagnerova J, Dore-Duffy P (1996) CNS microvascular pericytes express macrophage-like function, cell surface integrin alpha M, and macrophage marker ED-2. Microvasc Res 52(2):127–142

Balabanov R, Beaumont T, Dore-Duffy P (1999) Role of central nervous system microvascular pericytes in activation of antigen-primed splenic T-lymphocytes. J Neurosci Res 55(5):578–587

Ballabh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 16(1):1–13

Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic BV (2010) Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. Neuron 68 (3):409–427

Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. Nat Rev Cancer 3(6):401–410

Bergers G, Song S (2005) The role of pericytes in blood-vessel formation and maintenance. Neuro-Oncology 7 (4):452–464.

Bergwerff M, Verberne ME, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC (1998) Neural crest cell contribution to the developing circulatory system: implications for vascular morphology? Circ Res 82 (2):221–231

Betsholtz C (2004) Insight into the physiological functions of PDGF through genetic studies in mice. Cytokine Growth Factor Rev 15(4):215–228

Birbrair A, Zhang T, Files DC, Mannava S, Smith T, Wang ZM, Messi ML, Mintz A, Delbono O (2014a) Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. Stem Cell Res Ther 5(6):122

Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, Delbono O (2014b) Type-2 pericytes participate in normal and tumoral angiogenesis. Am J Physiol Cell Physiol 307(1):C25–C38

Bouchard BA, Shatos MA, Tracy PB (1997) Human brain pericytes differentially regulate expression of procoagulant enzyme complexes comprising the extrinsic pathway of blood coagulation. Arterioscler Thromb Vasc Biol 17(1):1–9

Cai CL, Martin JC, Sun Y, Cui L, Wang L, Ouyang K, Yang L, Bu L, Liang X, Zhang X et al (2008) A myocardial lineage derives from Tbx18 epicardial cells. Nature 454(7200):104–108

Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 213(2):341–347

Caplan AI (2008) All MSCs are pericytes? Cell Stem Cell 3(3):229–230

Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H et al (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 7(5):575–583

Chang H, Huylebroeck D, Verschueren K, Guo Q, Matzuk MM, Zwijsen A (1999) Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. Development 126 (8):1631–1642

Chen S, Kulik M, Lechleider RJ (2003) Smad proteins regulate transcriptional induction of the SM22alpha gene by TGF-beta. Nucleic Acids Res 31 (4):1302–1310

Chen CW, Montelatici E, Crisan M, Corselli M, Huard J, Lazzari L, Peault B (2009) Perivascular multi-lineage progenitor cells in human organs: regenerative units, cytokine sources or both? Cytokine Growth Factor Rev 20(5–6):429–434

Chen CW, Okada M, Proto JD, Gao X, Sekiya N, Beckman SA, Corselli M, Crisan M, Saparov A, Tobita K et al (2013) Human pericytes for ischemic heart repair. Stem Cells 31(2):305–316

Chen WC, Baily JE, Corselli M, Diaz ME, Sun B, Xiang G, Gray GA, Huard J, Peault B (2015) Human myocardial pericytes: multipotent mesodermal precursors exhibiting cardiac specificity. Stem Cells 33(2):557–573

Cleaver O, Melton DA (2003) Endothelial signaling during development. Nat Med 9(6):661–668

Creazzo TL, Godt RE, Leatherbury L, Conway SJ, Kirby ML (1998) Role of cardiac neural crest cells in cardiovascular development. Annu Rev Physiol 60:267–286

Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L et al (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3(3):301–313

Cuevas P, Gutierrez-Diaz JA, Reimers D, Dujovny M, Diaz FG, Ausman JI (1984) Pericyte endothelial gap junctions in human cerebral capillaries. Anat Embryol (Berl) 170(2):155–159

da Silva Meirelles L, Caplan AI, Nardi NB (2008) In search of the in vivo identity of mesenchymal stem cells. Stem Cells 26(9):2287–2299

Daneman R, Zhou L, Kebede AA, Barres BA (2010) Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature 468(7323):562–566

Dar A, Domev H, Ben-Yosef O, Tzukerman M, Zeevi-Levin N, Novak A, Germanguz I, Amit M, Itskovitz-Eldor J (2012) Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb. Circulation 125(1):87–99

DeRuiter MC, Poelmann RE, VanMunsteren JC, Mironov V, Markwald RR, Gittenberger-de Groot AC (1997) Embryonic endothelial cells transdifferentiate into mesenchymal cells expressing smooth

muscle actins in vivo and in vitro. Circ Res 80 (4):444–451

Dettman RW, Denetclaw W Jr, Ordahl CP, Bristow J (1998) Common epicardial origin of coronary vascular smooth muscle, perivascular fibroblasts, and intermyocardial fibroblasts in the avian heart. Dev Biol 193(2):169–181

Diaz-Flores L, Gutierrez R, Madrid JF, Varela H, Valladares F, Acosta E, Martin-Vasallo P, Diaz-Flores L Jr (2009) Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol 24(7):909–969

Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ (1995) Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. Development 121(6):1845–1854

Dohgu S, Takata F, Yamauchi A, Nakagawa S, Egawa T, Naito M, Tsuruo T, Sawada Y, Niwa M, Kataoka Y (2005) Brain pericytes contribute to the induction and up-regulation of blood-brain barrier functions through transforming growth factor-beta production. Brain Res 1038(2):208–215

Dore-Duffy P (2008) Pericytes: pluripotent cells of the blood brain barrier. Curr Pharm Des 14 (16):1581–1593

Dore-Duffy P, Katychev A, Wang X, Van Buren E (2006) CNS microvascular pericytes exhibit multipotential stem cell activity. J Cereb Blood Flow Metab 26 (5):613–624

Dulauroy S, Di Carlo SE, Langa F, Eberl G, Peduto L (2012) Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. Nat Med 18(8):1262–1270

Eliasberg CD, Dar A, Jensen AR, Murray IR, Hardy WR, Kowalski TJ, Garagozlo CA, Natsuhara KM, Khan AZ, McBride OJ et al (2017) Perivascular stem cells diminish muscle atrophy following massive rotator cuff tears in a small animal model. J Bone Joint Surg Am 99(4):331–341

Ema M, Faloon P, Zhang WJ, Hirashima M, Reid T, Stanford WL, Orkin S, Choi K, Rossant J (2003) Combinatorial effects of Flk1 and Tal1 on vascular and hematopoietic development in the mouse. Genes Dev 17(3):380–393

Etchevers HC, Couly G, Le Douarin NM (2002) Morphogenesis of the branchial vascular sector. Trends Cardiovasc Med 12(7):299–304

Fabry Z, Fitzsimmons KM, Herlein JA, Moninger TO, Dobbs MB, Hart MN (1993) Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes. J Neuroimmunol 47(1):23–34

Farrington-Rock C, Crofts NJ, Doherty MJ, Ashton BA, Griffin-Jones C, Canfield AE (2004) Chondrogenic and adipogenic potential of microvascular pericytes. Circulation 110(15):2226–2232.

Fisher M (2009) Pericyte signaling in the neurovascular unit. Stroke 40(3 Suppl):S13–S15

Foster K, Sheridan J, Veiga-Fernandes H, Roderick K, Pachnis V, Adams R, Blackburn C, Kioussis D, Coles M (2008) Contribution of neural crest-derived cells in the embryonic and adult thymus. J Immunol 180 (5):3183–3189

Friedman SL, Arthur MJ (1989) Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. J Clin Invest 84 (6):1780–1785

Gaengel K, Genove G, Armulik A, Betsholtz C (2009) Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol 29(5):630–638

Gerhardt H, Betsholtz C (2003) Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res 314 (1):15–23

Gerhardt H, Wolburg H, Redies C (2000) N-cadherin mediates pericytic-endothelial interaction during brain angiogenesis in the chicken. Dev Dyn 218 (3):472–479

Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P (2002) Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. EMBO J 21(7):1743–1753

Hart CE, Forstrom JW, Kelly JD, Seifert RA, Smith RA, Ross R, Murray MJ, Bowen-Pope DF (1988) Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240(4858):1529–1531

Hayashi K, Nakao S, Nakaoke R, Nakagawa S, Kitagawa N, Niwa M (2004) Effects of hypoxia on endothelial/pericytic co-culture model of the blood-brain barrier. Regul Pept 123(1–3):77–83

He W, Nieponice A, Soletti L, Hong Y, Gharaibeh B, Crisan M, Usas A, Peault B, Huard J, Wagner WR et al (2010) Pericyte-based human tissue engineered vascular grafts. Biomaterials 31(32):8235–8244

Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126(14):3047–3055

Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H, Betsholtz C (2001) Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J Cell Biol 153(3):543–553

Herrmann M, Bara JJ, Sprecher CM, Menzel U, Jalowiec JM, Osinga R, Scherberich A, Alini M, Verrier S (2016) Pericyte plasticity – comparative investigation of the angiogenic and multilineage potential of pericytes from different human tissues. Eur Cell Mater 31:236–249

Hirschi KK, D'Amore PA (1996) Pericytes in the microvasculature. Cardiovasc Res 32(4):687–698.

Hung CF, Mittelsteadt KL, Brauer R, McKinney BL, Hallstrand TS, Parks WC, Chen P, Schnapp LM,

Liles WC, Duffield JS et al (2017) Lung pericyte-like cells are functional interstitial immune sentinel cells. Am J Physiol Lung Cell Mol Physiol 312(4):L556–L567

Ishitsuka K, Ago T, Arimura K, Nakamura K, Tokami H, Makihara N, Kuroda J, Kamouchi M, Kitazono T (2012) Neurotrophin production in brain pericytes during hypoxia: a role of pericytes for neuroprotection. Microvasc Res 83(3):352–359

Jansson D, Rustenhoven J, Feng S, Hurley D, Oldfield RL, Bergin PS, Mee EW, Faull RL, Dragunow M (2014) A role for human brain pericytes in neuroinflammation. J Neuroinflammation 11:104

Jung KH, Chu K, Lee ST, Bahn JJ, Jeon D, Kim JH, Kim S, Won CH, Kim M, Lee SK et al (2011) Multipotent PDGFRbeta-expressing cells in the circulation of stroke patients. Neurobiol Dis 41(2):489–497

Kamouchi M, Ago T, Kitazono T (2011) Brain pericytes: emerging concepts and functional roles in brain homeostasis. Cell Mol Neurobiol 31(2):175–193

Karow M, Sanchez R, Schichor C, Masserdotti G, Ortega F, Heinrich C, Gascon S, Khan MA, Lie DC, Dellavalle A et al (2012) Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. Cell Stem Cell 11(4):471–476

Katare R, Riu F, Mitchell K, Gubernator M, Campagnolo P, Cui Y, Fortunato O, Avolio E, Cesselli D, Beltrami AP et al (2011) Transplantation of human pericyte progenitor cells improves the repair of infarcted heart through activation of an angiogenic program involving micro-RNA-132. Circ Res 109 (8):894–906

Kida Y, Duffield JS (2011) Pivotal role of pericytes in kidney fibrosis. Clin Exp Pharmacol Physiol 38 (7):467–473

Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ (2005) SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 121 (7):1109–1121

Kim JA, Tran ND, Li Z, Yang F, Zhou W, Fisher MJ (2006) Brain endothelial hemostasis regulation by pericytes. J Cereb Blood Flow Metab 26(2):209–217

Kirton JP, Crofts NJ, George SJ, Brennan K, Canfield AE (2007) Wnt/beta-catenin signaling stimulates chondrogenic and inhibits adipogenic differentiation of pericytes: potential relevance to vascular disease? Circ Res 101(6):581–589

Knittel T, Dinter C, Kobold D, Neubauer K, Mehde M, Eichhorst S, Ramadori G (1999) Expression and regulation of cell adhesion molecules by hepatic stellate cells (HSC) of rat liver: involvement of HSC in recruitment of inflammatory cells during hepatic tissue repair. Am J Pathol 154(1):153–167

Konig MA, Canepa DD, Cadosch D, Casanova E, Heinzelmann M, Rittirsch D, Plecko M, Hemmi S, Simmen HP, Cinelli P et al (2016) Direct transplantation of native pericytes from adipose tissue: a new perspective to stimulate healing in critical size bone defects. Cytotherapy 18(1):41–52

Korn J, Christ B, Kurz H (2002) Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. J Comp Neurol 442(1):78–88

Kristensson K, Olsson Y (1973) Accumulation of protein tracers in pericytes of the central nervous system following systemic injection in immature mice. Acta Neurol Scand 49(2):189–194

Krueger M, Bechmann I (2010) CNS pericytes: concepts, misconceptions, and a way out. Glia 58(1):1–10

Kutcher ME, Herman IM (2009) The pericyte: cellular regulator of microvascular blood flow. Microvasc Res 77(3):235–246

Lan Y, Liu B, Yao H, Li F, Weng T, Yang G, Li W, Cheng X, Mao N, Yang X (2007) Essential role of endothelial Smad4 in vascular remodeling and integrity. Mol Cell Biol 27(21):7683–7692

Larson DM, Carson MP, Haudenschild CC (1987) Junctional transfer of small molecules in cultured bovine brain microvascular endothelial cells and pericytes. Microvasc Res 34(2):184–199

Larsson J, Goumans MJ, Sjostrand LJ, van Rooijen MA, Ward D, Leveen P, Xu X, ten Dijke P, Mummery CL, Karlsson S (2001) Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. EMBO J 20(7):1663–1673

Lauvrud AT, Kelk P, Wiberg M, Kingham PJ (2017) Characterization of human adipose tissue-derived stem cells with enhanced angiogenic and adipogenic properties. J Tissue Eng Regen Med 11(9):2490–2502

Lebrin F, Srun S, Raymond K, Martin S, van den Brink S, Freitas C, Breant C, Mathivet T, Larrivee B, Thomas JL et al (2010) Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. Nat Med 16 (4):420–428

Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C (1994) Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev 8(16):1875–1887

Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP (1999) Defective angiogenesis in mice lacking endoglin. Science 284 (5419):1534–1537

Li J, Zhang YP, Kirsner RS (2003) Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc Res Tech 60(1):107–114

Lin SL, Kisseleva T, Brenner DA, Duffield JS (2008) Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 173(6):1617–1627

Lindahl P, Johansson BR, Leveen P, Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277(5323):242–245

Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N et al (1997)

Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277 (5322):55–60

Majesky MW (2007) Developmental basis of vascular smooth muscle diversity. Arterioscler Thromb Vasc Biol 27(6):1248–1258

Majesky MW, Dong XR, Regan JN, Hoglund VJ (2011) Vascular smooth muscle progenitor cells: building and repairing blood vessels. Circ Res 108(3):365–377

Mandarino LJ, Sundarraj N, Finlayson J, Hassell HR (1993) Regulation of fibronectin and laminin synthesis by retinal capillary endothelial cells and pericytes in vitro. Exp Eye Res 57(5):609–621

Marra F, Choudhury GG, Pinzani M, Abboud HE (1994) Regulation of platelet-derived growth factor secretion and gene expression in human liver fat-storing cells. Gastroenterology 107(4):1110–1117

Maxwell PH, Osmond MK, Pugh CW, Heryet A, Nicholls LG, Tan CC, Doe BG, Ferguson DJ, Johnson MH, Ratcliffe PJ (1993) Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 44(5):1149–1162

Mendel TA, Clabough EB, Kao DS, Demidova-Rice TN, Durham JT, Zotter BC, Seaman SA, Cronk SM, Rakoczy EP, Katz AJ et al (2013) Pericytes derived from adipose-derived stem cells protect against retinal vasculopathy. PLoS One 8(5):e65691

Mikawa T, Gourdie RG (1996) Pericardial mesoderm generates a population of coronary smooth muscle cells migrating into the heart along with ingrowth of the epicardial organ. Dev Biol 174(2):221–232

Miller FN, Sims DE, Schuschke DA, Abney DL (1992) Differentiation of light-dye effects in the microcirculation. Microvasc Res 44(2):166–184

Mills SJ, Cowin AJ, Kaur P (2013) Pericytes, mesenchymal stem cells and the wound healing process. Cells 2 (3):621–634

Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A (2008) Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 3(3):265–278

Mohle R, Green D, Moore MA, Nachman RL, Rafii S (1997) Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. Proc Natl Acad Sci U S A 94(2):663–668

Montiel-Eulefi E, Nery AA, Rodrigues LC, Sanchez R, Romero F, Ulrich H (2012) Neural differentiation of rat aorta pericyte cells. Cytometry A 81(1):65–71

Nakagawa S, Deli MA, Nakao S, Honda M, Hayashi K, Nakaoke R, Kataoka Y, Niwa M (2007) Pericytes from brain microvessels strengthen the barrier integrity in primary cultures of rat brain endothelial cells. Cell Mol Neurobiol 27(6):687–694

Nakamura K, Kamouchi M, Kitazono T, Kuroda J, Matsuo R, Hagiwara N, Ishikawa E, Ooboshi H, Ibayashi S, Iida M (2008) Role of NHE1 in calcium signaling and cell proliferation in human CNS pericytes. Am J Physiol Heart Circ Physiol 294(4): H1700–H1707

Nehls V, Drenckhahn D (1991) Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J Cell Biol 113(1):147–154

Obara N, Suzuki N, Kim K, Nagasawa T, Imagawa S, Yamamoto M (2008) Repression via the GATA box is essential for tissue-specific erythropoietin gene expression. Blood 111(10):5223–5232

Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donahoe PK, Li L, Miyazono K, ten Dijke P, Kim S et al (2000) Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. Proc Natl Acad Sci U S A 97 (6):2626–2631

Olson LE, Soriano P (2011) PDGFRbeta signaling regulates mural cell plasticity and inhibits fat development. Dev Cell 20(6):815–826

Oshima M, Oshima H, Taketo MM (1996) TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. Dev Biol 179 (1):297–302

Ota T, Fujii M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, Miyazono K (2002) Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor-beta in human umbilical vein endothelial cells. J Cell Physiol 193(3):299–318

Paquet-Fifield S, Schluter H, Li A, Aitken T, Gangatirkar P, Blashki D, Koelmeyer R, Pouliot N, Palatsides M, Ellis S et al (2009) A role for pericytes as microenvironmental regulators of human skin tissue regeneration. J Clin Invest 119(9):2795–2806

Park TS, Gavina M, Chen CW, Sun B, Teng PN, Huard J, Deasy BM, Zimmerlin L, Peault B (2011) Placental perivascular cells for human muscle regeneration. Stem Cells Dev 20(3):451–463

Popescu FC, Busuioc CJ, Mogosanu GD, Pop OT, Parvanescu H, Lascar I, Nicolae CI, Mogoanta L (2011) Pericytes and myofibroblasts reaction in experimental thermal third degree skin burns. Romanian J Morphol Embryol 52(3 Suppl):1011–1017

Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, Rowe D, Nourshargh S (2012) Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. J Exp Med 209(6):1219–1234

Que J, Wilm B, Hasegawa H, Wang F, Bader D, Hogan BL (2008) Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. Proc Natl Acad Sci U S A 105 (43):16626–16630

Rajantie I, Ilmonen M, Alminaite A, Ozerdem U, Alitalo K, Salven P (2004) Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood 104(7):2084–2086

Rajkumar VS, Shiwen X, Bostrom M, Leoni P, Muddle J, Ivarsson M, Gerdin B, Denton CP, Bou-Gharios G, Black CM et al (2006) Platelet-derived growth factor-

beta receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. Am J Pathol 169(6):2254–2265

Risau W, Engelhardt B, Wekerle H (1990) Immune function of the blood-brain barrier: incomplete presentation of protein (auto-)antigens by rat brain microvascular endothelium in vitro. J Cell Biol 110 (5):1757–1766

Rucker HK, Wynder HJ, Thomas WE (2000) Cellular mechanisms of CNS pericytes. Brain Res Bull 51 (5):363–369

Sato Y, Rifkin DB (1989) Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture. J Cell Biol 109(1):309–315

Sato M, Suzuki S, Senoo H (2003) Hepatic stellate cells: unique characteristics in cell biology and phenotype. Cell Struct Funct 28(2):105–112

Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, Roysam B, Temple S (2008) Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 3 (3):289–300

Shepro D, Morel NM (1993) Pericyte physiology. FASEB J 7(11):1031–1038

Shimizu F, Sano Y, Maeda T, Abe MA, Nakayama H, Takahashi R, Ueda M, Ohtsuki S, Terasaki T, Obinata M et al (2008) Peripheral nerve pericytes originating from the blood-nerve barrier expresses tight junctional molecules and transporters as barrier-forming cells. J Cell Physiol 217(2):388–399

Sims DE (1986) The pericyte–a review. Tissue Cell 18 (2):153–174

Sims DE (1991) Recent advances in pericyte biology–implications for health and disease. Can J Cardiol 7 (10):431–443

Sims DE (2000) Diversity within pericytes. Clin Exp Pharmacol Physiol 27(10):842–846.

Sims DE, Miller FN, Horne MM, Edwards MJ (1994) Interleukin-2 alters the positions of capillary and venule pericytes in rat cremaster muscle. J Submicrosc Cytol Pathol 26(4):507–513

Soriano P (1994) Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev 8(16):1888–1896

Suematsu M, Aiso S (2001) Professor Toshio Ito: a clairvoyant in pericyte biology. Keio J Med 50(2):66–71

Sundberg C, Ljungstrom M, Lindmark G, Gerdin B, Rubin K (1993) Microvascular pericytes express platelet-derived growth factor-beta receptors in human healing wounds and colorectal adenocarcinoma. Am J Pathol 143(5):1377–1388

Tavazoie M, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F (2008) A specialized vascular niche for adult neural stem cells. Cell Stem Cell 3(3):279–288

Thomas WE (1999) Brain macrophages: on the role of pericytes and perivascular cells. Brain Res Rev 31 (1):42–57

Tilton RG, Kilo C, Williamson JR (1979) Pericyte-endothelial relationships in cardiac and skeletal muscle capillaries. Microvasc Res 18(3):325–335

Tonnesen MG, Feng X, Clark RA (2000) Angiogenesis in wound healing. J Investig Dermatol Symp Proc 5 (1):40–46

Tu Z, Li Y, Smith DS, Sheibani N, Huang S, Kern T, Lin F (2011) Retinal pericytes inhibit activated T cell proliferation. Invest Ophthalmol Vis Sci 52 (12):9005–9010

Urness LD, Sorensen LK, Li DY (2000) Arteriovenous malformations in mice lacking activin receptor-like kinase-1. Nat Genet 26(3):328–331

Verbeek MM, Westphal JR, Ruiter DJ, de Waal RM (1995) T lymphocyte adhesion to human brain pericytes is mediated via very late antigen-4/vascular cell adhesion molecule-1 interactions. J Immunol 154 (11):5876–5884

Verbeek MM, de Waal RM, Schipper JJ, Van Nostrand WE (1997) Rapid degeneration of cultured human brain pericytes by amyloid beta protein. J Neurochem 68(3):1135–1141

Wang S, Voisin MB, Larbi KY, Dangerfield J, Scheiermann C, Tran M, Maxwell PH, Sorokin L, Nourshargh S (2006) Venular basement membranes contain specific matrix protein low expression regions that act as exit points for emigrating neutrophils. J Exp Med 203(6):1519–1532

Wang S, Cao C, Chen Z, Bankaitis V, Tzima E, Sheibani N, Burridge K (2012) Pericytes regulate vascular basement membrane remodeling and govern neutrophil extravasation during inflammation. PLoS One 7(9):e45499

Wessels A, Perez-Pomares JM (2004) The epicardium and epicardially derived cells (EPDCs) as cardiac stem cells. Anat Rec A Discov Mol Cell Evol Biol 276(1):43–57

Wilm B, Ipenberg A, Hastie ND, Burch JB, Bader DM (2005) The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. Development 132(23):5317–5328

Wong L, Yamasaki G, Johnson RJ, Friedman SL (1994) Induction of beta-platelet-derived growth factor receptor in rat hepatic lipocytes during cellular activation in vivo and in culture. J Clin Invest 94 (4):1563–1569

Yamashima T, Tonchev AB, Vachkov IH, Popivanova BK, Seki T, Sawamoto K, Okano H (2004) Vascular adventitia generates neuronal progenitors in the monkey hippocampus after ischemia. Hippocampus 14 (7):861–875

Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, Naito M, Nakao K, Nishikawa S (2000) Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. Nature 408 (6808):92–96

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J (2000) Vascular-specific growth factors and blood vessel formation. Nature 407 (6801):242–248

Yang X, Castilla LH, Xu X, Li C, Gotay J, Weinstein M, Liu PP, Deng CX (1999) Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. Development 126(8):1571–1580

Zhang X, Peault B, Chen W, Li W, Corselli M, James AW, Lee M, Siu RK, Shen P, Zheng Z et al (2011) The Nell-1 growth factor stimulates bone formation by purified human perivascular cells. Tissue Eng Part A 17(19–20):2497–2509

Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J, Jiang D, von Gise A, Ikeda S, Chien KR et al (2008) Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature 454(7200):109–113

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Stem Cell Therapy: Repurposing Cell-Based Regenerative Medicine Beyond Cell Replacement

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Abstract

Stem cells exhibit simple and naive cellular features, yet their exact purpose for regenerative medicine continues to elude even the most elegantly designed research paradigms from developmental biology to clinical therapeutics. Based on their capacity to divide indefinitely and their dynamic differentiation into any type of tissue, the advent of transplantable stem cells has offered a potential treatment for aging-related and injury-mediated diseases. Recent laboratory evidence has demonstrated that transplanted human neural stem cells facilitate endogenous reparative mechanisms by initiating multiple regenerative processes in the brain neurogenic areas. Within these highly proliferative niches reside a myriad of potent regenerative molecules, including anti-inflammatory cytokines, proteomes, and neurotrophic factors, altogether representing a biochemical cocktail vital for restoring brain function in the aging and diseased brain. Here, we advance the concept of therapeutically repurposing stem cells not towards cell replacement *per se*, but rather exploiting the cells' intrinsic properties to serve as the host brain regenerative catalysts.

Keywords

Cell transplantation · Neurological disorders · Endogenous brain repair · Neurogenic niches

Abbrevaitions

PD	Parkinson's Disease
hNSCs	human neural stem cells
SVZ	Subventricular zone
SGZ	Subgranular zone
6-OHDA	6-hydroxydopamine

Stem cell therapy has reached clinical trials as an experimental treatment for many neurological disorders, including Parkinson's disease (PD) and stroke (Kondziolka et al. 2000; Lindvall et al. 1990). Whether it may provide insights into the complexity of the development of cells into tissues and organs, or be equally therapeutic in retarding aging and even reversing the death of brain cells, cell transplantation represents a milestone in translating basic science research into relevant clinical indications (Alexi et al. 2000; Borlongan 2000; Borlongan et al. 1999). Largely due to the well-established PD pathological

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hallmark of depleted nigrostriatal dopaminergic system, a direct dopaminergic cell replacement was deemed a feasible approach, thereby logistically positioning this neurological disorder as the initial test bed for safety and efficacy assessment of cell therapy (Brundin and Bjorklund 1998; Herrera-Marschitz et al. 2010; Perlow et al. 1979; Ungerstedt and Arbuthnott 1970; Ungerstedt et al. 1974). The pioneering fetal dopaminergic cell transplantation in PD patients (Freed et al. 1992; Kordower et al. 1995; Lindvall et al. 1989; Wenning et al. 1997) revealed survival of the transplanted cells, but more importantly the integration of the grafted cells with the host environment (Kordower et al. 1995). Transplanted PD patients displayed modest clinical improvements that subsequently diminished over time (Freeman et al. 1995; Li et al. 2016), with some of the patients presenting with worsening dyskinesias, although such adverse effects have been debated (Dunnett et al. 2001; Freed et al. 2001; Hagell et al. 2002). This first-to-human cell transplantation in PD paved the way for cell therapy applications to other neurological disorders (Borlongan 2016a, b; Cox et al. 2011; Freeman et al. 2000; Kalladka et al. 2016; Kondziolka et al. 2000; Napoli and Borlongan 2016, 2017b), with optimized safety and efficacy of cell dose, route of delivery, and timing of transplantation tailored to each disease.

Based on the major pathological symptom of neurodegeneration in aging and brain diseases (Borlongan 2016a, b; Cox et al. 2011; Freeman et al. 2000; Kalladka et al. 2016; Kondziolka et al. 2000; Napoli and Borlongan 2016, 2017b), turning stem cells into neurons to replace the already dead, or dying, brain cells became a key tenet for catering cell therapy to neurological disorders. To this end, the quest for obtaining a neural cell phenotype free from the ethical and logistical controversies associated with fetal cells is a logical choice for cell therapy, and human neural stem cells (hNSCs) appear to satisfy these transplantable caveats. Building on this cell replacement approach, many of the cell transplantation studies for PD have been designed to reconstruct the denervated nigrostriatal dopaminergic system. However, equally compelling evidence supports an alternative hypothesis stipulating that stem cells may mobilize the brain neurogenic niches to trigger an endogenous repair mechanism (Napoli and Borlongan 2017a). This non-dopaminergic cell replacement strategy may target known neurogenic niches, such as the subventricular zone (SVZ) and the subgranular zone (SGZ). Indeed, the SVZ has been implicated in the initiation of an endogenous repair mechanism after experimental brain insult, such as the PD 6-hydroxydopamine (6-OHDA)-induced dopamine lesions (Zuo et al. 2017). Employing behavioral, imaging, immunohistochemical, and proteomic outcome measures, hNSC transplants have been observed to target the SVZ in ameliorating the 6-OHDA-induced neurotoxic symptoms, as revealed by improved motor and cognitive functions that paralleled a therapeutic profile of proteomes, neurotrophic factors and cytokines in the SVZ. Intriguingly, these functional improvements occurred with no detectable significant proliferation of the transplanted hNSCs, indicating that a non-cell replacement therapy via a graft-induced stimulation of the neurogenic SVZ could have prompted the endogenous stem cells to confer a multi-pronged brain repair process (Napoli and Borlongan 2017a).

Transplantation of hNSCs, or for that matter any type of stem cells, even of non-neural phenotypes, may work cooperatively with SVZ through a by-stander effect in affording a beneficial outcome in PD, as well as other neurological disorders, beyond the traditional cell replacement mechanism (Alexi et al. 2000; Borlongan 2000; Borlongan et al. 1999). Although standardized animal models, such as the 6-OHDA for PD (Zuo et al. 2017), middle cerebral artery occlusion for stroke (Acosta et al. 2015b), controlled cortical impact approach for traumatic brain injury (Acosta et al. 2015a) may replicate many of the human pathological correlates, their routine application has inappropriately limited the field to cell replacement mechanism when testing effects and

mode of action of cell therapy. The observation that transplanted stem cells may harness the neurogenic niche to mount a brain repair process suggests that this by-stander effect is an equally robust cell therapy mechanism that may involve the remodeling of the neurogenic niche's proteomes, growth factors, and anti-inflammatory cytokines, among many other therapeutic molecules.

A key concept that arises when probing this alternative cell therapy mechanism is that undifferentiated stem cells are as effective as neurally differentiated stem cells, advancing the idea that the need for ample supply of differentiated cells for transplantation (Freeman et al. 1995; Kefalopoulou et al. 2014; Lindvall et al. 1992) can now be circumvented by merely using naive stem cells. Additionally, although the regenerative process may originate from the SVZ, the observed improvements in behavioral functions include cognitive performance, which is a hippocampal-dependent task (Costa et al. 2012; Kitamura et al. 2017; Redondo et al. 2014), indicating that the other neurogenic niche, SGZ, also has an active role in the regenerative process. Interestingly, a pathological link between the hippocampus and the dopaminergic system exists and modulates PD non-motor symptoms (Calabresi et al. 2013; Hall et al. 2014). Moreover, while PD symptoms have been overwhelmingly ascribed to the nigrostriatal dopaminergic depletion, synaptic plasticity dysfunctions unrelated to this dopaminergic system have been shown to mediate cognitive deficits in PD (Bagetta et al. 2011). Accordingly, expanding PD treatment to extra-nigrostriatal non-dopaminergic tissues, i.e., SVZ and SGZ host neurogenic niche, represents a novel and robust approach for cell therapy. Remarkably, similar by-stander effects have already been demonstrated in PD rats (Yasuhara et al. 2006) and primates (Redmond et al. 2007). In the end, a combination of both dopaminergic and non-dopaminergic regenerative mechanisms will likely emerge optimal cell transplant regimen. In addition, other non-cell replacement strategies including the graft formation of cellular biobridges (Tajiri et al. 2013, Tajiri et al. 2014b) and secretion of exosomes and microvesicles (Crowley et al. 2017; Tajiri et al. 2014a) may further optimize this by-stander effect-mediated brain repair process.

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References

Acosta SA, Tajiri N, de la Pena I, Bastawrous M, Sanberg PR, Kaneko Y, Borlongan CV (2015a) Alpha-synuclein as a pathological link between chronic traumatic brain injury and Parkinson's disease. J Cell Physiol 230(5):1024–1032

Acosta SA, Tajiri N, Hoover J, Kaneko Y, Borlongan CV (2015b) Intravenous bone marrow stem cell grafts preferentially migrate to spleen and abrogate chronic inflammation in stroke. Stroke 46(9):2616–2627

Alexi T, Borlongan CV, Faull RL, Williams CE, Clark RG, Gluckman PD, Hughes PE (2000) Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. Prog Neurobiol 60(5):409–470

Bagetta V et al (2011) Dopamine-dependent long-term depression is expressed in striatal spiny neurons of both direct and indirect pathways: implications for Parkinson's disease. J Neurosci 31(35):12513–12522

Borlongan CV (2000) Transplantation therapy for Parkinson's disease. Expert Opin Investig Drugs 9 (10):2319–2330

Borlongan CV (2016a) Age of PISCES: stem-cell clinical trials in stroke. Lancet 388(10046):736–738

Borlongan CV (2016b) Preliminary reports of stereotaxic stem cell transplants in chronic stroke patients. Mol Ther 24(10):1710–1711

Borlongan CV, Sanberg PR, Freeman TB (1999) Neural transplantation for neurodegenerative disorders. Lancet 353(Suppl 1):SI29–SI30

Brundin P, Bjorklund A (1998) Survival of expanded dopaminergic precursors is critical for clinical trials. Nat Neurosci 1(7):537

Calabresi P, Castrioto A, Di Filippo M, Picconi B (2013) New experimental and clinical links between the hippocampus and the dopaminergic system in Parkinson's disease. Lancet Neurol 12(8):811–821

Costa C et al (2012) Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease. Brain 135(Pt 6):1884–1899

Cox CS Jr et al (2011) Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. Neurosurgery 68(3):588–600

Crowley MG, Liska MG, Borlongan CV (2017) Stem cell therapy for sequestering neuroinflammation in traumatic brain injury: an update on exosome-targeting to the spleen. J Neurosurg Sci 61(3):291–302

Dunnett SB, Bjorklund A, Lindvall O (2001) Cell therapy in Parkinson's disease – stop or go? Nat Rev Neurosci 2(5):365–369

Freed CR et al (1992) Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease. N Engl J Med 327(22):1549–1555

Freed CR et al (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 344(10):710–719

Freeman TB et al (1995) Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. Ann Neurol 38(3):379–388

Freeman TB et al (2000) Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. Proc Natl Acad Sci U S A 97 (25):13877–13882

Hagell P et al (2002) Dyskinesias following neural transplantation in Parkinson's disease. Nat Neurosci 5 (7):627–628

Hall H et al (2014) Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. Brain 137(Pt 9): 2493–2508

Herrera-Marschitz M, Arbuthnott G, Ungerstedt U (2010) The rotational model and microdialysis: significance for dopamine signalling, clinical studies, and beyond. Prog Neurobiol 90(2):176–189

Kalladka D et al (2016) Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet 388 (10046):787–796

Kefalopoulou Z et al (2014) Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. JAMA Neurol 71(1):83–87

Kitamura T et al (2017) Engrams and circuits crucial for systems consolidation of a memory. Science 356 (6333):73–78

Kondziolka D et al (2000) Transplantation of cultured human neuronal cells for patients with stroke. Neurology 55(4):565–569

Kordower JH et al (1995) Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N Engl J Med 332 (17):1118–1124

Li W et al (2016) Extensive graft-derived dopaminergic innervation is maintained 24 years after transplantation in the degenerating parkinsonian brain. Proc Natl Acad Sci U S A 113(23):6544–6549

Lindvall O et al (1989) Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. Arch Neurol 46 (6):615–631

Lindvall O et al (1990) Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. Science 247(4942):574–577

Lindvall O et al (1992) Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. Ann Neurol 31(2):155–165

Napoli E, Borlongan CV (2016) Recent advances in stem cell-based therapeutics for stroke. Transl Stroke Res 7 (6):452–457

Napoli E, Borlongan CV (2017a) Cell therapy in Parkinson's disease: host brain repair machinery gets a boost from stem cell grafts. Stem Cells 35 (6):1443–1445

Napoli E, Borlongan CV (2017b) Stem cell recipes of bone marrow and fish: just what the stroke doctors ordered. Stem Cell Rev 13(2):192–197

Perlow MJ, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ (1979) Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science 204(4393):643–647

Redmond DE Jr et al (2007) Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. Proc Natl Acad Sci U S A 104(29):12175–12180

Redondo RL, Kim J, Arons AL, Ramirez S, Liu X, Tonegawa S (2014) Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature 513(7518):426–430

Tajiri N et al (2013) Stem cell recruitment of newly formed host cells via a successful seduction? Filling the gap between neurogenic niche and injured brain site. PLoS One 8(9):e74857

Tajiri N et al (2014a) Intravenous transplants of human adipose-derived stem cell protect the brain from traumatic brain injury-induced neurodegeneration and motor and cognitive impairments: cell graft biodistribution and soluble factors in young and aged rats. J Neurosci 34(1):313–326

Tajiri N et al (2014b) Stem cell-paved biobridge facilitates neural repair in traumatic brain injury. Front Syst Neurosci 8:116

Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res 24(3):485–493

Ungerstedt U, Ljungberg T, Steg G (1974) Behavioral, physiological, and neurochemical changes after

6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. Adv Neurol 5:421–426

Wenning GK et al (1997) Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. Ann Neurol 42 (1):95–107

Yasuhara T et al (2006) Transplantation of human neural stem cells exerts neuroprotection in a rat model of Parkinson's disease. J Neurosci 26(48):12497–12511

Zuo F, Xiong F, Wang X, Li X, Wang R, Ge W, Bao X (2017) Intrastriatal transplantation of human neural stem cells restores the impaired subventricular zone in parkinsonian mice. Stem Cells 35(6):1519–1531

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Stem Cells in Alzheimer's Disease: Current Standing and Future Challenges

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Abstract

To date, there is no definitive treatment for Alzheimer's disease (AD). The realm of stem cells is very promising in regenerative medicine, particularly for neurodegenerative diseases. Various types of stem cells have been used in preclinical/clinical trials for AD aiming the development of an elusive disease modifying therapy. Over the last decade, much knowledge has been gained in this field regarding types of cells, routes and timing of administration, and outcomes of stem cells-based strategies for AD. In this chapter, we will trace the state of art and the challenges facing the use of stem cells in AD.

Keywords

Alzheimer's disease · Mesenchymal stem cells · Neuroinflammation · Neuronal stem cells

Abbreviation

AD	Alzheimer's disease
ADSCs	Adipose tissue derived stem cells
Aβ	Amyloid-beta plaques
BDNF	Brain derived neurotropic factor
Epi-NCSCs	Epidermal neural crest derived stem cells
ESCs	Embryonic stem cells
HUCBCs	Human umbilical cord blood cells
IA	Intra-arterial
IC	Intracerebral
IL-10	Interleukin-10
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
iPSCs	Induced pluripotent stem cells
IV	Intravenous
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
NGF	Nerve growth factor
NIRFI	Near-infrared fluorescence imaging
NPCs	Neuronal precursor cells
NSCs	Neuronal stem cells
SPECT	Single-photon emission computed tomography
TNF-α	Tumor necrosis factor alpha

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1 Introduction

Alzheimer's disease (AD) is the most common form of neurodegenerative disease accounting for about 60–70% of cases of dementia worldwide. In the United States alone, the prevalence of AD is estimated at 4.7 million cases and is

expected a significant increase of this number (Hebert et al. 2013).

AD has a very complex pathogenesis with distinct neuropathological features, including extracellular amyloid-beta (Aβ) plaques, intracellular neurofibrillary tangles due to deposition of hyperphosphorylated tau protein, neuronal loss with preferential degeneration of the temporoparietal lobes and parts of the frontal cortex and cingulate gyrus. Clinically, AD is characterized by progressive impairment of memory, visuospatial and language skills, behavioral problems, and ultimately death. All the available treatment modalities for AD are only symptomatic, being unable to alter its natural course (Wenk 2003; Burns and Iliffe 2009; Desikan et al. 2009). It became an imperative to develop new treatments, including unconventional forms of management, to ameliorate the crippling effects of AD (Rosemann 2015).

Stem cells appeared to be specifically promising in the field of AD. Different forms of stem cell lines were applied in numerous studies in the past decade, ranging from basic laboratory research to preclinical studies and clinical trials, showing very promising results on the path for an innovative treatment for AD (Kimbrel and Lanza 2015).

The objectives of the current chapter are: (i) to present the different stem cell lines used in pre-clinical models of AD; (ii) to explore their potential mechanisms of action in AD; (iii) to highlight the challenges and future perspectives of stem cell therapy in AD.

2 MSCs and NSCs Studies in AD: Evidence and Mechanisms

Studies have used different types of stem cells for transplantation. However, the most frequently used types are mesenchymal stem cells (MSCs) and neuronal stem cells (NSCs) (Table 1).

Since their discovery by Friedenstein and co-workers in the bone marrow, MSCs have been the most studied adult stem cell type, especially in age-related conditions (Peng et al. 2013; Bash et al. 2004). MSCs are currently being harvested from almost all tissues, such as adipose tissue (Mahmoudifar and Doran 2015), olfactory bulb (Marei et al. 2013), placenta (Yun et al. 2013), cord blood (Yang et al. 2013), and amniotic fluid (Kim et al. 2013).

When transplanted, MSCs rapidly home to sites of injury and inflammation (Majka et al. 2003). The mechanisms of action of MSC-related improvements in healing have not been fully elucidated and are hypothesized to be due to both local and systemic effects. MSCs have been shown to enhance the local angiogenic response after injury through the release of proangiogenic factors. In addition, they trigger the immune responses via microglia activation, which in turn triggers an anti-inflammatory response (Birch et al. 2014).

Actually, MSCs have been revealed to switch microglia phenotype from classically activated to alternatively activated, resulting in decreased levels of pro-inflammatory cytokines such as IL-1β and TNF-α, and increased levels of anti-inflammatory cytokines like IL-4 and IL-10 in experimental models of AD. Moreover, the modified microglia was found to possess higher capability to degrade Aβ deposits because of increased expression of the Aβ-degrading enzyme neprilysin. Therefore, MSC-mediated regulation on microglial cells leads to markedly reduced Aβ deposition and improved memory dysfunction. Although MSCs derived from different sources such as bone marrow, umbilical cord, adipose tissue and Wharton's Jelly have been demonstrated to exert similar effects on microglia in the context of AD, the underlying mechanism still remains elusive (Chen et al. 2017).

In a very interesting study, it was shown that a negative correlation exists between MSC-induced microglial activation with high anti-inflammatory cytokines expression and Aβ deposits decrease and tau phosphorylation reduction, mostly in the hippocampus, in mice (Lee et al. 2012). MSCs direct transplantation into the hippocampus was also shown to improve cognition in mice, as well as augmentation of hippocampal neurogenesis through Wnt pathway, thus enhancing endogenous repair (Oh et al. 2015). MSCs were also shown to improve neovascularization, leading to

Table 1 Stem cells used in AD research with their advantages and disadvantages

Cell type	Advantages	Disadvantages
MSCs	Easily harvested from all tissues	Heterogeneity issues
	Abundant	Long culture times
	Enhance anti-inflammatory and angiogenic response	Homing problems
		Immunogenicity and tumorigenicity issues
NSCs	Better for neurogenesis	Most challenging to harvest
	Neuroprotective	Not effective in late cases
	Robust migratory capacity	
	Mitochondrial biogenesis	
	Proven to be safe and effective	
UCBCs	Promising in neurodegenerative disease	Mostly MSCs in nature
	Very popular in source of stem cells in clinical trials	
ADSCs	Readily abundant	Very heterogeneous group
	Easiest to harvest	Potential tumorigenicity
	Lower senescence rates	
iPSCs	Eliminate the need for immunosuppression use	Still limited to basic laboratory trials
	Molecularly and functionally similar to ESCs	
	No ethical concerns of their origin	
Hair follicle stem cells	Neurogenesis	Unknown fate of their newly formed neurons
	Accessible with no issues in their harvest unlike NSCs	
	Easily available	

memory and learning deficits recovery (Garcia et al. 2014). High-risk pre-dementia mice models were treated with MSC from the bone marrow, and reduction in Aβ deposits and changes in key proteins required for synaptic transmission were detected 2 months post injection, suggesting a potential early interventional therapy in prodromal AD by bone marrow-MSC transplantation (Bae et al. 2013).

Despite the huge potential and promise of MSCs, there are many issues regarding their unwanted proliferation or differentiation and the consequent tumorigenicity, long culture preparation times, the need for irradiation and/or other toxic preconditioning to achieve proper engraftment, and immunogenicity (Shin et al. 2011; Barkholt et al. 2015; Rohban and RudolfPieber 2017). In part due to these concerns, there are no available clinical data so far. On the clinicaltrials.gov website, there are only 10 registered clinical human studies at early stages of recruitment, most of them using the umbilical cord derived stem cells.

Recently, NSCs transplantation became one of the main lines of AD research. It must be mentioned that active adult neurogenesis is spatially restricted to two specific "neurogenic" brain regions: (i) the subgranular zone located in the dentate gyrus of the hippocampus where new dentate granule cells are generated, and (ii) the subventricular zone of the lateral ventricles where new neurons are generated before migrating through the rostral stream to the olfactory bulb to become interneurons. The subventricular zone of the lateral ventricles is considered the largest source of NSCs in the adult mammalian brain (Capilla-Gonzalez et al. 2015). NSCs from adult brains have proved in multiple settings to be safe and effective when transplanted into the hippocampus, improving cognitive functions, synaptic connectivity and neuronal survival in animal models (Hampton et al. 2010). Genetically modified NSCs expressing the proteolytic enzyme neprilysin – that underlies the endogenous degradation of Aβ – was shown to lead to marked and significant reduction in the AD pathology in transgenic mice.

Other studies combined the effect of both nerve growth factor (NGF) and brain derived neurotropic factor (BDNF) on NSCs differentiation showing an increased neurogenesis rate, which is interesting for future clinical applications, especially in dosage optimization (Liu et al. 2014). In addition, NSCs also showed significant neuroprotective effect against AD inflammation by suppressing glial and toll-like receptor-4 mediated inflammatory pathway activation, down-regulating pro-inflammatory mediators and ameliorating the cognitive deficits (Zhang et al. 2016).

NSCs were able to enhance mitochondrial biogenesis via increasing the number of mitochondria and mitochondrial proteins expression (dynamin related protein 1, mitochondrial fission 1 protein, optic atrophy 1) in mice models, therefore improving cognitive outcome (Zhang et al. 2015). These results are important as mitochondrial bioenergetics deficits usually precede AD pathogenesis (Kim et al. 2015a, b). However, it should be mentioned that in late stages of AD, NSCs failed to restore behavioral/cognitive deficits.

As an alternative, human neural stem cell populations were used in transplantation experiments in animal models of neurodegeneration. These human NSCs succeeded to ameliorate context and place dependent learning with proper cell survival and engraftment detected by immunofluorescence and confocal microscopy. Interestingly, cognitive improvement was not associated with altered Aβ or tau pathology (as seen with murine NSCs transplantation), indicating that other mechanisms, such as synaptogenesis, would be implicated in cognitive improvement (Ager et al. 2015).

Neuronal precursor cells (NPCs) derived from mouse embryonic stem cells (ESCs) was shown to promote behavioral recovery in rat models of AD following commitment to a cholinergic cell phenotype (Moghadam et al. 2009). It was shown clearly that their transplantation led to an increase in the number of cholinergic neurons after treatment. Other less frequently used stem cells sources include Human umbilical cord blood cells (HUCBCs) that have been therapeutically beneficial in many neurodegenerative diseases (Romanov et al. 2003; Ende et al. 2001; Vendrame et al. 2004). Adipose tissue derived stem cells (ADSCs) appeared as a new attractive source for stem cells therapy that is readily abundant, easily accessible, with a detected lower senescence ratio compared to bone marrow-MSCs (Huang et al. 2007; Anghileri et al. 2008; Ra et al. 2011). Hair follicle stem cells or Epidermal neural crest derived stem cells (Epi-NCSCs) were also used in AD models with promising results (Esmaeilzade et al. 2012).

Induced pluripotent stem cells (iPSCs) have been used in the neuroregenerative field giving their high capacity for scaling up and the ability to use autologous cells, thereby reducing or eliminating the need for immune-suppression (Takahashi and Yamanaka 2006). It is worth mentioning that iPSCs exhibit molecular and functional characteristic similar to those of the ESCs, but unlike ESCs, iPSCs raise no major ethical concerns regarding their source. Moreover, they provide autologous cells for cell-based therapies (Menon et al. 2016). Despite the very promising preliminary results, iPSCs trials are still limited to the basic laboratory boundaries (Kondo et al. 2013; Smith et al. 2016). Several research groups have used iPSCs modeling patient-specific AD pathology features *in vitro*. However, issues regarding donor-to-donor variability and intercellular heterogeneity are hurdling the models. Also, in most studies iPSCs derived from healthy individuals or family members were used as controls. Such control groups are not the most appropriate approach in disease modeling assays, as they may have a totally different genetic background from the AD-iPSCs (Juan et al. 2016).

In summary, while a huge progress has been accomplished in the last decade, the best cell line to be used in neurodegenerative disease research is still debatable. Noteworthy, MSCs and NSCs together accounted for approximately 75% of all the used stem cell lines.

3 Practical and Theoretical Challenges for Clinical Translation

Choosing a cell delivery route for clinical translation is a daunting challenge. The best way to deliver stem cells to the target brain region remains to be established. More than two thirds of the published studies so far used the intravenous (IV) route. Nevertheless, peripheral delivery of cells and proteins appears to be less effective than intracerebral injections (IC). For instance, IV delivery route succeeded in reducing plasma Aβ, but failed to clear Aβ plaques within the brain (Walker et al. 2013). Based on the theory of blood-brain barrier disruption and leakage in AD patients, different groups have built their preclinical experiments using the IV route for cell delivery, being an easier and less invasive method (Zipser et al. 2007; Jeynes and Provias 2011).

It is intuitive to assume that direct implantation techniques to deliver cells to a particular organ should be the best strategy. The safety and tolerability of stereotactic administration was proven to be feasible, safe, and well tolerated by a phase 1 clinical trial in humans (Rafii et al. 2014; Kim et al. 2015a, b). In cases of IV injected stem cells, most of the injected cells were found in the splenic marginal zone, rather than homing in the target brain tissues, highlighting their unpredictable fate upon peripheral administration (Lee et al. 2008; Ehrhart et al. 2016). Direct intra-arterial (IA) stem cells transplantation has also been used, but mainly for stroke and ischemic diseases. Moreover, this technique is far from optimization for the clinical use (Kamiya et al. 2013; Toyoshima et al. 2015; Cerri et al. 2015; Poduslo et al. 2011; Poduslo et al. 2007). Other less common alternative routes in AD models like direct intra-cardiac injection (Yang et al. 2013), and intranasal (IN) route (Danielyan et al. 2014) were tried, without a clear or convincing justification for their use.

The lack of a reliable method to evaluate the biological interactions of transplanted cells is another hurdle (Chung and Yang 2010). Several multimodal imaging techniques have been developed to overcome the limitations of individual methods (Wegner and Hildebrandt 2015; Von der Haar et al. 2015; Zhao et al. 2016), such as: nanomedicine and nanoparticles-based delivery systems, superparamagnetic iron oxide, magnetic resonance imaging (MRI) (Villa et al. 2011; Li et al. 2015; Cheng et al. 2015), positron emission tomography (PET), or single-photon emission computed tomography (SPECT) (Kim et al. 2012; Yang et al. 2016), fluorescence imaging (Nagarajan and Zhang 2011; Rosenholm et al. 2016), Near-infrared fluorescence imaging (NIRFI) (Page et al. 2015; Lehtivuori et al. 2015). Still, proper methods of in vivo cell tracking are far from optimization for reliable use in human subjects.

Several mechanisms have been postulated for the stem cells actions *in vivo*. As discussed above, stem cells have been shown to activate microglia (Lee et al. 2009, 2010). Activated microglia delay the progression of AD by clearing Aβ through phagocytosis and releasing enzymes responsible for Aβ degradation. Moreover, microglia also secrete growth factors and anti-inflammatory cytokines which are neuroprotective (Hickman et al. 2008). It is worth mentioning that with aging microglia become steadily less efficient at these processes, and they tend to be over-activated in response to stimulation, which may cause further neuronal damage.

BDNF, a stem cell-induced neurotrophic factor, is postulated to be responsible for the improvement in cognition in AD mice model (Blurton-Jones et al. 2009). NSCs were shown to induce an elevation in the hippocampal levels of BDNF, thus increasing synaptic density and restoring hippocampal-dependent cognition. In multiple studies, stem cells were shown to increase the expression of anti-inflammatory cytokines while decreasing pro-inflammatory cytokines (Ennis et al. 2013; Zhang et al. 2013; Kyurkchiev et al. 2014), in addition to the ability to increase the expression of vascular endothelial growth factor (VEGF), known for its neuroprotective and neurogenic roles (Ruiz de Almodovar et al. 2009).

The role of the extracellular vesicles (or 'secretomes') as key components of stem cells paracrine has also been proposed. Studies have shown that extracellular vesicles deliver bio-active cargo to the neighboring diseased or injured cells, inducing immune modulation, angiogenesis, neurogenesis and synaptogenesis (Paul and Anisimov 2013; Han et al. 2016 Konala et al. 2016; Cassar and Blundell 2016). The clinical application of 'secretomes' still faces several challenges, including their heterogeneity, and isolation and production techniques. Their use in AD models needs to be explored.

4 Future Perspectives

Stem cell based therapies had promising results in preclinical models of AD, but further studies are needed to solve the multiple practical and theoretical problems. For instance, we are still uncertain about the best way to deliver the cells to the brain. The complexity of AD, in which there is no uniform disease progression with multiple levels of pathological changes, results in difficulty in optimizing a single cell type based therapy to reverse all the cognitive and behavioral symptoms. This is one of the major hurdles that face a potential cell based therapy (Salem et al. 2016).

We do not know the exact mechanisms of action of stem cells. More specifically, detecting them *in vivo* after transplantation is a major challenge for their clinical application. Tracing them *in vivo* will allow treatment follow-up, dosage optimization, avoiding toxicity and unwanted cellular interactions. Secretomes need to be explored in AD.

Regarding the complex structure of the human brain with its highly interconnected neuronal circuits, it seems unlikely that a single stem cell type (or another single treatment modality) will effectively treat patients with AD, modifying the disease course. Most likely, the 'ultimate cure' of AD will depend on a combination of multimodal approaches at different phases of the disease (early, intermediate, and late), probably using different stem cells and/or secretomes, in addition to other strategies to prevent pathological protein aggregation (Aβ and tau) and to foster neurogenesis/synaptogenesis.

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References

Ager RR, Davis JL, Agazaryan A, Benavente F, Poon WW, LaFerla FM, Blurton-Jones M (2015) Human neural stem cells improve cognition and promote synaptic growth in two complementary transgenic models of Alzheimer's disease and neuronal loss. Hippocampus 25:813–826

Anghileri E, Marconi S, Pignatelli A, Cifelli P, Galie M, Sbarbati A, Krampera M, Belluzzi O, Bonetti B (2008) Neuronal differentiation potential of human adiposederived mesenchymal stem cells. Stem Cells Dev 17:909–916

Bae JS, Jin HK, Lee JK, Richardson JC, Carter JE (2013) Bone marrow-derived mesenchymal stem cells contribute to the reduction of amyloid-β deposits and the improvement of synaptic transmission in a mouse model of pre-dementia Alzheimer's disease. Curr Alzheimer Res 10:524–531

Barkholt L, Flory E, Jekerle V, Lucas-Samuel S, Ahnert P, Bisset L, Buscher D, Fibbe W, Foussat A, Kwa M, Lantz O, Maciulaitis R, Palomaki T, Schneider CK, Sensebe L, Tachdjian G, Tarte K, Tosca L, Salmikangas P, Capilla-Gonzalez V, Lavell E, Quinones-Hinojosa A, Guerrero-Cazares H (2015) Regulation of subventricular zone-derived cells migration in the adult brain. Adv Exp Med Biol 853:1–21

Bash D, Song L, Tuan RS (2004) Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 8:301–316

Birch AM, Katsouri L, Sastre M (2014) Modulation of inflammation in transgenic models of Alzheimer's disease. Neuroinflammation 11:25

Blurton-Jones M, Kitazawa M, Martinez-Coria H, Castello NA, Muller FJ, Loring JF, Yamasaki TR, Poon WW, Green KN, LaFerla FM (2009) Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. Proc Natl Acad Sci U S A 106:13594–13599

Burns A, Iliffe S (2009) Alzheimer's disease. BMJ 338:b158

Capilla-Gonzalez V, Herranz-Perez V, Garcia-Verdugo JM (2015) The aged brain: genesis and fate of residual progenitor cells in the subventricular zone. Front Cell Neurosci 9:365

Cassar P, Blundell R (2016) The use of umbilical stem cells. Open J Pathol 6:41–56

Cerri S, Greco R, Levandis G, Ghezzi C, Mangione AS, Fuzzati-Armentero MT, Bonizzi A, Avanzini MA, Maccario R, Blandini F (2015) Intracarotid infusion of mesenchymal stem cells in an animal model of Parkinson's disease, focusing on cell distribution and neuroprotective and behavioral effects. Stem Cells Transl Med 4:1073–1085

Chen X, Wang S, Cao W (2017) Mesenchymal stem cell medicated immunomodulation in cell therapy of neurodegenerative diseases. Cell Immunol. pii: S0008–8749(17)30101–6. doi: https://doi.org/10.1016/j.cellimm.2017.06.006. [Epub ahead of print]

Cheng SH, Yu D, Tsai HM, Morshed RA, Kanojia D, Lo LW, Leoni L, Govind Y, Zhang L, Aboody KS, Lesniak MS, Chen CT, Balyasnikova IV (2015) Dynamic in vivo SPECT imaging of neural stem cells functionalized with radiolabeled nanoparticles for tracking of glioblastoma. J Nucl Med 57:279–284

Chung J, Yang PC (2010) Molecular imaging of stem cell transplantation in myocardial disease. Curr Cardiovasc Imaging Rep 3:106–112

Danielyan L, Beer-Hammer S, Stolzing A, Schafer R, Siegel G, Fabian C, Kahle P, Biedermann T, Lourhmati A, Buadze M, Novakovic A, Proksch B, Gleiter CH, Frey WH, Schwab M (2014) Intranasal delivery of bone marrow-derived mesenchymal stem cells, macrophages, and microglia to the brain in mouse models of Alzheimer's and Parkinson's disease. Cell Transplant 23(Suppl 1):S123–S139

Desikan RS, Cabral HJ, Hess CP, Dillon WP, Glastonbury CM, Weiner MW, Schmansky NJ, Greve DN, Salat DH, Buckner RL, Fischl B (2009) Alzheimer's Disease Neuroimaging Initiative. Automated MRI measures identify individuals with mild cognitive impairment and Alzheimer's disease. Brain 132:2048–2057

Ehrhart J, Darlington D, Kuzmin-Nichols N, Sanberg CD, Sawmiller DR, Sanberg PR, Tan J (2016) Biodistribution of infused human umbilical cord blood cells in Alzheimer's disease-like murine model. Cell Transplant 25:195–199

Ende N, Chen R, Ende-Harris D (2001) Human umbilical cord blood cells ameliorate Alzheimer's disease in transgenic mice. J Med 32:241–247

Ennis WJ, Sui A, Bartholomew A (2013) Stem cells and healing: impact on inflammation. Adv Wound Care (New Rochelle) 2:369–378

Esmaeilzade B, Nobakht M, Joghataei MT, Rahbar Roshandel N, Rasouli H, Samadi Kuchaksaraei A, Hosseini SM, Najafzade N, Asalgoo S, Hejazian LB, Moghani GF (2012) Delivery of epidermal neural crest stem cells (EPI-NCSC) to hippocamp in Alzheimer's disease rat model. Iran Biomed J 16:1–9

Garcia KO, Ornellas FL, Martin PK, Patti CL, Mello LE, Frussa-Filho R, Han SW, Longo BM (2014) Therapeutic effects of the transplantation of VEGF overexpressing bone marrow mesenchymal stem cells in the hippocampus of murine model of Alzheimer's disease. Front Aging Neurosci 6:30

Hampton DW, Webber DJ, Bilican B, Goedert M, Spillantini MG, Chandran S (2010) Cell-mediated neuroprotection in a mouse model of human tauopathy. J Neurosci 30:9973–9983

Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, Li J, Zhang G, Huang J, Lin Z, Xiong N, Wang T (2016) Exosomes and their therapeutic potentials of stem cells. Stem Cells Int 7653489

Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology 80:1778–1783

Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci 28:8354–8360

Huang T, He D, Kleiner G, Kuluz J (2007) Neuron-like differentiation of adipose-derived stem cells from infant piglets in vitro. J Spinal Cord Med 30(Suppl 1):S35–S40

Jeynes B, Provias J (2011) The case for blood-brain barrier dysfunction in the pathogenesis of Alzheimer's disease. J Neurosci Res 89:22–28

Juan Yang, Song Li, Xi-Biao He, Cheng Cheng, Weidong Le (2016) Induced pluripotent stem cells in Alzheimer's disease: applications for diseasemodeling and cell-replacement therapy. Mol Neurodegener. 2016; 11: 39. Published online May 17. doi: https://doi.org/10.1186/s13024-016-0106-3

Kamiya N, Ueda M, Igarashi H, Nishiyama Y, Suda S, Okubo S, Inaba T, Katayama Y (2013) In vivo monitoring of arterially transplanted bone marrow mononuclear cells in a rat transient focal brain ischemia model using magnetic resonance imaging. Neurol Res 35:573–579

Kim JS, Kim YH, Kim JH, Kang KW, Tae EL, Youn H, Kim D, Kim SK, Kwon JT, Cho MH, Lee YS, Jeong JM, Chung JK, Lee DS (2012) Development and in vivo imaging of a PET/MRI nanoprobe with enhanced NIR fluorescence by dye encapsulation. Nanomedicine (Lond) 7:219–229

Kim KS, Kim HS, Park JM, Kim HW, Park MK, Lee HS, Lim DS, Lee TH, Chopp M, Moon J (2013) Long-term immunomodulatory effect of amniotic stem cells in an Alzheimer's disease model. Neurobiol Aging 34:2408–2420

Kim JA, Ha S, Shin KY, Kim S, Lee KJ, Chong YH, Chang KA, Suh YH (2015a) Neural stem cell transplantation at critical period improves learning and memory through restoring synaptic impairment in Alzheimer's disease mouse model. Cell Death Dis 6: e1789

Kim HJ, Seo SW, Chang JW, Lee JI, Kim CH, Chin J, Choi SJ, Kwon H, Yun HJ, Lee JM, Kim ST, Choe YS, Lee KH, Na DL (2015b) Stereotactic brain injection of human umbilical cord blood mesenchymal stem cells

in patients with Alzheimer's disease dementia: a phase 1 clinical trial. Alzheimers Dement (NY) 1:95–102
Kimbrel EA, Lanza R (2015) Current status of pluripotent stem cells: moving the first therapies to the clinic. Nat Rev Drug Discov 14:681–692
Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R (2016) The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. Cytotherapy 18:13–24
Kondo T, Asai M, Tsukita K, KutokuY OY, Sunada Y, Imamura K, Egawa N, Yahata N, Okita K, Takahashi K, Asaka I, Aoi T, Watanabe A, Watanabe K, Kadoya C, Nakano R, Watanabe D, Maruyama K, Hori O, Hibino S, Choshi T, Nakahata T, Hioki H, Kaneko T, Naitoh M, Yoshikawa K, Yamawaki S, Suzuki S, Hata R, Ueno S, Seki T, Kobayashi K, Toda T, Murakami K, Irie K, Klein WL, Mori H, Asada T, Takahashi R, Iwata N, Yamanaka S, Inoue H (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. Cell Stem Cell 12:487–496
Kyurkchiev D, Bochev I, Ivanova-Todorova E, Mourdjeva M, Oreshkova T, Belemezova K, Kyurkchiev S (2014) Secretion of immunoregulatory cytokines by mesenchymal stem cells. World J Stem Cells 6:552–570
Lee ST, Chu K, Jung KH, Kim SJ, Kim DH, Kang KM, Hong NH, Kim JH, Ban JJ, Park HK, Kim SU, Park CG, Lee SK, Kim M, Roh JK (2008) Anti-inflammatory mechanism of intravascular neural stem cell transplantation in hemorrhagic stroke. Brain 131:616–629
Lee JK, Jin HK, Bae JS (2009) Bone marrow-derivedmesenchymal stem cells reduce brain amyloid-beta deposition and accelerate the activation of microglia in an acutely induced Alzheimer's disease mouse model. Neurosci Lett 450:136–141
Lee JK, Jin HK, Endo S, Schuchman EH, Carter JE, Bae JS (2010) Intracerebral transplantation of bone marrow derived mesenchymal stem cells reduces amyloid-beta deposition and rescues memory deficits in Alzheimer's disease mice by modulation of immune responses. Stem Cells 28:329–343
Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, OhW YYS, Suh JG, Lee BH, Jin HK, Bae JS (2012) Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiol Aging 33:588–602
Lehtivuori H, Bhattacharya S, Angenent-Mari NM, Satyshur KA, Forest KT (2015) Removal of chromophoreproximal polar atoms decreases water content and increases fluorescence in a near infrared phytofluor. Front Mol Biosci 2:65
Li X, Liu X, Shi D, Wen X (2015) Particle systems for stem cell applications. J Biomed Nanotechnol 11:1107–1123
Liu F, Xuan A, Chen Y, Zhang J, Xu L, Yan Q, Long D (2014) Combined effect of nerve growth factor and brainderived brainderived neurotrophic factor on neuronal differentiation of neural stem cells and the potential molecular mechanisms. Mol Med Rep 10:1739–1745
Mahmoudifar N, Doran PM (2015) Mesenchymal stem cells derived from human adipose tissue. Methods Mol Biol 1340:53–64. https://doi.org/10.1007/978-1-4939-2938-2_4
Majka SM, Jackson KA, Kienstra KA, Majesky MW, Goodell MA, Hirschi KK (2003) Distinct progenitor populations in skeletal muscle are bone marrow derived and exhibit different cell fates during vascular regeneration. J Clin Invest 111:71–79
Marei HES, Althani A, Afifi N, Abd-Elmaksoud A, Bernardini CFM, Pescatori M, Maira G, Paldino E, Manni L, Casalbore P, Cenciarelli C (2013) Over-expression of hNGF in adult human olfactory bulb neural stem cells promotes cell growth and oligodendrocytic differentiation. PLoS One 8:e82206
Menon S, Shailendra S, Renda A, Longaker M, Quarto N (2016) An overview of direct somatic reprogramming: the ins and outs of iPSCs. Int J Mol Sci 17. pii, E141
Moghadam FH, Alaie H, Karbalaie K, Tanhaei S, Nasr Esfahani MH, Baharvand H (2009) Transplantation of primed or unprimed mouse embryonic stem cell-derived neural precursor cells improves cognitive function in Alzheimerian rats. Differentiation 78:59–68
Nagarajan S, Zhang Y (2011) Upconversion fluorescent nanoparticles as a potential tool for in-depth imaging. Nanotechnology 22:395101
Oh SH, Kim HN, Park HJ, Shin JY, Lee PH (2015) Mesenchymal stem cells increase hippocampal neurogenesis and neuronal differentiation by enhancing the Wnt signaling pathway in an Alzheimer's disease model. Cell Transplant 24:1097–1109
Page MJ, AL L¸o, David T, LeBeau AM, Cattaruzza F, Castro HC, VanBrocklin HF, Coughlin SR, Craik CS (2015) Non-invasive imaging and cellular tracking of pulmonary emboli by near-infrared fluorescence and positron-emission tomography. Nat Commun 6:8448
Paul G, Anisimov SV (2013) The secretome of mesenchymal stem cells: potential implications for neuroregeneration. Biochimie 95:2246–2256
Peng Y, Huang S, Cheng B, Nie X, Enhe J, Feng C, Fu X (2013) Mesenchymal stem cells: a revolution in therapeutic strategies of age-related diseases. Ageing Res Rev 12:103–115
Poduslo JF, Ramakrishnan M, Holasek SS, Ramirez-Alvarado M, Kandimalla KK, Gilles EJ, Curran GL, Wengenack TM (2007) In vivo targeting of antibody fragments to the nervous system for Alzheimer's disease immunotherapy and molecular imaging of amyloid plaques. J Neurochem 102:420–433

Poduslo JF, Hultman KL, Curran GL, Preboske GM, Chamberlain R, Marjanska M, Garwood M, Jack CR Jr, Wengenack TM (2011) Targeting vascular amyloid in arterioles of Alzheimer disease transgenic mice with amyloid β protein antibody-coated nanoparticles. J Neuropathol Exp Neurol 70, 653–661

Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E (2011) Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev 20:1297–1308

Rafii MS, Baumann TL, Bakay RA, Ostrove JM, Siffert J, Fleisher AS, Herzog CD, Barba D, Pay M, Salmon DP, ChuY KJH, Bishop K, Keator D, Potkin S, Bartus RT (2014) A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease. Alzheimers Dement 10:571–581

Rohban R, RudolfPieber T (2017) Mesenchymal stem and progenitor cells in regeneration: tissue specificity and regenerative potential. Stem Cells Int 2017:1–16. https://doi.org/10.1155/2017/5173732

Romanov YA, Svintsitskaya VA, Smirnov VN (2003) Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 21:105–110

Rosemann A (2015) Stem cell treatments for neurodegenerative diseases: challenges from a science, business and healthcare perspective. Neurodegener Dis Manag 5:85–87

Rosenholm JM, Gulin-Sarfraz T, Mamaeva V, Niemi R, Ozliseli E, Desai D, Antfolk D, von Haartman E, Lindberg D, Prabhakar N, Nareoja T, Sahlgren C (2016) Prolonged dye release from mesoporous silica-based imaging probes facilitates long-term optical tracking of cell populations in vivo. Small 12:1578–1592

Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P (2009) Role and therapeutic potential of VEGF in the nervous system. Physiol Rev 89:607–648

Salem H, Pesso N, Colpo G, Teixeira AL (2016) Moving from the dish to the clinical practice: a decade of lessons and perspectives from the pre-clinical and clinical stem cell studies for Alzheimer's disease. J Alzheimers Dis 53(3):1209–1230. https://doi.org/10.3233/JAD-160250

Shin JW, Lee JK, Lee JE, Min WK, Schuchman EH, Jin HK, Bae JS (2011) Combined effects of hematopoietic progenitor cell mobilization from bone marrow by granulocyte colony stimulating factor and AMD3100 and chemotaxis into the brain using stromal cell-derived factor-1_ in an Alzheimer's disease mouse model. Stem Cells 29:1075–1089

Smith DK, He M, Zhang CL, Zheng JC (2016) The therapeutic potential of cell identity reprogramming for the treatment of aging-related neurodegenerative disorders. Prog Neurobiol 157:212–229. https://doi.org/10.1016/j.pneurobio.2016.01.006

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676

Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, Sasaki T, Sasada S, Shinko A, Wakamori T, Okazaki M, Kondo A, Agari T, Borlongan CV, Date I (2015) Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: analyses of therapeutic time window and its mechanisms. PLoS One 10:e0127302

Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE (2004) Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. Stroke 35:2390–2395

Villa C, Erratico S, Razini P, Farini A, Meregalli M, Belicchi M, Torrente Y (2011) In vivo tracking of stem cell by nanotechnologies: future prospects for mouse to human translation. Tissue Eng Part B Rev 17:1–11

Von der Haar K, Lavrentieva A, Stahl F, Scheper T, Blume C (2015) Lost signature: progress and failures in in vivo tracking of implanted stem cells. Appl Microbiol Biotechnol 99:9907–9922

Walker JR, Pacoma R, Watson J, Ou W, Alves J, Mason DE, Peters EC, Urbina HD, Welzel G, Althage A, Liu B, Tuntland T, Jacobson LH, Harris JL, Schumacher AM (2013) Enhanced proteolytic clearance of plasma A_ by peripherally administered neprilysin does not result in reduced levels of brain Aβ in mice. J Neurosci 33:2457–2464

Wegner KD, Hildebrandt N (2015) Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors. Chem Soc Rev 44:4792–4834

Wenk GL (2003) Neuropathologic changes in Alzheimer's disease. J Clin Psychiatry 64(Suppl 9):7–10

Yang H, Yang H, Xie Z, Wei L, Bi J (2013) Systemic transplantation of human umbilical cord derived mesenchymal stem cells-educated T regulatory cells improved the impaired cognition in A_PPswe/PS1dE9 transgenic mice. PLoS One 8:e69129

Yang B, Brahmbhatt A, Nieves Torres E, Thielen B, McCall DL, Engel S, Bansal A, Pandey MK, Dietz AB, Leof EB, DeGrado TR, Mukhopadhyay D, Misra S (2016) Tracking and therapeutic value of human adipose tissue derived mesenchymal stem cell transplantation in reducing venous neo-intimal hyperplasia associated with arterio-venous fistula. Radiology 279:513–522

Yun HM, Kim HS, Park KR, Shin JM, Kang AR, il Lee K, Song S, Kim YB, Han SB, Chung HM, Hong JT (2013) Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1-42-infused mouse model of Alzheimer's disease. Cell Death Dis 4:e958

Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, Chen FF, Jiang XD (2013) Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation 10:106

Zhang W, Gu GJ, Shen X, Zhang Q, Wang GM, Wang PJ (2015) Neural stem cell transplantation enhances mitochondrial biogenesis in a transgenic mouse model of Alzheimer's disease-like pathology. Neurobiol Aging 36:1282–1292

Zhang Q, Wu HH, Wang Y, Gu GJ, Zhang W, Xia R (2016) Neural stem cell transplantation decreases neuroinflammation in a transgenic mouse model of Alzheimer's disease. J Neurochem 136:815–825

Zhao JY, Chen G, Gu YP, Cui R, Zhang ZL, Yu ZL, Tang B, Zhao YF, Pang DW (2016) Ultrasmall magnetically engineered Ag2Se quantum dots for instant efficient labeling and whole-body high-resolution multimodal real-time tracking of cell-derived microvesicles. J Am Chem Soc 138:1893–1903

Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, Vitek MP, Hovanesian V, Stopa EG (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging 28:977–986

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Developments in Hematopoietic Stem Cell Expansion and Gene Editing Technologies

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Abstract

Hematopoietic stem cells (HSCs) are rare cells, which housed in the adult bone marrow. They maintain all types of differentiated blood cells throughout life. Due to limited availability of HSCs for transplantation, treatment of various inherited bone marrow disorders and anemia requires the development of HSC expansion and gene editing technologies. To this end, various studies addressed the use of cytokines and growth factors for HSC expansion. Major hurdle with these studies was found to be spontaneous differentiation of HSCs into different lineages during ex vivo procedure. In addition, cost efficient approaches were needed. Thus, studies move on to the identification of small molecules and development of RNA interference technologies with potential to enhance cell cycle progression and block inhibitory signaling mechanisms during ex vivo HSC expansion as well as single cell expansion of HSCs following gene editing studies. This review aims to highlight developments in hematopoietic stem cells expansion and gene editing technologies.

Keywords

HSC expansion · Small molecules · Cell cycle progression · Single cell expansion · Gene editing

Highlights

- Small molecules targeting HSC quiescence provide robust ex vivo HSC expansion
- miRNAs and their inhibition could be further exploited to prevent of HSC differentiation post expansion
- HSCs expansion from umbilical cord blood and transplantation to patients proved to be clinically safe.
- Single cell selection post gene editing in blood disorders associated with HSCs and single HSC expansion from human bone marrow HSCs require development of HSC expansion technologies to be employed for autologous gene-edited HSC transplantations

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1 Introduction

Hematopoietic stem cells (HSCs) have the capability to differentiate into all specialized blood

cell types via a process called hematopoiesis. Self-renewal of HSCs prevents depletion of HSCs and supports lifetime hematopoiesis. HSCs help in maintaining body functions via altering the composition of specialized blood cell types such as B cells or T cells after an environmental response (Eaves 2015). In addition, studies showed the differentiation capacity of HSCs into non-hematopoietic cell types like cardiomyocytes (Wu et al. 2015). It is substantial to keep HSCs in their steady state to prevent unnecessary differentiation and depletion. In an adult mammalian system, stem cells are preserved in stem cell niches, microenvironments that preserve HSCs with undifferentiated and self-renewal characteristics. This preservation in the stem cell niche is mediated through cellular interactions with osteoblasts or endothelial cells via adhesion proteins and signaling pathways (Morrison and Scadden 2014).

Since HSCs could repopulate whole blood cells, these cells have been used for treatment of malignancies and hematological diseases (Mo et al. 2015; Talano and Cairo 2015). These diseases include but not limited to leukemia, myelodysplastic syndrome, anemia, immune system or metabolic disorders (Kurtzberg 2009; Kim et al. 2014). HSC transplantation is also known as bone marrow transplantation (BMT). However, for an efficient transplantation, donor's human leukocyte antigen (HLA) profile should match with the recipient's (Morishima et al. 2015). This requirement hinders the efficiency of bone marrow transplantation and could cause loss of patients. Historically, transplantations have generally been performed bone marrow since HSCs are at a density of 1 in 10,000 bone marrow cells while 1 in every 100,000 blood cell (Ng et al. 2009). However, in recent years, collection of HSCs from peripheral blood following mobilization from bone marrow into blood have been preferred and become widely accepted procedure for HSC transplantations. Several advantages of HSCs derived from peripheral blood (PB) and umbilical cord blood (UCB) have been shown when compared to bone marrow transplantation; which made them to be used as the common sources for HSCs in clinical applications. Rate of graft versus host disease and possibility of viral infection in UCB transplantation are lower in comparison to BMT. However, the number of HSC yield isolated from UCB is insufficient for many cases (Narimatsu et al. 2005; MacMillan et al. 2009). With current technologies, UCB is still promising but an inadequate source for HSC transplantation (Delaney et al. 2010a, b). Lack of sufficient HSCs from UCB drove researchers to develop new systems for expansion of HSCs. Although growth factors and cytokines have been widely studied, disadvantages such as inefficiency in maintaining of HSCs in undifferentiated state have been reported (Allouche and Bikfalvi 1995). Thus, identification of hematopoietic stem cell factors that take role in cell cycle progression and maintaining HSCs in an undifferentiated state become a crucial step. These factors may represent new targets for *ex vivo* HSC expansion that were isolated from any source to overcome limitations of current clinical practices.

2 Hematopoietic Stem Cell Expansion

HSC expansion involves HSCs isolation from any source and stimulation of proliferation HSC *ex vivo* (Aggarwal et al. 2012). Human HSCs express markers of CD34, CD133, CD90 and lack CD38, that are used to separate them from rest of the bone marrow cell populations (Notta et al. 2011; Wisniewski et al. 2011). HSC cultures have been established and optimized for *ex vivo* expansion after stimulation of different factors (Sauvageau et al. 2004). In culture conditions, HSCs demonstrate the capability to form long-term culture initiating cells (LTC-ICs). In 1996, LTC-IC assay has been introduced to measure quantity of HSCs. In addition to LTC-IC assay, quantification of HSCs after expansion can be mediated by using flow cytometry and competitive repopulation methods (Frisch and Calvi 2014).

Several cell-extrinsic and cell-intrinsic factors have been identified so far as effective regulators

of HSC self-renewal and expansion *in vivo* (Wagner et al. 2016). These extrinsic and intrinsic effects are imitated by *in vitro* studies to generate signals leading to *ex vivo* expansion of HSCs as well. Homeobox B4 (HoxB4) gene is one of the intrinsic factors known as a regulator of HSC fate determination. Previous studies revealed that *in vivo* overexpression of HoxB4 results in about 50-fold increase in HSC expansion (Sauvageau et al. 1995) while the *ex vivo* overexpression of the same gene leads to 40-fold increased expansion of murine HSCs (Antonchuk et al. 2002). In addition to the studies displaying its role in the regulation of the self-renewal of HSCs, Hoxb4$^{-/-}$ mice showed a reduced cellularity in bone marrow and spleen, as well as a defective proliferative ability in another study (Brun et al. 2004).

However, cell-intrinsic gene manipulation studies should avoid direct introduction of the genetic material inducing HSC expansion, because the self-renewal mechanism of HSCs could not be controlled properly and continuous activation of self-renewal may eventually cause some abnormalities like HSC exhaustion and malignant transformation[88]. Therefore, instead of a direct manipulation of the genetic material, administration of hematopoietic cytokines, growth factors, miRNAs or small molecules adjusting the activity of these intrinsic factors might be a safer method for the expansion of HSCs (Allouche and Bikfalvi 1995).

Although cytokines and growth factors have been used widely, it is also known that they induce HSC differentiation. Therefore, it is necessary to identify factors that target only HSC expansion without causing HSC differentiation. Another disadvantage of cytokines and growth factors is that productions of these proteins are costly and time-consuming. Thus, studies regarding identification of hematopoietic small molecules (HSM) have emerged since they can be easily produced and cost efficient. HSMs are capable of penetrating into the cell and target cytoplasmic components of an intrinsic pathway unlike growth factors or cytokines. Besides, miRNA induced HSC expansion has been explored recently. Although some of the studies regarding application of these miRNAs showed efficient HSC expansion, more studies are needed for these components to take role in regular HSC expansion procedures.

3 Cytokines and Growth Factors Involved in HSC Expansion

Cytokines and growth factors constitute a large group of signaling molecules that regulate proliferation, differentiation, migration, growth and apoptosis (Park 1996; Seita and Weissman 2010; Endele et al. 2014). In addition to common features of cytokines and growth factors, they can mediate mobilization of HSCs (Shaughnessy et al. 2013). However, they generally fail to stimulate self-renewal by themselves (Lui et al. 2014). In addition, they cause HSCs to differentiate into blood cell lineages, which may eventually result in a decrease in the number of HSCs in ex vivo expansion pool (Zumkeller 2002; Isufi et al. 2007; Jenkins et al. 2012).

To compensate the undesired outcomes in the culture, researchers have developed the HSC expansion mixtures that include a set of growth factors, cytokines and other components (Table 1). As one of the first identified cytokines used in HSC expansion, stem cell factor (SCF) remained as an essential component in many mixtures (Zsebo et al. 1990). Its proliferative effect on hematopoietic cells was firstly identified in hematopoietic progenitor cells (HPCs) *in vitro* and its effect on HSC has been further investigated *in vivo* studies (de Vries et al. 1991; Migliaccio et al. 1991; Andrews et al. 1992; Galli et al. 1993). Although there have been results suggesting that SCF can be used for HSC expansion, several other studies opposed to this idea due to low efficiency of SCF when administrated alone (de Vries et al. 1991; Metcalf and Nicola 1991; Cicuttini et al. 1992). However, this inefficiency has been reported to be compensated when SCF is administrated with other cytokines such as erythropoietin (EPO), interleukin-3 (IL-3) or interleukin-6 (IL-6) (Mohamed et al. 2006). Taken together, idea of SCF as an important

Table 1 Composition of HSC expansion cocktails that passed or being evaluated in clinical trials

Composition of HSC Expansion Cocktail	Expansion of TNC	Expansion of CD34+ cells	Days in culture	References
SCF, G-CSF, MGDF	56-fold (Up to 278- fold)	4-fold (Up to 20- fold)	10	Shpall et al. (2002)
FBS, horse serum, PIXY321, Flt-3 ligand, erythropoietin	Up to 8.5-fold	Up to 2.45-fold	12	Jaroscak et al. (2003)
SCF, FLT-3 ligand, IL-6, thrombopoietin, TEPA	219-fold (Up to 620- fold)	6-fold	21	de Lima et al. (2008)
SCF, Flt-3 ligand, thrombopoietin, IL6, NAM	486-fold (Up to 643- fold)	72-fold (Up to 186- fold)	21	Horwitz et al. (2014)
SCF, FLT-3 ligand, thrombopoietin, IL6, SR1	854-fold (Up to 2121- fold)	330 fold (Up to 848- fold)	15	Wagner et al. (2016)
IL-3, IL6, thrombopoietin, Flt-3 ligand, SCF, fibronectin, Notch ligand ($Delta^{ext\text{-}IgG}$)	789-fold (Up to 2161- fold)	184-fold (Up to 688- fold)	16	Delaney (2010a, b)

TNC total nucleated cells. *SCF* stem cell factor, *G-CSF* granulocyte colony-stimulating factor, *MGDF* megakaryocyte growth and differentiation factor

factor HSC expansion is still controversial since many procedures involves use of SCF with other cytokines and these applications trigger hematopoiesis and eventually decrease number of long-term HSCs (Ema et al. 2000).

Thrombopoietin (TPO) like interleukin cytokines and SCF have been used in HSC expansion mixtures as well. Similar to SCF, it has the disadvantage of insufficiently induce HSC expansion when administrated alone. Thus, its activity is dependent on the presence of other cytokines (Sitnicka et al. 1996; Goff et al. 1998; Murray et al. 1999). In addition, TPO suppresses apoptosis and promote megakaryocyte generation (Kaushansky 1995; Eaton and de Sauvage 1997; Yagi et al. 1999; Gilmore et al. 2000; Nikougoftar Zarif et al. 2011). Several studies reported that TPO and other cytokines induced proliferation of adult repopulating stem cells (SRCs). Thus, TPO is not selectively induce proliferation of HSCs, rather it induces proliferation of wide range of hematopoietic cells (Gammaitoni et al. 2003; Kaushansky 2003; Wang et al. 2004). Moreover, it has been reported that, expanded HSCs following TPO treatment is not sufficient to restore HSC populations following transplantation (Von Drygalski et al. 2004). Many interleukin family members have been used for HSC expansion *in vitro*. Among the interleukin cytokine family members, IL-6 and IL-3 have been used widely. Effect of IL-11 have also been investigated and its ability to expand HSCs have been reported (Audet et al. 2002). However, contribution of IL-11 to HSC proliferation has been lower in comparison to IL-6 and IL-3 factors (Breems et al. 1997; Von Drygalski et al. 2004; Bordeaux-Rego et al. 2010). Nonetheless, these cytokines take role as co-activators of other cytokines and inefficient in stimulation of HSC expansion by themselves.

Granulocyte colony-stimulating factor (G-CSF) have also been applied for *ex vivo* expansion of HSCs. When administrated with several other cytokines, HSC expansion has been induced (Breems et al. 1997). In addition, it promoted mobilization and proliferation of HSCs *in vivo* (Morrison et al. 1997; Uchida et al. 1997). However, it has been hypothesized that long-term application of G-CSF could lead to cancer formation (Rosenberg et al. 2006). This hypothesis has been supported with another study in which DNA damage after G-CSF treatment has been detected (Souza et al. 2014). G-CSF has not been identified as a suitable agent for HSC expansion, it is rather preferred for HSC mobilization (Ott et al. 2002). The important use of G-CSF is the mobilization of stem cells for use in allogeneic hematopoietic stem cell transplantation for clinical uses.

Wnt signaling pathway has been previously shown as an effective mediator of HSC self-

renewal and repopulation, as well as human HSC engraftment in mice (Murdoch et al. 2003; Reya et al. 2003). Wnt signaling is an essential pathway that regulates the fate of various types of cells including HSCs (Logan and Nusse 2004). Wnt3a and Wnt5a are the common Wnt signaling proteins that are involved in the regulation of HSC fate. Although HSC self-renewal can be induced by the addition of Wnt3a to HSC culture (Audet et al. 2002; Breems et al. 1997; Bordeaux-Rego et al. 2010; Uchida et al. 1997; Morrison et al. 1997), which may lead to an increase in the number of HSCs in the stem cell pool, constitutive activation of the canonical Wnt signaling pathway (or known as Wnt/β-catenin signaling pathway) through binding of Wnt proteins to its receptor ends up with the defects in multilinege differentiation and in repopulation capacity of HSCs (Rosenberg et al. 2006). However, downregulation of Wnt/β-catenin signaling by Wnt5a, a member of non-canonical Wnt pathway, leads to the recovery of the repopulating ability of HSC (Souza et al. 2014). On the other hand, Reya *et al*. defined an intercourse between Wnt and Notch signaling pathways, showing induced self-renewal of HSCs by activation of Wnt signaling is linked to increased expression levels of HoxB4 and Notch-1 (Audet et al. 2002), suggesting that different signaling pathways work cooperatively in the regulation of HSC expansion.

Another important signaling mechanism that takes role in hematopoiesis and HSC proliferation is Notch signaling. Over-activation of Notch signaling cascade often results with blockage in differentiation process coupled with enhanced proliferation (Yuan et al. 2015). Notch ligands Delta-1 and Jagged-1 have been shown take role in hematopoiesis and proliferation of HSCs. Delta-1 reduced number of myeloid cells in culture leading to increased rate of proliferation and recovery of HSCs *in vivo* and *in vitro* (Karanu et al. 2001; Pelus and Singh 2014). This decrease in myeloid cells is likely originated from decrease in monocyte population since it was reported that increased Delta-1 activity drove monocytes into apoptosis (Ohishi et al. 2000). At the same time Delta-1 promotes the development of cytoplasmic CD3 + T / NK cell precursors and multifold expansion of common lymphoid progenitor cells (CD34 + CD7 + CD45RA +) (Ohishi et al. 2000). Delta-1 and HOXB4 stimulation has been demonstrated to enhance lymphoid engraftment after cord blood transplantation (Watts et al. 2013). Delta1 inhibits the thymocytes differentiated to B-cells but stimulate to consisting of T-cell population or natural killer precursors (Lehar et al. 2005). In addition, it was reported that osteoblasts increase activity of Notch signaling cascade by expressing Jagged-1 and Delta-1 genes to induce hematopoiesis in stem cell niches (Chitteti et al. 2010). Various cell lines including 3T3 have been used to synthesize Notch ligand Jagged-1, which is normally produced by stromal cells in the stem cell niche. As a result, number of primitive HSCs and progenitors increased several folds *in vitro* (Varnum-Finney et al. 1998; Jones et al. 1998). In 2000, it was demonstrated that addition of Jagged-1 directly to the HSC culture without mimicking stem cell niche-like environment increased number of HSCs and HPCs *in vitro* (Karanu et al. 2000). Despite such studies suggesting that Jagged-1 induce HSC proliferation, several studies demonstrated that Jagged-1 has no effect on HSC proliferation *in vivo* or *in vitro* (Walker et al. 1999; Mancini et al. 2005; Kertesz et al. 2006; Toda et al. 2011; Negishi et al. 2014).

Sonic hedgehog (Shh) proteins have been implicated as developmental factors regulating HSC function. Bhardwaj *et al*. reported that inhibition of hedgehog (Hh) alters cytokine-induced proliferation of human HSCs, while Shh treatment allows expansion of the repopulating human HSCs (Bhardwaj et al. 2001). Interestingly, the inhibition of bone morphogenic protein 4 (BMP4) leads to inhibition of HSC proliferation. Therefore, it has been suggested that the regulatory effect of Shh on HSC proliferation is linked to BMP4 signaling. This is consistent with the regulatory role of BMP4 on differentiation and proliferation of primitive human HSCs (Bhatia et al. 1999). Intriguingly, BMP4 has been indicated as one of the factors providing maintaining of primitive HSCs *in vitro* (Hutton et al. 2006; Grassinger et al. 2007).

Fibroblast growth factor receptors (FGFR) are highly expressed in HSCs, which increases the likelihood of fibroblast growth factor (FGF) signaling proteins to regulate HSC fate (Walasek et al. 2012). Effects of FGF on the proliferation of hematopoietic cells have been firstly demonstrated in HPCs. In the study, basic FGF (FGF-2) application have been shown to enhance colony formation of HPCs and provide radioprotection until HPCs became sensitive to FGF (Gallicchio et al. 1991). In the following years, FGF-2 has been found to induce hematopoiesis (Allouche and Bikfalvi 1995). Although colony formation of HPCs have been stimulated after FGF-2 treatment, some studies could not detect any proliferative response in HSCs. Instead of HSCs, it appears to act on lin^+ myeloid progenitors (Berardi et al. 1995). One of the very first studies regarding *in vitro* generation of HSCs via FGF has been carried out by using FGF-1. Addition of FGF-1 into serum free media elevated the number of HSCs in the culture (de Haan et al. 2003). Since several studies confirmed proliferative ability of FGF family members, researchers continued to use these growth factors for HSC expansion (Rossi et al. 2004; Zhang and Lodish 2005; Yeoh et al. 2006). However, like most of the growth factors, FGF is generally used with other cytokines to induce proliferation of HSCs.

Insulin-like growth factor (IGF) family members have also been marked as one of the growth factors that induce HSC cell cycle progression. To date, several studies showed IGF proteins promote hematopoietic cell or hematopoietic stem cell expansion with or without stromal environment (Sanders et al. 1993; Okajima et al. 1998; DiFalco et al. 2003; Zhang and Lodish 2005). These findings are correlated with oncogenic properties of IGF proteins determined in leukemia (Xie et al. 2015a, b). Angiopoietin-like proteins (Angptls) have been added to usual HSC expansion mixtures to enhance the efficiency of HSC expansion in some studies. Introduction of Angptl2, Angptl3, Angptl5 and Angptl7 to the usual mixtures were resulted with up to 30-fold increase in HSCs whereas use of the same mixture without any Angptl caused only fivefold expansion (Zhang et al. 2006, Zheng et al. 2011). Since the first impression of use of Angptls was promising, studies regarding the effect of these proteins on HSC expansion have been continued (Zhang et al. 2008). It was revealed that Angptl3 is expressed by endothelial cells in hematopoietic stem cell niche to maintain proliferative ability of HSCs (Zheng et al. 2011). Similar to Angptl3, Angptl7 has also been marked as an essential factor for HSC repopulation (Xiao et al. 2015). However, it has also been demonstrated that Angptls rely on activity of other growth factor for stimulation of HSCs proliferation since they could not expand HSCs by themselves (Akhter et al. 2013). Studies suggested that Angptls regulate a signaling pathway that cannot be regulated by other cytokines or developmental factors. Still, these proteins remarkably increase HSC expansion when administrated with expansion mixtures (Farahbakhshian et al. 2014).

Growth factors and cytokines are still used for HSCs expansion. However, none of these factors could show a remarkable impact on HSC proliferation when administrated alone. Thus, they are generally administrated as expansion mixtures. Moreover, use of these mixtures has the possibility to induce HSC differentiation or increase sensitivity to the growth factors in the mixture.

4 Small Molecule Induced HSC Expansion

As mentioned previously, *ex vivo* expansion of hematopoietic stem cells using cytokines and growth factors generally ends up with differentiation or exhaustion of HSCs. Therefore, replacing or supplementing these factors with hematopoietic small molecules that have the effect of increasing self-renewal, but do not alter the functional properties of HSCs, may solve this problem. To date, a number of small molecules have been studied for their role in HSC fate and have been identified as effective compounds inducing *ex vivo* expansion of HSCs (Table 2). One of the first identified HSM is **StemRegenin1** (SR1), which targets aryl

Table 2 Hematopoietic small molecules, their targets and corresponding concentrations that used in the studies

Small molecule	Effective concentration	Target	Fold expansion	References
StemReginin1	0.75 μM	AHR	2.6 fold at d7 ($CD34^+$)	Boitano et al. (2010)
UM171	35 nM	AHR	5 fold at d12 ($CD34^+$)	Fares et al. (2014)
Garcinol	5 μM	HAT	4.5 fold at d7 ($CD34^+$)	Nishino et al. (2011)
Isogarcinol	2 μM	HAT	7.4 fold at d7 ($CD34^+$)	Nishino et al. (2011)
Valproic acid	1 mM	HDAC	2 fold at d7 ($CD34^+$)	De Felice et al. (2005)
Trichostatin A	5 ng/ml	HDAC	2 fold at d3 ($CD34^+$)	Araki et al. (2006)
5-aza-2′ deoxycytidine	10 μM	Dnmt	2 fold at d3 ($CD34^+$)	Araki et al. (2006)
Nicotine-amide	5 μM	SIRT1	−0.7 fold at d7 ($CD34^+$)	Peled et al. (2012)
UNC0638	1 μM	G9a, GLP	3 fold at day11 ($CD34^+$)	Chen et al. (2012)
NR-101	1 μg/ml	c-MPL	10 fold at d7 ($CD34^+$)	Nishino et al. (2009)
Caffeic acid phenethyl	1 μg/ml	NF-KB	2.5 fold at d7 ($CD34^+$)	Liu et al. (2014)
SB203580	5 μM	p38	8 fold at d7 (LSK)	Wang et al. (2011)
N-acetylcysteine	0.1 mM	p38	1.5 fold at d7 ($CD133^+$)	Zou et al. (2011)
Compound 40	100 nM	p18	2.61fold at d5 ($Lin^-Sca1^+CD48^-CD150^+$)	Xie et al. (2015a, b)
XIE18-6	105.5 nM	p18	1.5 fold at d5 ($Lin^-Sca1^+CD48^-CD150^+$)	Xie et al. (2015a, b)
P18IN003	20 μM	p18	3 fold at w16 (LSK)	Gao et al. (2015)
P18IN011	20 μM	p18	3.92 fold at w16 (LSK)	Gao et al. (2015)
Tetraethylenepentamine	5 μM	Copper	8.5 fold at w7 ($CD34^+$)	Peled et al. (2004)
All-trans retinoic acid	1 μM	RAR	4.3-fold increase in Sca-1^+ cell percentage	Purton et al. (1999)
CHIR-911	1 μM	GSK-3β	3 fold at d9 (LSK)	Trowbridge et al. (2006)
CHIR-837	1 mM	GSK-3β	3 fold at d9 (LSK)	Trowbridge et al. (2006)
Rapamycin	20 ng/ml	mTOR	–	Luo et al. (2014)
α-Tocopherol	40 mg/kg	ERK1/2, PKC, STAT5	2 fold w2 (LSK)	Nogueira-Pedro et al. (2011)
PD98059	10 mg/kg	AHR	No change	Nogueira-Pedro et al. (2011)
L-NIL	500 μM	NOS	5.2 fold d7 ($CD34^+$)	Reykdal et al. (1999)
Dasatinib	15 mg/kg	Tyrosine Kinase	2 fold at d4 ($CD150^+$, $CD48^-$ LSK cells)	Duyvestyn et al. (2016)
PGE_2	1 μM	CXCR4, SDF-1	4-fold	Hoggatt et al. (2009)

LSK murine Lin^- c-kit^+ Sca-1^+, *LT-HSC* murine $CD150^+$ $CD48^-$ Sca-1^+ Lin^-

hydrocarbon receptor (AHR) and inhibits its activity. This inhibition by SR1 have caused up to 73-fold increase in human BM, UCB and PB-derived CD34 expressing HSCs *ex vivo* (Boitano et al. 2010). To develop this SR1 mediated HSC expansion system, HSCs were cultured in presence of SR1 and other factors (Gori et al. 2012). In a study, it has been demonstrated that addition of Delta-1 along with SR1 increased percentage of primitive HSCs in comparison to treatment with SR1 alone. SR1 acts like a cytokine and do not

significantly suppress differentiation of HSCs that was compensated by presence of Delta-1 (Dahlberg et al. 2014). Ability of SR1 in promoting differentiation of HSCs has been shown later (Thordardottir et al. 2014; Roeven et al. 2015). SR1 has been compared with another small molecule **UM171**, and it was found that SR1 is more effective on expansion of primitive HSCs (Fares et al. 2014). Currently SR1 has been tested in phase I-II trials (Wagner et al. 2016).

An antagonist of histone acetyltransferase, **Garcinol** has also been used for HSC expansion. This plant derived molecule, along with its derivative **Isogarcinol** caused HSC expansion with 4.5 and 7.4-fold, respectively (Nishino et al. 2011). **Garcinol** has been reported as promoting HSPC expansion through histone acetyl transferase (HAT) inhibition (Araki et al. 2006). In addition, **valproic acid** (VPA), an antagonist of histone deacetylase (HDAC), has been used along with a cytokine mixture. Although mixtures including VPA showed lower total cell number *ex vivo*, addition of VPA into the expansion mixture preserved $CD34^+$ hematopoietic cells in the culture. When administrated along with Flt-3 ligand, stem cell factor and IL-3, VPA strongly preserved CD34+ cell population (32.5%) in comparison to cytokines alone (0.6%) post 3 weeks (De Felice et al. 2005). This data have confirmed by another group that VPA treatment along with growth factors increased the percentage of $CD34^+$ cells by approximately twofolds in comparison to cells cultured without VPA. In addition, it has been demonstrated that knockdown the transcription of histone deacetylase 3, a target of VPA, expanded $CD34^+$ cells *in vitro* (Elizalde et al. 2012). Another histone deacetylase inhibitor, **Trichostatin A** (TSA) has also been tested for ex vivo HSC expansion (Walasek et al. 2012; Araki et al. 2006). In this study, TSA has been added into the cytokine containing expansion mixtures with DNA methyltransferase (Dnmt) inhibitor **5-aza-2′-deoxycytidine** (5azaD). Interestingly, these small molecules enhance the proliferation of human UCB $CD34^+$ $CD90^+$ cells more than ninefold. It was demonstrated that this proliferative response could be triggered by 5azaD/TSA's alteration in methylation patterns of CpG sites of γ-globin promoter and acetylation of histone 4 (Araki et al. 2006). Another HDAC inhibitor, **Nicotinamide** (NAM) was also identified as a potential small molecule for HSC expansion via suppressing the activity of SIRT1. After addition of this compound into the cytokine mixture, total cell number increased by 400-fold and $CD34^+$ cells increase 80-fold (Peled et al. 2012). Moreover, the number of differentiated cells was significantly decreased; differentiated hematopoietic cells showed lower proliferative response compared to non-NAM treated cells. Homing of NAM-treated $CD34^+$ HPCs following transplantation has increased by threefold (Peled et al. 2012). Like HDAC or HAT proteins, methyl transferase enzymes take role in HSC proliferation and hematopoiesis. G9a and GLP methyltransferase proteins have been marked with their ability to induce differentiation. It was observed that inhibition of these proteins by **UNC063** in a cytokine mixture delayed differentiation and increased proportion of $CD34^+$ in HSPC pool by more than twofold in comparison to cell population treated with only cytokines after day 11 (Chen et al. 2012). Intriguingly, *in vitro* treatment with SR1 and UNC0638 together doubled the expanded $CD34^+$ cells at the end of day 14 (Chen et al. 2012).

Proliferative ability of TPO in HSCs has been shown previously via binding to c-MPL (Gilmore et al. 2000). Based on this information, **NR-101**, a small molecule that is agonist to c-MPL has been used for HSCs expansion. Interestingly, HSCs have been expanded more than twofold with NR-101 treatment (Nishino et al. 2009). NR-101 promotes expression of Hif-1α that induces HSC survival and proliferation. Same effect has been achieved by using another small molecule, **Caffeic acid phenethyl** (CAPE) whose administration with other cytokines increased the percentage of $CD34^+$ cells more than threefold without changing total number of cells significantly (Liu et al. 2014). I was found that CAPE increased expression of SCF and HIF-1α that led to expansion of HSPCs (Liu et al. 2014).

Since p38 is a tumor suppressor protein and can cause cell cycle arrest, it has been hypothesized that inhibition of p38 may result in HSC expansion. Treatment with **SB203580, an inhibitor of p38,** elevated the murine LSK^+ HSPC number more than eightfold (Wang et al. 2011). In addition, decreased activity of p38 and reduced levels of the reactive oxygen species (ROS) by treatment of **N-acetylcysteine** (NAC-an anti oxidant) has been achieved. Addition of NAC into the HSC culture caused approximately 15-fold increase of $CD133^+$ cells (Zou et al. 2011). Studies indicated that p18 upregulation is associated with HSC quiescence (Sirin et al. 2010) p18, which inhibits various cyclin dependent kinases, has been targeted to induce HSC expansion. Inhibition of p18 activity has been achieved by using **compound 40,** an analog of another p18 inhibitor **XIE18-6**. Inhibition via addition of compound 40 was resulted with more than twofold increase in long term hematopoietic stem cells specifically by regulating CDK4/6 activity (Xie et al. 2015a, b). Other p18 inhibitors have been tested *in vitro* include **P18IN003** and **P18IN011.** They increased the frequency of LSK cells by up to 4.2-fold (Gao et al. 2015).

Copper (Cu) has been indicated as a potential effector of HSC fate. **Tetraethylenepentamine** (TEPA) has been discovered to induce HSC proliferation via Cu chelation. This ability has been related to suppression of differentiation and increased engraftment of HSCs since both $CD34^+CD38^-$ and $CD34^+CD38^-Lin^-$ cells were higher after TEPA treatment (Peled et al. 2004).

TGF-β has also targeted by its inhibitor, **SB431542,** to increase HSPC yield in a fed-batch system. Addition of cytokines with **SB431542** enhanced the proliferation of HSPCs in early culture whereas this effect is lost after day 12 which could be an effect of fed-batch system (Csaszar et al. 2012).

All-trans retinoic acid (ATRA) is the retinoic acid receptor agonist, effects of which on HSC fate have been reported previously (Purton et al. 1999, 2000). Presence of ATRA in the culture media containing murine $Lin^-Sca\text{-}1^+$ hematopoietic precursors showed increased repopulating ability after day 7 and 14. Moreover, addition of RAR antagonist AGN193109 significantly decreased this ability. These findings suggest that ATRA can be used for HSC expansion not as a proliferation stimulating factor but as an inhibitor of differentiation (Purton et al. 2000). Furthermore, the inhibition of the retinoid signaling induced self-renewal of human HSCs *in vitro,* suggesting retinoic acid as a negative regulator of *ex vivo* HSC expansion (Chute et al. 2006; Szatmari et al. 2010).

We have previously mentioned that activation of Wnt pathway results with HSC expansion by inactivation of GSK3β. Instead of treating HSCs with Wnt proteins, the same effect has been achieved by using small molecule **CHIR-911** and **CHIR-837** (Trowbridge et al. 2006). *In vivo* application of CHIR-911 following GFP labeled HSC transplantation enhanced the ratio and number of $CD34^+$ cells by more than fourfold (ref). This treatment without transplantation has increased LSK cell population among pre-existing bone marrow cells. In addition to CHIR-911, effect of CHIR-837 was also tested on in vitro culture of murine LSK HSPCs. In presence of CHIR-837, murine LSK cells expanded 1.5-fold. After application of these inhibitors, it has been observed that Wnt, Notch and Hedgehog pathways were activated compared to controls whereas Hedgehog pathway has been repressed and become normalized in long term (Trowbridge et al. 2006).

It has been suggested that inhibition of mTOR pathway may be used for HSC expansion by preventing differentiation (Huang et al. 2012). Following rapamycin treatment, frequency of phosphorylated p70 ribosomal protein s6 kinase 1 (S6K1), which is an indicator for mTOR activation, has been reduced in a time-depended manner. In addition, number of LSK cells ex vivo increased significantly after day 10 without changing the total number of cells in comparison to DMSO treated controls (Luo et al. 2014).

α-Tocopherol has been marked as a proliferative HSM. It has been demonstrated that application of this molecule *in vivo* increases

percentage of murine LSK cells over total cells by twofold. However, it has been associated with an increase in Mac-1^+Gr-1^+ cell populations through ERK1/2-mediated differentiation. Production of differentiated cells has been abolished by **PD98059** treatment. Following treatment with both of these HSMs, frequency of LSK cells increased significantly compared with only α-Tocopherol treated mice. Besides, differentiation rate has been normalized (Nogueira-Pedro et al. 2011).

After it has been demonstrated that production of free radical Nitric Oxide (NO) is resulted with inhibition of hematopoiesis, its effect on proliferation has also been tested (Maciejewski et al. 1995). By using nitric oxide synthase inhibitor (iNOS) **L-N^6-(1-iminoethyl)-lysine hydrochloride** (L-NIL), $CD34^+$ cell composition has been increased slightly following decrease in nitric oxide. In conclusion, handling of oxygen tension and NO inhibition can induce HSC expansion (Reykdal et al. 1999).

Dasatinib, previously known as **BMS-354825**, is another small molecule that shows promising results in HSC expansion. Dasatinib is known with its anti-tyrosine kinase abilities and used to treat myeloid leukemia. Intriguingly, following administration of this compound in vivo with doses of 15 mg/kg, it has been observed that this application increased proportion of Ki-67^+ LT-HSCs by twofold after 4 days. However, this effect is lost significantly after day 8 (Duyvestyn et al. 2016). As a result Dasatinib promotes the transient activation of quiescent HSCs. This finding is surprising because of the dasatinib generally cause c-kit inhibition and cell cycle arrest in many cell types. More studies needed to understand underlying mechanism of Dasatinib's effect on HSCs.

Prostaglandin E2 (PGE_2) is a physiological mediator that has shown regulatory effects on hematopoiesis such as inhibiting myelopoiesis in vivo, stimulating erythroid and multilineage progenitor cells (Hoggatt et al. 2009). HSCs express PGE_2 receptors and treatment with PGE_2 can increase homing, survival and proliferation, emerging in reduced long-term repopulating cell and competitive unit frequency. All these results give competitive advantages to HSC's for maintaining characteristic properties among serial transplantations.

Cytokines and growth factors or small molecules seem more effective when they used as mixtures. SR1 has been widely used for many applications. However, it should also be noted that SR1 induces proliferation of $CD45RA^+$ cells, suggesting that SR1 shows the similar side effects as cytokine mixtures. UM171 and SR1 cotreatment have provided more promising results than SR1 alone since SR1 mediated $CD45RA^+$ cell proliferation has been inhibited following addition of UM171. As a result, $CD34^-$ cell proliferation has been diminished significantly without altering total cell population in comparison to only SR1 or UM171 added cultures (Fares et al. 2014). Similar relationship has been established between α-Tocopherol and PD98059. Without PD98059, α-Tocopherol increases the proliferation and promotes differentiation. To attenuate hematopoiesis, PD98059 has been used (Nogueira-Pedro et al. 2011). These two studies show the necessity of combination of small molecules that take different roles in HSC expansion. Other factors that inhibit cell differentiation include Garcinol and Isogarcinol that caused significant change in $CD34^+CD38^-$ cell proliferation. One of the most interesting results had been obtained after NAM treatment. Among all of the mentioned compounds, this compound may be the most effective in induction of proliferation of HSCs without altering cell cycle activity of other cell types. Treatment with NAM decreased total cell number by twofold but it also significantly increased HSC proliferation. Decreasing the rate of differentiation has also been achieved by targeting mTOR (Kalaitzidis et al. 2012). GSK3β antagonists have been shown to increase cell cycle activity of HSCs and induce hematopoiesis in primates (D'Souza et al. 2016). Thus, this approach may not be reliable. Inhibition of tumor suppressors such as p38 and p18 can also be considered as an efficient approach since such studies gave promising results. Not only in vitro proliferation,

but also radiation induced HSC apoptosis following p38 activation can be inhibited by using these compounds. Inhibition of ROS has also been a target to stimulate HSC expansion. Studies regarding lowering oxidative stress via decreasing ROS levels have been thought to not only helps HSC expansion but also give protection against DNA mutations.

HSC expansion can be achieved by using three major types of HSMs. First group involves antagonists of chromatin remodeling enzymes. By applying these HSMs into the culture media, differentiation following chromatin remodeling has been inhibited, which leads an increase in the HSCs pool. Second group of molecules are regulators of oxidative stress. These HSMs cause scavenging of ROS and inhibit ROS-mediated HSM quiescence. The last group involves antagonists of components of substantial pathways that are activated during hematopoiesis or HSC culture, which are inhibitory to HSC expansion.

5 miRNAs in HSC Expansion Procedures

Since their discovery, miRNAs have been shown to regulate various cellular pathways. Functions of these small RNA particles include regulation of cell metabolism, proliferation, differentiation, which all are closely related to the HSC fate (Choi et al. 2013). Their relatively small size brought excitement if they can be applied to the clinical application (Gentner et al. 2010). Studies showed that various miRNAs are upregulated or downregulated in proliferating hematopoietic cells, especially during the course of HSC expansion or formation of leukemias (Gal et al. 2006). If activation of cell cycle progression in HSCs is mediated by the miRNAs, it is likely these hematopoietic miRNAs (HematoMIRs) can be used for HSC expansion (Table 3).

First miRNA that mediates HSC self-renewal was identified in 2009 as **miR-155**. Intriguingly, as a repressor of src homology 2 domain-containing inositol-5-phosphatase (SHIP), miR-155 overexpressing transgenic mice developed leukemia by accumulation of undifferentiated lymphocytes (Costinean et al. 2009). This outcome correlates with the studies suggesting expansion of HSCs via SHIP downregulation and occurrence of myeloproliferative disorder after miR-155 upregulation (Desponts et al. 2006; O'Connell et al. 2008). Taken together, these studies suggest that the expansion of HSCs can be mediated with miR-155 treatments. Another miRNA that modulates proliferation kinetics of HSCs is **miR29a**. It was discovered that the overexpression of miR29a catalyze G1 to S transitions in HSCs (Han et al. 2010a, b). This miRNA is also overexpressed in acute myeloid leukemia (AML) (Xu et al. 2014). It was discovered that the p53 promotes quiescence and inhibits cell cycle progression in HSCs (Asai et al. 2011). Similar functions have also been identified for **miR34a** but these functions depend on p53 activity (Choi et al. 2011). However, recent studies suggested that the miR34a downregulation leads to inhibition of cell proliferation and differentiation of K562 and TIG3 cell line without depending on p53 expression (Christoffersen et al. 2010, Chung et al. 2011). miR34a takes role in regulation in senescent TIG3 cells without p53 control. However p53 somehow has a control over miR34a levels in cells (Song et al. 2013). These two studies suggest that induction of cell proliferation in HSCs may be achieved with downregulation of miR34a. Another study indicated that BM failure coupled with loss of self-renewal of HSCs after downregulation of **miRNA-124** by increased EVI1 expression (Dickstein et al. 2010). Another putative HematoMIR is **miR-125b**. miR-125b upregulation leads to myelopoiesis (Surdziel et al. 2011). Along with other members of the same family, **miR-125a**, **miR-125b1** and **miR-125b2** were overexpressed in mice. Results showed that these HematoMIRs aid conservation of HSCs in their primitive state (Wojtowicz et al. 2014).

In 2011, upregulation of four HematoMIRs **mir-292**, **miR-17**, **miR-20**, **miR-93** and **miR-106** was discovered to induce HSC expansion (Gentner et al. 2010). Moreover, identification

Table 3 List of putative HematoMIRs and their effect on HSCs and hematopoietic cells

HematoMIR	Status	Outcome	References
miR-155	Upregulation	Myeproliferative disease, B-cell proliferation	Desponts et al. (2006 and Costinean et al. (2009)
miR-29a	Upregulation	Expansion of HSC, decrease of B-cell and T-cell frequency	Han et al. (2010a, b) and Xu et al. (2014)
miR-34a	Upregulation	Senescence, megakaryogenesis	Christoffersen et al. (2010) and Chung et al. (2011)
miR-124	Downregulation	HSC proliferation, myelodysplastic syndrome	Dickstein et al. (2010)
miR-125b	Upregulation	Proliferation, inhibition of granulocyte differentiation	Surdziel et al. (2011)
miR-125a, b1, b2	Upregulation	Preservation of stemness	Wojtowicz et al. (2014)
miR-292	Upregulation	Proliferation, inhibition of myeloid differentiation	Meenhuis et al. (2011)
miR-17	Upregulation	Proliferation of myeloid progenitors	Meenhuis et al. (2011)
miR-20	Upregulation	Proliferation of myeloid progenitors	Meenhuis et al. (2011)
miR-93	Upregulation	Proliferation of myeloid progenitors	Meenhuis et al. (2011)
miR-106	Upregulation	Proliferation of myeloid progenitors	Meenhuis et al. (2011)
miR-130a	Upregulation	Loss of TGF induced proliferation in granulocytes	Hager et al. (2011)
miR-17/92	Upregulation	Expansion of HSCs, leukemia	Li et al. (2012)
miR-126	Downregulation	HSC expansion	Lechman et al. (2012)
miR-142	Upregulation	Inhibition of HSC proliferation	Sun et al. (2010)
miR-22	Upregulation	HSC self-renewal	Song et al. (2013)
miR-139-3p	Upregulation	Inhibition of proliferation of progenitors	Alemdehy et al. (2015)
miR-199a-3p	Downregulation	Myeloid proliferation	Alemdehy et al. (2015)
miR-223	Upregulation	Leukemia	Gentner et al. (2015)
miR-30e	Upregulation	Tumor suppression	Hershkovitz-Rokah et al. (2015)

of the same AAAGUGC sequence within these HematoMIRs suggested that SQSTM1 is the main target whose downregulation is resulted in HSC proliferation via MAPK activation. Among these identified miRNA molecules, miR-17, miR-93, miR-20 or miR-106 have been detected to enhance myeloid differentiation (Meenhuis et al. 2011).

SMAD4 is known as an essential component for maintaining of HSC renewal (Karlsson et al. 2007). Downregulation of SMAD4 blocks TGF-β1 signaling and inhibit expansion of granulocytic cell lines following overexpression of **miR-130a** (Hager et al. 2011). Since TGF- β1 signaling is also required for HSC self-renewal, miR-130a may represent a potential target for expansion of HSCs. Cluster of **miR-17** and **miR-92** overexpression has also shown stimulation of HSC production among with leukemia formation with intervene by p53 in mice (Li et al. 2012). **miR-126** has also been identified as a HematoMIR since it contributes to proliferation process in HSCs confirmed in both in vivo and in vitro studies (Lechman et al. 2012). **miR-142** may also induce proliferation of HSCs when downregulated as it does in mesenchymal stem cells since it is also highly expressed in HSCs (Skarn et al. 2013). Moreover, its upregulation has been resulted with decrease of K562 cell number (Sun et al. 2010).

HematoMIRs do not only target cytokines and proliferation promoting proteins but also target tumor suppressors such as TET2. In 2013, **miR-22** was identified as one of these HematoMIRs that downregulates TET2 production in HSCs and induces HSCs proliferation and self-renewal

(Song et al. 2013). Both in fetal and adult HSCs, Hmga2 and Lin28b expressions were required to maintain the self-renewal activity. These two genes crosstalk between each other via HematoMIR called **let-7**. Lin28b downregulates the let-7 expression whereas increased let-7 activity is resulted with Hmga2 inhibition (Copley et al. 2013). Thus, let-7 limits the capacity of self-renewal property of HSCs in both adult and fetal stages. Another study was carried out and added bantam miRNA as HematoMIR to the literature. This miRNA provides regeneration capacity of HSCs in the stem cell niche in Drosophila (Lam et al. 2014). Recently two other HematoMIRs were identified as **miR-139-3p** and **miR-199a-3p** that have proliferation inhibiting and enhancing properties, respectively (Alemdehy et al. 2015). Other candidates identified as HematoMIRs include **miR-30e** and **miR-223** since they show similar tumor suppressor and onco-miRNA properties (Gentner et al. 2015; Hershkovitz-Rokah et al. 2015; Tian et al. 2015).

There are not many studies regarding induction of HSC expansion via affecting miRNA functions. However, upon identification, these factors may be applied on HSC culture to induce cell cycle progression like small molecules and cytokines. Since identified HematoMIRs are less in number compared with small molecules, use of these factors in ex vivo procedures also not prevalent. These studies suggest that transient introduction of HematoMIRs with proliferative phenotype may induce HSC self-renewal and expansion ex vivo.

6 Potential Use of Hematopoietic Stem Cell Expansion Technologies

HSC expansion could be used for the development of HSC transfusion products, especially for adult recipients. The first transfusion study of hematopoietic stem and progenitor cells from umbilical cord blood (UCB) were established by Broxmeyer et al. 1989). The UCB sample was obtained at the birth of a baby and safe stored by cryopreservation. The recipient of UCB was a 5 year old patient with severe aplastic anemia (Gluckman et al. 1989). The patient had no signs of graft-versus-host-disease (GVHD) and is currently healthy more than 25 years after the treatment (Ballen et al. 2013). This successful transplantation leads to new UCB transplantation strategies since it shows that UCB can be collected at birth. In addition, a single UCB contains enough HSCs for reconstruction of the host HSCs in young patients (Ballen et al. 2013). However the UCB transplantation was not successful enough for treatment of adult patients due to insufficient amount of stem cell number obtained from a single UCB unit (Barker 2007).

HSC expansion technologies could be used in establishment of erythroid transfusion products as an alternative blood source. Erythroid cell transfusion is an important alternative for treatment of several diseases as they include low expression of HLA and being a well characterized single cell suspension lacking nucleated cells (Mountford et al. 2010). Considering the risks of infection, insufficient supply and absence of required blood type, in vitro generation of red blood cells (RBCs) is one of the important alternatives to classic transfusion products. It is well known that, 0 RhD negative erythrocytes reduce the requirement of immunological matching. Therefore, generation of 0 RhD negative erythrocytes favor the in vitro generation of RBCs. The in vitro production of enucleated erythrocytes from hematopoietic stem and progenitor cells in the inexistence of feeder layers was reported by (Miharada et al. 2006). However, in large scale production of erythrocytes from HSC is challenging due to lack of such replication capacity. Nevertheless, ex vivo expansion of CD34+ HSCs derived from cord blood and ex vivo production of RBCs is a promising field of study. However, clinical applications related to ex vivo RBC generation from HSCs is still difficult due to complex production processes, difficulties in HSC expansion, and high production costs (Migliaccio et al. 2009).

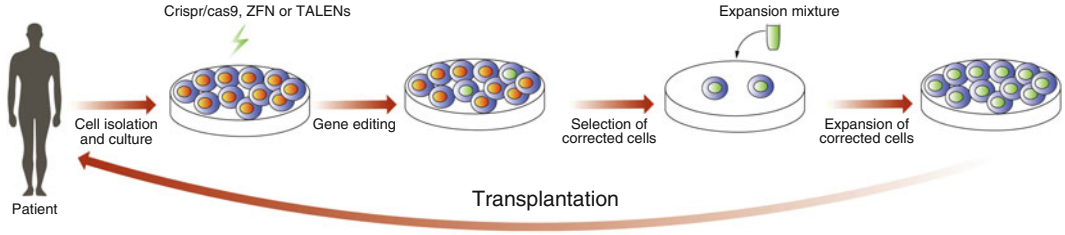

Fig. 1 Schematic representation of gene edited HSC expansion. After expansion of genetically corrected single HSC, HSCs could be transplanted to the patient for engraftment and repopulation

Robust expansion of patients own HSCs from single HSC is especially needed for the development of gene editing technologies towards treatments of inherited disorders affecting hematopoietic system. Idea of replacement of defective gene sequence with a wild type sequence drove scientists to discover simple methods for "gene editing"(Friedmann and Roblin 1972). However, in terms of genetic stability and safety, classic methods could not be applied in clinical applications. On the other hand, in recent years, various methods have been improved and developed that are considered as promising in comparison to the previous version of the gene editing methods (Maeder and Gersbach 2016). CRISPR/Cas9 is recently developed and became one of the widely used gene editing method in a short period of time (Wei et al. 2013). This approach involves CRISPR-associated protein Cas9 that is not modified or engineered depending on the process. In other gene editing approaches like transcription activator-like effector nucleases (TALENs) or zinc finger nucleases (ZFNs), nucleases are designed and engineered to target specific sequences (Finotti et al. 2015). In CRISPR/Cas9 approach, same nuclease is used but the guide RNA that binds to targeted sequence varies. During the process, Cas9 nuclease is introduced to the DNA sequence with a guide RNA that is synthesized depending on the process and binds to the targeted sequence. On the complementary site of the DNA strand that is bound to the guide RNA there should be 5′ NGG 3′ sequence that is also called protospacer-adjacent motif (PAM) (Wright et al. 2015; Gaj and Gersbach 2013). Moreover, gene editing using Cas9, TALEN or ZFN approaches are still need to be improved since they require single cell selection and expansion of gene edited HSCs.

It is known that a substantial proportion of hematological diseases originate from genetic complications. Gene therapy applications aimed to treat these diseases by gene therapy based largely on use of viral vectors (Nienhuis 2013). Introducing these viral components into the patient's cells is risky since insertional oncogenesis may occur as in previous cases of gene therapy studies in HSCs (Sadelain 2004; Cavazzana-Calvo et al. 2012). On the other hand, conventional HSC transplantation may not be possible without proper HLA matching status (Park and Seo 2012). Considering these two restrictions, correction of HSC genome of the patients *in vitro* coupled with HSC expansion via expansion cocktails containing HSMs, growth factors or cytokines following selection of corrected cells might be the safest and the most effective approach with our current technologies (Fig. 1).

7 Final Remarks

BMT is a commonly used procedure for the treatment of wide range of diseases including cancer, myeloproliferative disorders and anemias. However, problems such as low availability of a HLA matched donor, limit the usage of this technique efficiently. To overcome these disadvantages, ex vivo expansion of HSCs has been studied in the past decades. Although first trials focused on expansion of mostly CD34+ cells, today's ultimate goal is expansion without triggering differentiation process.

Several expansion mixtures have been evaluated in clinical trials. These mixtures mostly included SCF, several IL family proteins and TPO (see Table 1). Although significant contribution to HSC proliferation of these mixtures have been verified in clinical trials, increased rate of hematopoiesis has also been observed in the expansion cultures. One of the first clinical trials was studied by using CD34+ cells in an expansion mixture containing SCF, G-CSF and megakaryocyte growth and differentiation factor. The study was important as it demonstrated that ex vivo expansion from CD34+ cells prior transplantation is possible. However, CD34+ cells increased only by fourfold while total nucleated cell number was increased by 56-fold (Shpall et al. 2002). This study was followed by various other trials of expansion mixtures (Jaroscak et al. 2003, de Lima et al. 2008, Delaney et al. 2010a, b). As studies being carried out nowadays aim for expansion of immature HSCs instead of mature HSCs, other additives like small molecules have been included in the expansion systems. Although these small molecules may contribute to increase in proliferation rate of HSCs, mainly they have been used as hematopoiesis inhibitors. Among wide range of identified small molecules, NAM, TEPA and SR1 containing expansion systems are being used in the I/II clinical trials (de Lima et al. 2008; Horwitz et al. 2014; Wagner et al. 2016). Considering there are various different putative small molecules that can be used in expansion cultures, these clinical trials holds additional importance. Moreover, unlike growth factors, small molecules can easily be administrated *in vivo* in form of drugs without causing undesired side effects if designed properly.

As another putative additive of HSC expansion mixtures, miRNAs have also studied by several research teams. Although miRNAs have not been evaluated in any clinical trials yet in HSC expansion, they have an important role for determination of potential genes or pathways that may be involved in cell cycle of HSCs. Moreover, it is not impossible for HematoMIRs to be a part of expansion cultures in the future as currently there is one miRNA is being evaluated in preclinical trials (Hydbring and Badalian-Very 2013). Drugs such as SPC3649 can also be developed to target miRNAs without causing any delivery difficulties unlike miRNAs.

With the advances in gene editing technologies, it is now possible to correct the hematopoietic diseases such as anemia in clinic. Since yield of genetically corrected cells is low, expansion mixtures can be used to amplify the desired cells for further transplantation to the patient.

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References

Aggarwal R, Lu J, Pompili VJ, Das H (2012) Hematopoietic stem cells: transcriptional regulation, ex vivo expansion and clinical application. Curr Mol Med 12(1):34–49

Akhter S, Rahman MM, Lee HS, Kim HJ, Hong ST (2013) Dynamic roles of angiopoietin-like proteins 1, 2, 3, 4, 6 and 7 in the survival and enhancement of

ex vivo expansion of bone-marrow hematopoietic stem cells. Protein Cell 4(3):220–230

Alemdehy MF, Haanstra JR, de Looper HW, van Strien PM, Verhagen-Oldenampsen J, Caljouw Y, Sanders MA, Hoogenboezem R, de Ru AH, Janssen GM, Smetsers SE, Bierings MB, van Veelen PA, Lindern M v, Touw IP, Erkeland SJ (2015) ICL-induced miR139-3p and miR199a-3p have opposite roles in hematopoietic cell expansion and leukemic transformation. Blood 125(25):3937–3948

Allouche M, Bikfalvi A (1995) The role of fibroblast growth factor-2 (FGF-2) in hematopoiesis. Prog Growth Factor Res 6(1):35–48

Andrews RG, Bartelmez SH, Knitter GH, Myerson D, Bernstein ID, Appelbaum FR, Zsebo KM (1992) A c-kit ligand, recombinant human stem cell factor, mediates reversible expansion of multiple CD34+ colony-forming cell types in blood and marrow of baboons. Blood 80(4):920–927

Antonchuk J, Sauvageau G, Humphries RK (2002) HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell 109(1):39–45

Araki H, Mahmud N, Milhem M, Nunez R, Xu M, Beam CA, Hoffman R (2006) Expansion of human umbilical cord blood SCID-repopulating cells using chromatin-modifying agents. Exp Hematol 34(2):140–149

Asai T, Liu Y, Bae N, Nimer SD (2011) The p53 tumor suppressor protein regulates hematopoietic stem cell fate. J Cell Physiol 226(9):2215–2221

Audet J, Miller CL, Eaves CJ, Piret JM (2002) Common and distinct features of cytokine effects on hematopoietic stem and progenitor cells revealed by dose-response surface analysis. Biotechnol Bioeng 80 (4):393–404

Ballen KK, Gluckman E, Broxmeyer HE (2013) Umbilical cord blood transplantation: the first 25 years and beyond. Blood 122(4):491–498

Barker JN (2007) Umbilical Cord Blood (UCB) transplantation: an alternative to the use of unrelated volunteer donors? Hematology Am Soc Hematol Educ Program 2007:55–61

Berardi AC, Wang A, Abraham J, Scadden DT (1995) Basic fibroblast growth factor mediates its effects on committed myeloid progenitors by direct action and has no effect on hematopoietic stem cells. Blood 86 (6):2123–2129

Bhardwaj G, Murdoch B, Wu D, Baker DP, Williams KP, Chadwick K, Ling LE, Karanu FN, Bhatia M (2001) Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. Nat Immunol 2(2):172–180

Bhatia M, Bonnet D, Wu D, Murdoch B, Wrana J, Gallacher L, Dick JE (1999) Bone morphogenetic proteins regulate the developmental program of human hematopoietic stem cells. J Exp Med 189 (7):1139–1148

Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, Walker JR, Flaveny CA, Perdew GH, Denison MS, Schultz PG, Cooke MP (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329 (5997):1345–1348

Bordeaux-Rego P, Luzo A, Costa FF, Olalla Saad ST, Crosara-Alberto DP (2010) Both interleukin-3 and interleukin-6 are necessary for better ex vivo expansion of CD133+ cells from umbilical cord blood. Stem Cells Dev 19(3):413–422

Breems DA, Blokland EA, Ploemacher RE (1997) Stroma-conditioned media improve expansion of human primitive hematopoietic stem cells and progenitor cells. Leukemia 11(1):142–150

Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA (1989) Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci U S A 86(10):3828–3832

Brun AC, Bjornsson JM, Magnusson M, Larsson N, Leveen P, Ehinger M, Nilsson E, Karlsson S (2004) Hoxb4-deficient mice undergo normal hematopoietic development but exhibit a mild proliferation defect in hematopoietic stem cells. Blood 103(11):4126–4133

Cavazzana-Calvo M, Fischer A, Hacein-Bey-Abina S, Aiuti A (2012) Gene therapy for primary immunodeficiencies: Part 1. Curr Opin Immunol 24(5):580–584

Chen X, Skutt-Kakaria K, Davison J, Ou YL, Choi E, Malik P, Loeb K, Wood B, Georges G, Torok-Storb B, Paddison PJ (2012) G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. Genes Dev 26 (22):2499–2511

Chitteti BR, Cheng YH, Poteat B, Rodriguez-Rodriguez S, Goebel WS, Carlesso N, Kacena MA, Srour EF (2010) Impact of interactions of cellular components of the bone marrow microenvironment on hematopoietic stem and progenitor cell function. Blood 115 (16):3239–3248

Choi YJ, Lin CP, Ho JJ, He X, Okada N, Bu P, Zhong Y, Kim SY, Bennett MJ, Chen C, Ozturk A, Hicks GG, Hannon GJ, He L (2011) miR-34 miRNAs provide a barrier for somatic cell reprogramming. Nat Cell Biol 13(11):1353–1360

Choi E, Choi E, Hwang KC (2013) MicroRNAs as novel regulators of stem cell fate. World J Stem Cells 5 (4):172–187

Christoffersen NR, Shalgi R, Frankel LB, Leucci E, Lees M, Klausen M, Pilpel Y, Nielsen FC, Oren M, Lund AH (2010) p53-independent upregulation of miR-34a during oncogene-induced senescence represses MYC. Cell Death Differ 17(2):236–245

Chung SS, Hu W, Park CY (2011) The role of MicroRNAs in hematopoietic stem cell and leukemic stem cell function. Ther Adv Hematol 2(5):317–334

Chute JP, Muramoto GG, Whitesides J, Colvin M, Safi R, Chao NJ, McDonnell DP (2006) Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proc Natl Acad Sci U S A 103(31):11707–11712

Cicuttini FM, Begley CG, Boyd AW (1992) The effect of recombinant stem cell factor (SCF) on purified CD34-positive human umbilical cord blood progenitor cells. Growth Factors 6(1):31–39

Copley MR, Babovic S, Benz C, Knapp DJ, Beer PA, Kent DG, Wohrer S, Treloar DQ, Day C, Rowe K, Mader H, Kuchenbauer F, Humphries RK, Eaves CJ (2013) The Lin28b-let-7-Hmga2 axis determines the higher self-renewal potential of fetal haematopoietic stem cells. Nat Cell Biol 15(8):916–925

Costinean S, Sandhu SK, Pedersen IM, Tili E, Trotta R, Perrotti D, Ciarlariello D, Neviani P, Harb J, Kauffman LR, Shidham A, Croce CM (2009) Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. Blood 114(7):1374–1382

Csaszar E, Kirouac DC, Yu M, Wang W, Qiao W, Cooke MP, Boitano AE, Ito C, Zandstra PW (2012) Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. Cell Stem Cell 10(2):218–229

D'Souza SS, Maufort J, Kumar A, Zhang J, Smuga-Otto K, Thomson JA, Slukvin II (2016) GSK3beta inhibition promotes efficient myeloid and lymphoid hematopoiesis from non-human primate-induced pluripotent stem cells. Stem Cell Rep 6(2):243–256

Dahlberg A, Brashem-Stein C, Delaney C, Bernstein ID (2014) Enhanced generation of cord blood hematopoietic stem and progenitor cells by culture with StemRegenin1 and Delta1(Ext-IgG.) Leukemia 28(10):2097–2101

De Felice L, Tatarelli C, Mascolo MG, Gregorj C, Agostini F, Fiorini R, Gelmetti V, Pascale S, Padula F, Petrucci MT, Arcese W, Nervi C (2005) Histone deacetylase inhibitor valproic acid enhances the cytokine-induced expansion of human hematopoietic stem cells. Cancer Res 65 (4):1505–1513

de Haan G, Weersing E, Dontje B, van Os R, Bystrykh LV, Vellenga E, Miller G (2003) In vitro generation of long-term repopulating hematopoietic stem cells by fibroblast growth factor-1. Dev Cell 4(2):241–251

de Lima M, McMannis J, Gee A, Komanduri K, Couriel D, Andersson BS, Hosing C, Khouri I, Jones R, Champlin R, Karandish S, Sadeghi T, Peled T, Grynspan F, Daniely Y, Nagler A, Shpall EJ (2008) Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. Bone Marrow Transplant 41(9):771–778

de Vries P, Brasel KA, Eisenman JR, Alpert AR, Williams DE (1991) The effect of recombinant mast cell growth factor on purified murine hematopoietic stem cells. J Exp Med 173(5):1205–1211

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID (2010a) Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med 16 (2):232–236

Delaney C, Ratajczak MZ, Laughlin MJ (2010b) Strategies to enhance umbilical cord blood stem cell engraftment in adult patients. Expert Rev Hematol 3 (3):273–283

Desponts C, Hazen AL, Paraiso KH, Kerr WG (2006) SHIP deficiency enhances HSC proliferation and survival but compromises homing and repopulation. Blood 107(11):4338–4345

Dickstein J, Senyuk V, Premanand K, Laricchia-Robbio L, Xu P, Cattaneo F, Fazzina R, Nucifora G (2010) Methylation and silencing of miRNA-124 by EVI1 and self-renewal exhaustion of hematopoietic stem cells in murine myelodysplastic syndrome. Proc Natl Acad Sci U S A 107(21):9783–9788

DiFalco MR, Ali S, Congote LF (2003) The improved survival of hematopoietic cells cultured with a fusion protein of insulin-like growth factor II (IGF-II) and interleukin 3 (IL-3) is associated with increases in Bcl-xL and phosphatidylinositol-3 kinase activity. J Leukoc Biol 73(2):297–305

Duyvestyn JM, Taylor SJ, Dagger SA, Langdon WY (2016) Dasatinib promotes the activation of quiescent hematopoietic stem cells in mice. Exp Hematol 44 (5):410–421

Eaton DL, de Sauvage FJ (1997) Thrombopoietin: the primary regulator of megakaryocytopoiesis and thrombopoiesis. Exp Hematol 25(1):1–7

Eaves CJ (2015) Hematopoietic stem cells: concepts, definitions, and the new reality. Blood 125 (17):2605–2613

Elizalde C, Fernandez-Rueda J, Salcedo JM, Dorronsoro A, Ferrin I, Jakobsson E, Trigueros C (2012) Histone deacetylase 3 modulates the expansion of human hematopoietic stem cells. Stem Cells Dev 21 (14):2581–2591

Ema H, Takano H, Sudo K, Nakauchi H (2000) In vitro self-renewal division of hematopoietic stem cells. J Exp Med 192(9):1281–1288

Endele M, Etzrodt M, Schroeder T (2014) Instruction of hematopoietic lineage choice by cytokine signaling. Exp Cell Res 329(2):207–213

Farahbakhshian E, Verstegen MM, Visser TP, Kheradmandkia S, Geerts D, Arshad S, Riaz N, Grosveld F, van Til NP, Meijerink JP (2014) Angiopoietin-like protein 3 promotes preservation of stemness during ex vivo expansion of murine hematopoietic stem cells. PLoS One 9(8):e105642

Fares I, Chagraoui J, Gareau Y, Gingras S, Ruel R, Mayotte N, Csaszar E, Knapp DJ, Miller P, Ngom M, Imren S, Roy DC, Watts KL, Kiem HP, Herrington R, Iscove NN, Humphries RK, Eaves CJ, Cohen S, Marinier A, Zandstra PW, Sauvageau G (2014) Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science 345(6203):1509–1512

Finotti A, Breda L, Lederer CW, Bianchi N, Zuccato C, Kleanthous M, Rivella S, Gambari R (2015) Recent

trends in the gene therapy of beta-thalassemia. J Blood Med 6:69–85

Friedmann T, Roblin R (1972) Gene therapy for human genetic disease? Science 175(4025):949–955

Frisch BJ, Calvi LM (2014) Hematopoietic stem cell cultures and assays. Methods Mol Biol 1130:315–324

Gaj T, Gersbach CA, Barbas CF 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31(7):397–405

Gal H, Amariglio N, Trakhtenbrot L, Jacob-Hirsh J, Margalit O, Avigdor A, Nagler A, Tavor S, Ein-Dor L, Lapidot T, Domany E, Rechavi G, Givol D (2006) Gene expression profiles of AML derived stem cells; similarity to hematopoietic stem cells. Leukemia 20(12):2147–2154

Galli SJ, Iemura A, Garlick DS, Gamba-Vitalo C, Zsebo KM, Andrews RG (1993) Reversible expansion of primate mast cell populations in vivo by stem cell factor. J Clin Invest 91(1):148–152

Gallicchio VS, Hughes NK, Hulette BC, DellaPuca R, Noblitt L (1991) Basic fibroblast growth factor (B-FGF) induces early- (CFU-s) and late-stage hematopoietic progenitor cell colony formation (CFU-gm, CFU-meg, and BFU-e) by synergizing with GM-CSF, Meg-CSF, and erythropoietin, and is a radioprotective agent in vitro. Int J Cell Cloning 9 (3):220–232

Gammaitoni L, Bruno S, Sanavio F, Gunetti M, Kollet O, Cavalloni G, Falda M, Fagioli F, Lapidot T, Aglietta M, Piacibello W (2003) Ex vivo expansion of human adult stem cells capable of primary and secondary hemopoietic reconstitution. Exp Hematol 31(3):261–270

Gao Y, Yang P, Shen H, Yu H, Song X, Zhang L, Zhang P, Cheng H, Xie Z, Hao S, Dong F, Ma S, Ji Q, Bartlow P, Ding Y, Wang L, Liu H, Li Y, Cheng H, Miao W, Yuan W, Yuan Y, Cheng T, Xie XQ (2015) Small-molecule inhibitors targeting INK4 protein p18(INK4C) enhance ex vivo expansion of haematopoietic stem cells. Nat Commun 6:6328

Gentner B, Visigalli I, Hiramatsu H, Lechman E, Ungari S, Giustacchini A, Schira G, Amendola M, Quattrini A, Martino S, Orlacchio A, Dick JE, Biffi A, Naldini L (2010) Identification of hematopoietic stem cell-specific miRNAs enables gene therapy of globoid cell leukodystrophy. Sci Transl Med 2(58):58ra84

Gentner B, Pochert N, Rouhi A, Boccalatte F, Plati T, Berg T, Sun SM, Mah SM, Mirkovic-Hosle M, Ruschmann J, Muranyi A, Leierseder S, Argiropoulos B, Starczynowski DT, Karsan A, Heuser M, Hogge D, Camargo FD, Engelhardt S, Dohner H, Buske C, Jongen-Lavrencic M, Naldini L, Humphries RK, Kuchenbauer F (2015) MicroRNA-223 dose levels fine tune proliferation and differentiation in human cord blood progenitors and acute myeloid leukemia. Exp Hematol 43(10):858–868. e857

Gilmore GL, DePasquale DK, Lister J, Shadduck RK (2000) Ex vivo expansion of human umbilical cord blood and peripheral blood CD34(+) hematopoietic stem cells. Exp Hematol 28(11):1297–1305

Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P et al (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 321(17):1174–1178

Goff JP, Shields DS, Greenberger JS (1998) Influence of cytokines on the growth kinetics and immunophenotype of daughter cells resulting from the first division of single CD34(+)Thy-1(+)lin- cells. Blood 92(11):4098–4107

Gori JL, Chandrasekaran D, Kowalski JP, Adair JE, Beard BC, D'Souza SL, Kiem HP (2012) Efficient generation, purification, and expansion of CD34(+) hematopoietic progenitor cells from nonhuman primate-induced pluripotent stem cells. Blood 120 (13):e35–e44

Grassinger J, Simon M, Mueller G, Drewel D, Andreesen R, Hennemann B (2007) Bone morphogenetic protein (BMP)-7 but not BMP-2 and BMP-4 improves maintenance of primitive peripheral blood-derived hematopoietic progenitor cells (HPC) cultured in serum-free medium supplemented with early acting cytokines. Cytokine 40(3):165–171

Hager M, Pedersen CC, Larsen MT, Andersen MK, Hother C, Gronbaek K, Jarmer H, Borregaard N, Cowland JB (2011) MicroRNA-130a-mediated down-regulation of Smad4 contributes to reduced sensitivity to TGF-beta1 stimulation in granulocytic precursors. Blood 118(25):6649–6659

Han S, Wang Y, Wang B, Patel E, Okada S, Yang LJ, Moreb JS, Chang LJ (2010a) Ex vivo development, expansion and in vivo analysis of a novel lineage of dendritic cells from hematopoietic stem cells. J Immune Based Ther Vaccin 8:8

Han YC, Park CY, Bhagat G, Zhang J, Wang Y, Fan JB, Liu M, Zou Y, Weissman IL, Gu H (2010b) microRNA-29a induces aberrant self-renewal capacity in hematopoietic progenitors, biased myeloid development, and acute myeloid leukemia. J Exp Med 207(3):475–489

Hershkovitz-Rokah O, Modai S, Pasmanik-Chor M, Toren A, Shomron N, Raanani P, Shpilberg O, Granot G (2015) MiR-30e induces apoptosis and sensitizes K562 cells to imatinib treatment via regulation of the BCR-ABL protein. Cancer Lett 356(2 Pt B):597–605

Hoggatt J, Singh P, Sampath J, Pelus LM (2009) Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. Blood 113(22):5444–5455

Horwitz ME, Chao NJ, Rizzieri DA, Long GD, Sullivan KM, Gasparetto C, Chute JP, Morris A, McDonald C, Waters-Pick B, Stiff P, Wease S, Peled A, Snyder D, Cohen EG, Shoham H, Landau E, Friend E, Peleg I, Aschengrau D, Yackoubov D, Kurtzberg J, Peled T (2014) Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. J Clin Invest 124(7):3121–3128

Huang J, Nguyen-McCarty M, Hexner EO, Danet-Desnoyers G, Klein PS (2012) Maintenance of hematopoietic stem cells through regulation of Wnt and mTOR pathways. Nat Med 18(12):1778–1785

Hutton JF, Rozenkov V, Khor FS, D'Andrea RJ, Lewis ID (2006) Bone morphogenetic protein 4 contributes to the maintenance of primitive cord blood hematopoietic progenitors in an ex vivo stroma-noncontact co-culture system. Stem Cells Dev 15 (6):805–813

Hydbring P, Badalian-Very G (2013) Clinical applications of microRNAs. F1000Res 2:136

Isufi I, Seetharam M, Zhou L, Sohal D, Opalinska J, Pahanish P, Verma A (2007) Transforming growth factor-beta signaling in normal and malignant hematopoiesis. J Interferon Cytokine Res 27(7):543–552

Jaroscak J, Goltry K, Smith A, Waters-Pick B, Martin PL, Driscoll TA, Howrey R, Chao N, Douville J, Burhop S, Fu P, Kurtzberg J (2003) Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell system. Blood 101 (12):5061–5067

Jenkins CR, Shevchuk OO, Giambra V, Lam SH, Carboni JM, Gottardis MM, Holzenberger M, Pollak M, Humphries RK, Weng AP (2012) IGF signaling contributes to malignant transformation of hematopoietic progenitors by the MLL-AF9 oncoprotein. Exp Hematol 40(9):715–723. e716

Jones P, May G, Healy L, Brown J, Hoyne G, Delassus S, Enver T (1998) Stromal expression of Jagged 1 promotes colony formation by fetal hematopoietic progenitor cells. Blood 92(5):1505–1511

Kalaitzidis D, Sykes SM, Wang Z, Punt N, Tang Y, Ragu C, Sinha AU, Lane SW, Souza AL, Clish CB, Anastasiou D, Gilliland DG, Scadden DT, Guertin DA, Armstrong SA (2012) mTOR complex 1 plays critical roles in hematopoiesis and Pten-loss-evoked leukemogenesis. Cell Stem Cell 11(3):429–439

Karanu FN, Murdoch B, Gallacher L, Wu DM, Koremoto M, Sakano S, Bhatia M (2000) The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. J Exp Med 192 (9):1365–1372

Karanu FN, Murdoch B, Miyabayashi T, Ohno M, Koremoto M, Gallacher L, Wu D, Itoh A, Sakano S, Bhatia M (2001) Human homologues of Delta-1 and Delta-4 function as mitogenic regulators of primitive human hematopoietic cells. Blood 97(7):1960–1967

Karlsson G, Blank U, Moody JL, Ehinger M, Singbrant S, Deng CX, Karlsson S (2007) Smad4 is critical for self-renewal of hematopoietic stem cells. J Exp Med 204 (3):467–474

Kaushansky K (1995) Thrombopoietin: the primary regulator of platelet production. Blood 86(2):419–431

Kaushansky K (2003) Thrombopoietin: accumulating evidence for an important biological effect on the hematopoietic stem cell. Ann N Y Acad Sci 996:39–43

Kertesz Z, Vas V, Kiss J, Urban VS, Pozsonyi E, Kozma A, Paloczi K, Uher F (2006) In vitro expansion of long-term repopulating hematopoietic stem cells in the presence of immobilized Jagged-1 and early acting cytokines. Cell Biol Int 30(5):401–405

Kim SY, Le Rademacher J, Antin JH, Anderlini P, Ayas M, Battiwalla M, Carreras J, Kurtzberg J, Nakamura R, Eapen M, Deeg HJ (2014) Myelodysplastic syndrome evolving from aplastic anemia treated with immunosuppressive therapy: efficacy of hematopoietic stem cell transplantation. Haematologica 99(12):1868–1875

Kurtzberg J (2009) Update on umbilical cord blood transplantation. Curr Opin Pediatr 21(1):22–29

Lam V, Tokusumi T, Tokusumi Y, Schulz RA (2014) bantam miRNA is important for Drosophila blood cell homeostasis and a regulator of proliferation in the hematopoietic progenitor niche. Biochem Biophys Res Commun 453(3):467–472

Lechman ER, Gentner B, van Galen P, Giustacchini A, Saini M, Boccalatte FE, Hiramatsu H, Restuccia U, Bachi A, Voisin V, Bader GD, Dick JE, Naldini L (2012) Attenuation of miR-126 activity expands HSC in vivo without exhaustion. Cell Stem Cell 11 (6):799–811

Lehar SM, Dooley J, Farr AG, Bevan MJ (2005) Notch ligands Delta 1 and Jagged1 transmit distinct signals to T-cell precursors. Blood 105(4):1440–1447

Li Y, Vecchiarelli-Federico LM, Li YJ, Egan SE, Spaner D, Hough MR, Ben-David Y (2012) The miR-17-92 cluster expands multipotent hematopoietic progenitors whereas imbalanced expression of its individual oncogenic miRNAs promotes leukemia in mice. Blood 119(19):4486–4498

Liu Y, Zhang B, Zhang J, Wang S, Yao H, He L, Chen L, Yue W, Li Y, Pei X (2014) CAPE promotes the expansion of human umbilical cord blood-derived hematopoietic stem and progenitor cells in vitro. Sci China Life Sci 57(2):188–194

Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20:781–810

Lui WC, Chan YF, Chan LC, Ng RK (2014) Cytokine combinations on the potential for ex vivo expansion of murine hematopoietic stem cells. Cytokine 68 (2):127–132

Luo Y, Li L, Zou P, Wang J, Shao L, Zhou D, Liu L (2014) Rapamycin enhances long-term hematopoietic reconstitution of ex vivo expanded mouse hematopoietic stem cells by inhibiting senescence. Transplantation 97(1):20–29

Maciejewski JP, Selleri C, Sato T, Cho HJ, Keefer LK, Nathan CF, Young NS (1995) Nitric oxide suppression of human hematopoiesis in vitro. Contribution to inhibitory action of interferon-gamma and tumor necrosis factor-alpha. J Clin Invest 96(2):1085–1092

MacMillan ML, Weisdorf DJ, Brunstein CG, Cao Q, DeFor TE, Verneris MR, Blazar BR, Wagner JE (2009) Acute graft-versus-host disease after unrelated

donor umbilical cord blood transplantation: analysis of risk factors. Blood 113(11):2410–2415
Maeder ML, Gersbach CA (2016) Genome-editing technologies for gene and cell therapy. Mol Ther 24 (3):430–446
Mancini SJ, Mantei N, Dumortier A, Suter U, MacDonald HR, Radtke F (2005) Jagged1-dependent Notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation. Blood 105(6):2340–2342
Meenhuis A, van Veelen PA, de Looper H, van Boxtel N, van den Berge IJ, Sun SM, Taskesen E, Stern P, de Ru AH, van Adrichem AJ, Demmers J, Jongen-Lavrencic M, Lowenberg B, Touw IP, Sharp PA, Erkeland SJ (2011) MiR-17/20/93/106 promote hematopoietic cell expansion by targeting sequestosome 1-regulated pathways in mice. Blood 118(4):916–925
Metcalf D, Nicola NA (1991) Direct proliferative actions of stem cell factor on murine bone marrow cells in vitro: effects of combination with colony-stimulating factors. Proc Natl Acad Sci U S A 88 (14):6239–6243
Migliaccio G, Migliaccio AR, Druzin ML, Giardina PJ, Zsebo KM, Adamson JW (1991) Effects of recombinant human stem cell factor (SCF) on the growth of human progenitor cells in vitro. J Cell Physiol 148 (3):503–509
Migliaccio AR, Whitsett C, Migliaccio G (2009) Erythroid cells in vitro: from developmental biology to blood transfusion products. Curr Opin Hematol 16 (4):259–268
Miharada K, Hiroyama T, Sudo K, Nagasawa T, Nakamura Y (2006) Efficient enucleation of erythroblasts differentiated in vitro from hematopoietic stem and progenitor cells. Nat Biotechnol 24(10):1255–1256
Mo XD, Xu LP, Zhang XH, Liu DH, Wang Y, Chen H, Yan CH, Chen YH, Han W, Wang FR, Wang JZ, Liu KY, Huang XJ (2015) Haploidentical hematopoietic stem cell transplantation in adults with Philadelphia-negative acute lymphoblastic leukemia: no difference in the high- and low-risk groups. Int J Cancer 136 (7):1697–1707
Mohamed AA, Ibrahim AM, El-Masry MW, Mansour IM, Khroshied MA, Gouda HM, Riad RM (2006) Ex vivo expansion of stem cells: defining optimum conditions using various cytokines. Lab Hematol 12(2):86–93
Morishima Y, Kashiwase K, Matsuo K, Azuma F, Morishima S, Onizuka M, Yabe T, Murata M, Doki N, Eto T, Mori T, Miyamura K, Sao H, Ichinohe T, Saji H, Kato S, Atsuta Y, Kawa K, Kodera Y, Sasazuki T, Japan Marrow Donor P (2015) Biological significance of HLA locus matching in unrelated donor bone marrow transplantation. Blood 125(7):1189–1197
Morrison SJ, Scadden DT (2014) The bone marrow niche for haematopoietic stem cells. Nature 505 (7483):327–334
Morrison SJ, Wright DE, Weissman IL (1997) Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. Proc Natl Acad Sci U S A 94 (5):1908–1913
Mountford JC, Olivier E, Jordanides NE, de Sousa P, Turner ML (2010) Red blood cells from pluripotent stem cells for use in transfusion. Regen Med 5 (3):411–423
Murdoch B, Chadwick K, Martin M, Shojaei F, Shah KV, Gallacher L, Moon RT, Bhatia M (2003) Wnt-5A augments repopulating capacity and primitive hematopoietic development of human blood stem cells in vivo. Proc Natl Acad Sci U S A 100 (6):3422–3427
Murray LJ, Young JC, Osborne LJ, Luens KM, Scollay R, Hill BL (1999) Thrombopoietin, flt3, and kit ligands together suppress apoptosis of human mobilized CD34 + cells and recruit primitive CD34+ Thy-1+ cells into rapid division. Exp Hematol 27(6):1019–1028
Narimatsu H, Matsumura T, Kami M, Miyakoshi S, Kusumi E, Takagi S, Miura Y, Kato D, Inokuchi C, Myojo T, Kishi Y, Murashige N, Yuji K, Masuoka K, Yoneyama A, Wake A, Morinaga S, Kanda Y, Taniguchi S (2005) Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients. Biol Blood Marrow Transplant 11(6):429–436
Negishi N, Suzuki D, Ito R, Irie N, Matsuo K, Yahata T, Nagano K, Aoki K, Ohya K, Hozumi K, Ando K, Tamaoki N, Ito M, Habu S (2014) Effective expansion of engrafted human hematopoietic stem cells in bone marrow of mice expressing human Jagged1. Exp Hematol 42(6):487–494. e481
Ng YY, Baert MR, de Haas EF, Pike-Overzet K, Staal FJ (2009) Isolation of human and mouse hematopoietic stem cells. Methods Mol Biol 506:13–21
Nienhuis AW (2013) Development of gene therapy for blood disorders: an update. Blood 122(9):1556–1564
Nikougoftar Zarif M, Soleimani M, Abolghasemi H, Amirizade N, Abroun S, Kaviani S (2011) The high yield expansion and megakaryocytic differentiation of human umbilical cord blood CD133(+) cells. Cell J 13 (3):173–178
Nishino T, Miyaji K, Ishiwata N, Arai K, Yui M, Asai Y, Nakauchi H, Iwama A (2009) Ex vivo expansion of human hematopoietic stem cells by a small-molecule agonist of c-MPL. Exp Hematol 37(11):1364–1377. e1364
Nishino T, Wang C, Mochizuki-Kashio M, Osawa M, Nakauchi H, Iwama A (2011) Ex vivo expansion of human hematopoietic stem cells by garcinol, a potent inhibitor of histone acetyltransferase. PLoS One 6(9): e24298
Nogueira-Pedro A, Barbosa CM, Segreto HR, Lungato L, D'Almeida V, Moraes AA, Miranda A, Paredes-Gamero EJ, Ferreira AT (2011) alpha-Tocopherol induces hematopoietic stem/progenitor cell expansion

and ERK1/2-mediated differentiation. J Leukoc Biol 90(6):1111–1117

Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE (2011) Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science 333 (6039):218–221

O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP, Taganov KD, Nicoll J, Paquette RL, Baltimore D (2008) Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med 205(3):585–594

Ohishi K, Varnum-Finney B, Flowers D, Anasetti C, Myerson D, Bernstein ID (2000) Monocytes express high amounts of Notch and undergo cytokine specific apoptosis following interaction with the Notch ligand, Delta-1. Blood 95(9):2847–2854

Okajima Y, Matsumura I, Nishiura T, Hashimoto K, Yoshida H, Ishikawa J, Wakao H, Yoshimura A, Kanakura Y, Tomiyama Y, Matsuzawa Y (1998) Insulin-like growth factor-I augments erythropoietin-induced proliferation through enhanced tyrosine phosphorylation of STAT5. J Biol Chem 273 (36):22877–22883

Ott MG, Merget-Millitzer H, Ottmann OG, Martin H, Bruggenolte N, Bialek H, Seger R, Hossle JP, Hoelzer D, Grez M (2002) Mobilization and transduction of CD34(+) peripheral blood stem cells in patients with X-linked chronic granulomatous disease. J Hematother Stem Cell Res 11(4):683–694

Park JR (1996) Cytokine regulation of apoptosis in hematopoietic precursor cells. Curr Opin Hematol 3 (3):191–196

Park M, Seo JJ (2012) Role of HLA in hematopoietic stem cell transplantation. Bone Marrow Res 2012:680841

Peled T, Landau E, Mandel J, Glukhman E, Goudsmid NR, Nagler A, Fibach E (2004) Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. Exp Hematol 32(6):547–555

Peled T, Shoham H, Aschengrau D, Yackoubov D, Frei G, Rosenheimer GN, Lerrer B, Cohen HY, Nagler A, Fibach E, Peled A (2012) Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. Exp Hematol 40(4):342–355. e341

Pelus LM, Singh P (2014) Delta-1 provides pleasant stem cell environs. Blood 123(5):605–606

Purton LE, Bernstein ID, Collins SJ (1999) All-trans retinoic acid delays the differentiation of primitive hematopoietic precursors (lin-c-kit+Sca-1(+)) while enhancing the terminal maturation of committed granulocyte/monocyte progenitors. Blood 94(2):483–495

Purton LE, Bernstein ID, Collins SJ (2000) All-trans retinoic acid enhances the long-term repopulating activity of cultured hematopoietic stem cells. Blood 95(2):470–477

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R, Weissman IL (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 423(6938):409–414

Reykdal S, Abboud C, Liesveld J (1999) Effect of nitric oxide production and oxygen tension on progenitor preservation in ex vivo culture. Exp Hematol 27 (3):441–450

Roeven MW, Thordardottir S, Kohela A, Maas F, Preijers F, Jansen JH, Blijlevens NM, Cany J, Schaap N, Dolstra H (2015) The Aryl hydrocarbon receptor antagonist StemRegenin1 Improves In Vitro generation of highly functional natural killer cells from CD34(+) hematopoietic stem and progenitor cells. Stem Cells Dev 24(24):2886–2898

Rosenberg PS, Alter BP, Bolyard AA, Bonilla MA, Boxer LA, Cham B, Fier C, Freedman M, Kannourakis G, Kinsey S, Schwinzer B, Zeidler C, Welte K, Dale DC, R. Severe Chronic Neutropenia International (2006) The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood 107 (12):4628–4635

Rossi B, Zanolin E, Vincenzi C, Diani F, Pizzolo G, de Wynter E, Nadali G (2004) Effect of addition of FLT-3 ligand and megakaryocyte growth and development factor on hemopoietic cells in serum-free conditions. Stem Cells Dev 13(4):362–371

Sadelain M (2004) Insertional oncogenesis in gene therapy: how much of a risk? Gene Ther 11(7):569–573

Sanders M, Sorba S, Dainiak N (1993) Insulin-like growth factors stimulate erythropoiesis in serum-substituted umbilical cord blood cultures. Exp Hematol 21 (1):25–30

Sauvageau G, Thorsteinsdottir U, Eaves CJ, Lawrence HJ, Largman C, Lansdorp PM, Humphries RK (1995) Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo. Genes Dev 9 (14):1753–1765

Sauvageau G, Iscove NN, Humphries RK (2004) In vitro and in vivo expansion of hematopoietic stem cells. Oncogene 23(43):7223–7232

Seita J, Weissman IL (2010) Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdiscip Rev Syst Biol Med 2(6):640–653

Shaughnessy P, Chao N, Shapiro J, Walters K, McCarty J, Abhyankar S, Shayani S, Helmons P, Leather H, Pazzalia A, Pickard S (2013) Pharmacoeconomics of hematopoietic stem cell mobilization: an overview of current evidence and gaps in the literature. Biol Blood Marrow Transplant 19(9):1301–1309

Shpall EJ, Quinones R, Giller R, Zeng C, Baron AE, Jones RB, Bearman SI, Nieto Y, Freed B, Madinger N, Hogan CJ, Slat-Vasquez V, Russell P, Blunk B, Schissel D, Hild E, Malcolm J, Ward W, McNiece IK (2002) Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant 8(7):368–376

Sirin O, Lukov GL, Mao R, Conneely OM, Goodell MA (2010) The orphan nuclear receptor Nurr1 restricts the proliferation of haematopoietic stem cells. Nat Cell Biol 12(12):1213–1219

Sitnicka E, Lin N, Priestley GV, Fox N, Broudy VC, Wolf NS, Kaushansky K (1996) The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells. Blood 87 (12):4998–5005

Skarn M, Baroy T, Stratford EW, Myklebost O (2013) Epigenetic regulation and functional characterization of microRNA-142 in mesenchymal cells. PLoS One 8 (11):e79231

Song SJ, Ito K, Ala U, Kats L, Webster K, Sun SM, Jongen-Lavrencic M, Manova-Todorova K, Teruya-Feldstein J, Avigan DE, Delwel R, Pandolfi PP (2013) The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation. Cell Stem Cell 13 (1):87–101

Souza LR, Silva E, Calloway E, Kucuk O, Rossi M, McLemore ML (2014) Genistein protects hematopoietic stem cells against G-CSF-induced DNA damage. Cancer Prev Res (Phila) 7(5):534–544

Sun W, Shen W, Yang S, Hu F, Li H, Zhu TH (2010) miR-223 and miR-142 attenuate hematopoietic cell proliferation, and miR-223 positively regulates miR-142 through LMO2 isoforms and CEBP-beta. Cell Res 20(10):1158–1169

Surdziel E, Cabanski M, Dallmann I, Lyszkiewicz M, Krueger A, Ganser A, Scherr M, Eder M (2011) Enforced expression of miR-125b affects myelopoiesis by targeting multiple signaling pathways. Blood 117(16):4338–4348

Szatmari I, Iacovino M, Kyba M (2010) The retinoid signaling pathway inhibits hematopoiesis and uncouples from the Hox genes during hematopoietic development. Stem Cells 28(9):1518–1529

Talano JA, Cairo MS (2015) Hematopoietic stem cell transplantation for sickle cell disease: state of the science. Eur J Haematol 94(5):391–399

Thordardottir S, Hangalapura BN, Hutten T, Cossu M, Spanholtz J, Schaap N, Radstake TR, van der Voort R, Dolstra H (2014) The aryl hydrocarbon receptor antagonist StemRegenin 1 promotes human plasmacytoid and myeloid dendritic cell development from CD34+ hematopoietic progenitor cells. Stem Cells Dev 23(9):955–967

Tian C, You MJ, Yu Y, Zhu L, Zheng G, Zhang Y (2015) MicroRNA-9 promotes proliferation of leukemia cells in adult CD34-positive acute myeloid leukemia with normal karyotype by downregulation of Hes1. Tumour Biol 29(12):2442–2445

Toda H, Yamamoto M, Kohara H, Tabata Y (2011) Orientation-regulated immobilization of Jagged1 on glass substrates for ex vivo proliferation of a bone marrow cell population containing hematopoietic stem cells. Biomaterials 32(29):6920–6928

Trowbridge JJ, Xenocostas A, Moon RT, Bhatia M (2006) Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. Nat Med 12 (1):89–98

Uchida N, He D, Friera AM, Reitsma M, Sasaki D, Chen B, Tsukamoto A (1997) The unexpected G0/G1 cell cycle status of mobilized hematopoietic stem cells from peripheral blood. Blood 89 (2):465–472

Varnum-Finney B, Purton LE, Yu M, Brashem-Stein C, Flowers D, Staats S, Moore KA, Le Roux I, Mann R, Gray G, Artavanis-Tsakonas S, Bernstein ID (1998) The Notch ligand, Jagged-1, influences the development of primitive hematopoietic precursor cells. Blood 91(11):4084–4091

Von Drygalski A, Alespeiti G, Ren L, Adamson JW (2004) Murine bone marrow cells cultured ex vivo in the presence of multiple cytokine combinations lose radioprotective and long-term engraftment potential. Stem Cells Dev 13(1):101–111

Wagner JE Jr, Brunstein CG, Boitano AE, DeFor TE, McKenna D, Sumstad D, Blazar BR, Tolar J, Le C, Jones J, Cooke MP, Bleul CC (2016) Phase I/II trial of StemRegenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. Cell Stem Cell 18(1):144–155

Walasek MA, van Os R, de Haan G (2012) Hematopoietic stem cell expansion: challenges and opportunities. Ann N Y Acad Sci 1266:138–150

Walker L, Lynch M, Silverman S, Fraser J, Boulter J, Weinmaster G, Gasson JC (1999) The Notch/Jagged pathway inhibits proliferation of human hematopoietic progenitors in vitro. Stem Cells 17 (3):162–171

Wang YF, Li Q, Meng HX, Yu Z, Liu JH, Cui W, Zhou Y, Mai YJ, You SG, Qiu LG (2004) Preliminary study on extensive amplification of human dendritic cells differentiated from cord blood CD34+ progenitor cells by two-step culture. Zhonghua Xue Ye Xue Za Zhi 25(2):70–73

Wang Y, Kellner J, Liu L, Zhou D (2011) Inhibition of p38 mitogen-activated protein kinase promotes ex vivo hematopoietic stem cell expansion. Stem Cells Dev 20(7):1143–1152

Watts KL, Delaney C, Nelson V, Trobridge GD, Beard BC, Humphries RK, Kiem HP (2013) CD34(+) expansion with Delta-1 and HOXB4 promotes rapid engraftment and transfusion independence in a Macaca nemestrina cord blood transplant model. Mol Ther 21(6):1270–1278

Wei C, Liu J, Yu Z, Zhang B, Gao G, Jiao R (2013) TALEN or Cas9 - rapid, efficient and specific choices for genome modifications. J Genet Genomics 40 (6):281–289

Wisniewski D, Affer M, Willshire J, Clarkson B (2011) Further phenotypic characterization of the primitive lineage- CD34+CD38-CD90+CD45RA- hematopoietic stem cell/progenitor cell

sub-population isolated from cord blood, mobilized peripheral blood and patients with chronic myelogenous leukemia. Blood Cancer J 1(9):e36

Wojtowicz EE, Walasek MA, Broekhuis MJ, Weersing E, Ritsema M, Ausema A, Bystrykh LV, de Haan G (2014) MicroRNA-125 family members exert a similar role in the regulation of murine hematopoiesis. Exp Hematol 42(10):909–918. e901

Wright AV, Sternberg SH, Taylor DW, Staahl BT, Bardales JA, Kornfeld JE, Doudna JA (2015) Rational design of a split-Cas9 enzyme complex. Proc Natl Acad Sci U S A 112(10):2984–2989

Wu JM, Hsueh YC, Ch'ang HJ, Luo CY, Wu LW, Nakauchi H, Hsieh PC (2015) Circulating cells contribute to cardiomyocyte regeneration after injury. Circ Res 116(4):633–641

Xiao Y, Wei X, Jiang Z, Wang X, Ye W, Liu X, Zhang M, Xu Y, Wu D, Lai L, Yao H, Liu Z, Cao S, Liu P, Xu B, Li Y, Yao Y, Pei D, Li P (2015) Loss of Angiopoietin-like 7 diminishes the regeneration capacity of hematopoietic stem and progenitor cells. J Hematol Oncol 8:7

Xie J, Chen X, Zheng J, Li C, Stacy S, Holzenberger M, Hu X, Zhang CC (2015a) IGF-IR determines the fates of BCR/ABL leukemia. J Hematol Oncol 8:3

Xie XQ, Yang P, Zhang Y, Zhang P, Wang L, Ding Y, Yang M, Tong Q, Cheng H, Ji Q, McGuire T, Yuan W, Cheng T, Gao Y (2015b) Discovery of novel INK4C small-molecule inhibitors to promote human and murine hematopoietic stem cell ex vivo expansion. Sci Rep 5:18115

Xu L, Xu Y, Jing Z, Wang X, Zha X, Zeng C, Chen S, Yang L, Luo G, Li B, Li Y (2014) Altered expression pattern of miR-29a, miR-29b and the target genes in myeloid leukemia. Exp Hematol Oncol 3:17

Yagi M, Ritchie KA, Sitnicka E, Storey C, Roth GJ, Bartelmez S (1999) Sustained ex vivo expansion of hematopoietic stem cells mediated by thrombopoietin. Proc Natl Acad Sci U S A 96(14):8126–8131

Yeoh JS, van Os R, Weersing E, Ausema A, Dontje B, Vellenga E, de Haan G (2006) Fibroblast growth factor-1 and -2 preserve long-term repopulating ability of hematopoietic stem cells in serum-free cultures. Stem Cells 24(6):1564–1572

Yuan X, Wu H, Xu H, Xiong H, Chu Q, Yu S, Wu GS, Wu K (2015) Notch signaling: an emerging therapeutic target for cancer treatment. Cancer Lett 369(1):20–27

Zhang CC, Lodish HF (2005) Murine hematopoietic stem cells change their surface phenotype during ex vivo expansion. Blood 105(11):4314–4320

Zhang CC, Kaba M, Ge G, Xie K, Tong W, Hug C, Lodish HF (2006) Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. Nat Med 12(2):240–245

Zhang CC, Kaba M, Iizuka S, Huynh H, Lodish HF (2008) Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. Blood 111(7):3415–3423

Zheng J, Huynh H, Umikawa M, Silvany R, Zhang CC (2011) Angiopoietin-like protein 3 supports the activity of hematopoietic stem cells in the bone marrow niche. Blood 117(2):470–479

Zou J, Zou P, Lou Y, Xiao Y, Wang J, Liu L (2011) The cross-talk between ROS and p38MAPKalpha in the Ex Vivo expanded human umbilical cord blood CD133(+) cells. J Huazhong Univ Sci Technolog Med Sci 31(5):591–595

Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, Hsu RY, Birkett NC, Okino KH, Murdock DC et al (1990) Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 63(1):213–224

Zumkeller W (2002) The insulin-like growth factor system in hematopoietic cells. Leuk Lymphoma 43 (3):487–491

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Clinical Applications of Induced Pluripotent Stem Cells – *Stato Attuale*

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Abstract

In an adult human body, somatic stem cells are present in small amounts in almost all organs with the function of general maintenance and prevention of premature aging. But, these stem cells are not pluripotent and are unable to regenerate large cellular loss caused by infarctions or fractures especially in cells with limited replicative ability such as neurons and cardiomyocytes. These limitations gave rise to the idea of inducing pluripotency to adult somatic cells and thereby restoring their regeneration, replication and plasticity. Though many trials and research were focused on inducing pluripotency, a solid breakthrough was achieved by Yamanaka in 2006. Yamanaka's research identified 4 genes (OCT-4, SOX-2, KLF-4 and c-MYC) as the key requisite for inducing pluripotency in any somatic cells (iPSCs). Our study, reviews the major methods used for inducing pluripotency, differentiation into specific cell types and their application in both cell regeneration and disease modelling. We have also highlighted the current status of iPSCs in clinical applications by analysing the registered clinical trials. We believe that this review will assist the researchers to decide the parameters such as induction method and focus their efforts towards clinical application of iPSCs.

Keywords

Disease modelling · Non-integrative gene transfer · OSKM Factors · Regenerative therapy

Abbreviations

OCT-4	Octamer Transcription factor 4
SOX-2	SRY (Sex Determining Region-Y)-related high mobility group box protein 2
KLF-4	Kruppel-like factor -4
MYC	Myelocytomatosis oncogene
iPSCs	induced Pluripotent Stem Cells
OSKM factors	OCT4, SOX2, KLF4, MYC factors
PSCs	Pluripotent Stem Cells
ESCs	Embryonic Stem Cells
ICM	Inner Cell Mass
hES cells	human Embryonic Stem cells
bHLHZ	basic Helix-Loop-Helixzipper
LIF	Leukemia Inhibitory Factor
TAD	Transactivation Domain

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WNT	Wingless-type MMTV (Mouse Mammary Tumor Virus)
TGF-β 1	Transforming Growth Factor beta 1
NF-kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
mTOR	mechanistic Target of Rapamycin
NOTCH 1	Notch homologue 1
NGF	Nerve Growth Factor
ERK1 and ERK2	Extracellular Signal-Regulated Kinase 1/2
LET- 7	lethal-7
MuLV	Murine Leukemia virus
Tet	Tetracycline
DOX	Doxycycline
cDNA	complementary DNA
PB transposon	Piggybac transposon
MI	Myocardial infarction
EB	Embryoid Bodies
CSD	Cold Shock Domain
CCHC Zinc Fingers	$Cys_2HisCys$ Zinc Fingers
HIV	Human Immunodeficiency Virus
BMP2 and BMP4	Bone Morphogenetic Proteins 2 & 4
FGF	Fibroblast Growth Factor
FlK1	Foetal Liver Kinase 1
MCPs	Multipotent Cardiovascular Progenitors
CM	Cardiomyocytes
EC	Endothelial cells
SMC	Smooth Muscle Cells
IGF-1	Insulin like Growth Factor – 1
PIPAAm	Poly (N-isopropylacrylamide)
HNA	Human Nuclear Antigen
cTnT	Cardiac Troponin T
AD	Alzheimer's disease
PD	Parkinson's disease
GMEM	Glasgow's Minimum Essential Medium
KO-SR	KnockOut – Serum Replacer
NEAA	Non-essential amino acids
PDGF	Platelet-derived Growth Factor
PDAPP	PDGF promoter Driven Amyloid Precursor Protein
MGE	Medial ganglionic eminence
SOD1	Superoxide dismutase 1
ALS	Amyotrophic lateral sclerosis
MSCs	Mesenchymal Stem Cells
OPG/ RANKL	Osteoprotegerin/Receptor Activator of Nuclear Factor kappa-B Ligand
GF – iPSCs	Gingival Fibroblast – iPSCs
sAD	sporadic Alzheimer's Disease
APP^{DP}	Amyloid-β Precursor Protein gene Duplication
ER	Endoplasmic Reticulum
HCM	Hypertrophic Cardiomyopathy
DCM	Dilated Cardiomyopathy
BTHS	Barth Syndrome
iPSC-CMs	induced pluripotent derived cardiomyocytes
DM1	Muscular Dystrophy
COPD	Chronic Obstructive Pulmonary Disease
AMD	Age-related Macular Degeneration
CAD	Coronary Artery Disease
ATCC	American Type Culture Collection

1 Introduction

Stem cells can be classified into multipotent and pluripotent based on their differentiation ability. Multipotent stem cells can differentiate into other cell types which are usually limited to their germ layer of origin, most of the adult somatic stem cells are multipotent in nature. Pluripotent stem cells (PSCs) possess unique abilities like self-renewal and differentiation into multiple cell types constituting the human body not limited by the germ layer of origin. Initially, PSCs were derived from embryonic stem cells (ESCs) which were isolated from the inner cell mass at the blastocyst stage of the mammalian embryo (Thomson et al. 1998). This method of isolating ESCs inevitably destroyed the embryos which created ethical concerns. In spite of the ethical

challenges, many experiments were performed enumerating these cells as a functional replacement for dysfunctional cells and tissues causing disease. The increase in stem cell applications both in research and clinical fields and the relative rarity of their availability gave rise to the idea of reversing adult somatic cells to stem cells using nuclear reprogramming. This method of conferring pluripotency to adult somatic cells was demonstrated as early as 1997 where somatic cells were reprogrammed to pluripotent stem cells by transferring their nuclear content into oocytes (Wilmut et al. 1997). Later studies reported that fusion of somatic cells with embryonic stem cells also induced pluripotency traits in them (Cowan 2005; Tada et al. 2001). These reports inferred the fact that unfertilized oocytes and ESCs have factors which were able to induce pluripotency in adult somatic cells.

The pinnacle breakthrough in inducible pluripotency came up with the results obtained by Shinya Yamanaka (Takahashi and Yamanaka 2006). Yamanaka's research filtered the previously suggested 24 genes and reported 4 crucial genes OCT3/4 (octamer binding transcription factor), SOX-2 (SRY-related high mobility group box protein), KLF-4 (Kruppel-like factor), and c-MYC (avian myelocytomatosis virus oncogene cellular homolog) which were necessary for pluripotency. Later these genes were conveniently named as OSKM or Yamanaka factors. In 2007, humaninduced Pluripotent Stem Cells (hiPSCs) were generated from normal human lung cells by using a slightly different gene set which had Lin-28 instead of c-MYC (Yu et al. 2007). These "induced" somatic cells were found to be similar to embryonic stem cells (ESCs). This similarity was observed in global gene analysis experiments which confirmed that the induced cells exhibited both transcriptional and epigenetic signatures similar to that of ESCs (Maherali et al. 2007; Wernig et al. 2007). These factors that were responsible for pluripotency were transferred to the donor somatic cells using a viral (Fig. 1) or non-viral (Fig. 2) delivery system. These human pluripotent stem cells thus derived were ethically naïve and genetically matched to the patient. This breakthrough led to a cascade of studies employing different sources of donor cells like, fibroblasts (Takahashi et al. 2007a, b), peripheral blood (Loh et al. 2009), cord blood (Haase et al. 2009), adipose derived cells (Sugii et al. 2010), hepatocytes (Liu et al. 2010), keratinocytes (Aasen et al. 2008), neural stem cells (Kim et al. 2009) and so on. Parallel to these studies, experiments also revealed that iPSCs could be derived from patients with sporadic or genetically inherited diseases which faithfully recapitulate disease phenotypes (Ebert et al. 2009; Ku et al. 2010).

This ability to generate patient and disease-specific iPSCs enabled research in applications like disease etiology, novel drugs development and cell replacement therapies. In this review, we comprehensively describe the methods used for iPSC derivation, avenues in which these iPSCs were used in regenerative therapies and also the challenges encountered in such applications. We also report the current state of registered clinical trials and commercially available sources. We hope that this overview will help the readers to understand the basics of inducible pluripotency and will provide them withthe knowledge needed to select the parameters such as the method of induction/source of donor cells which are best suited for their research.

2 Genes Regulating Pluripotency

2.1 OCT-4

OCT-4 is an octamer binding transcription factor encoded by POU5fl gene and belongs to POU family of DNA-binding proteins (Scholer et al. 1990). Evidences indicate that OCT-4 is expressed exclusively in undifferentiated embryonic stem (ES) cells and embryonic germ cells. High OCT-4 expression drives ES cells towards mesoderm or endoderm lineages, while lower expression leads to the differentiation of trophoectodermal cellsand a balanced expression of OCT-4 is required to remain pluripotency of ES cells. (Nichols et al. 1998; Niwa 2001; Niwa et al. 2000; Ota et al. 1990; Pesce and Schöler 2000; Scholer et al. 1990). Thus OCT-4 activity must be tightly regulated to ensure the proper differentiation of various tissues and organs.

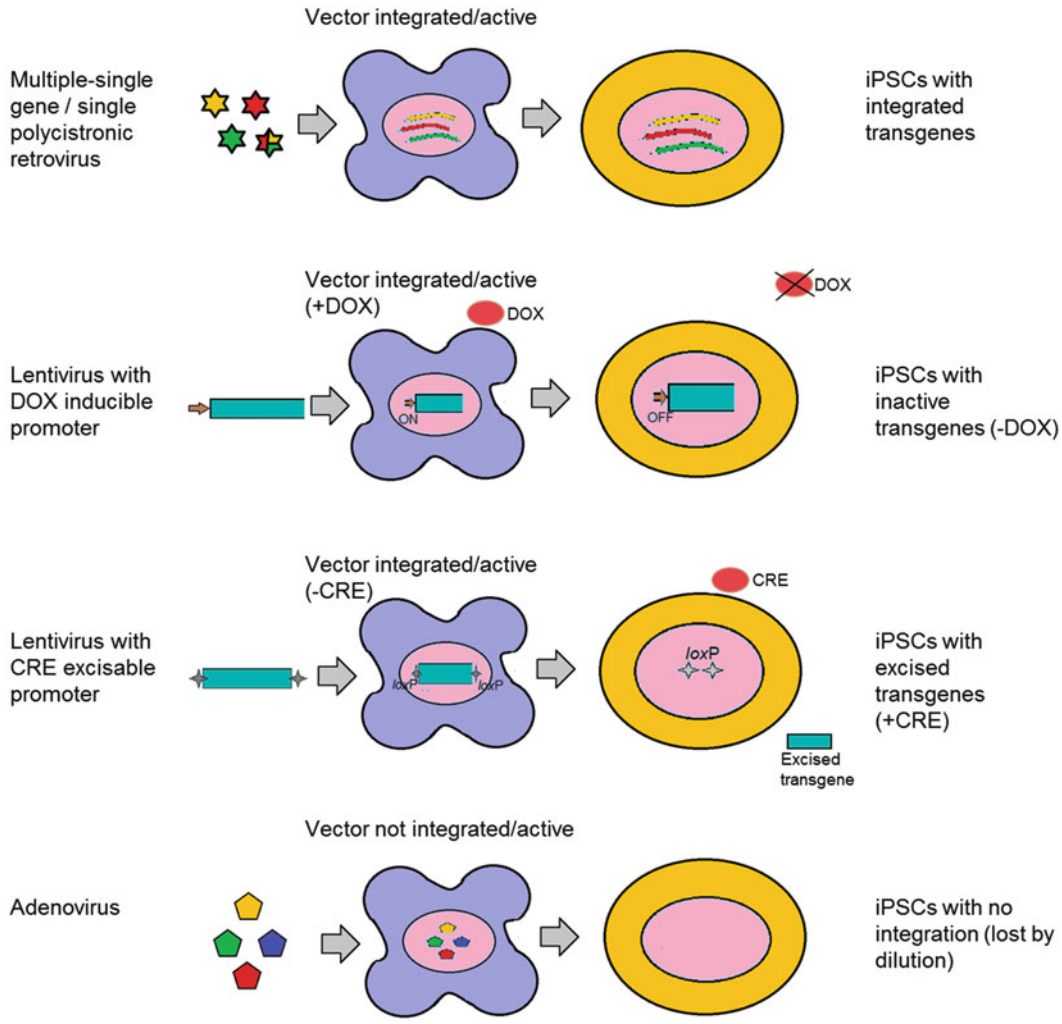


Fig. 1 iPSCs induction with viral delivery method. Schematic representation of major viral delivery methods used to transfer pluripotency factors

Knockdown of OCT-4 in mice and humans produced embryos devoid of a pluripotent inner cell mass (ICM) (Hay et al. 2004; Zafarana et al. 2009), suggesting that a critical level of Oct4 expression is necessary for the maintenance of pluripotency. Studies on OCT-4shows that it regulates the expression of 18,000 pluripotency-associated genes in human ES cells (Boyer et al. 2005). OCT-4 is known to act in unison with SOX-2 and NANOG to maintain the self-renewal and pluripotency of mouse and human ES cells (Fong et al. 2008; Loh et al. 2006; Niwa et al. 2000).

2.2 SOX-2

The SRY-related high mobility group box protein 2 (SOX-2) is a member of Sox family of transcription factors that plays a critical role in the maintenance of pluripotency. It is found expressed in undifferentiated pluripotent embryonic stem cells and germ cells. Like OCT-4, its downregulation leads to differentiation and restricted development potential (Avilion et al. 2003). Deletion of SOX-2 in embryonic cells leads to trophectodermal differentiation similar to that seen during the deletion of OCT-4

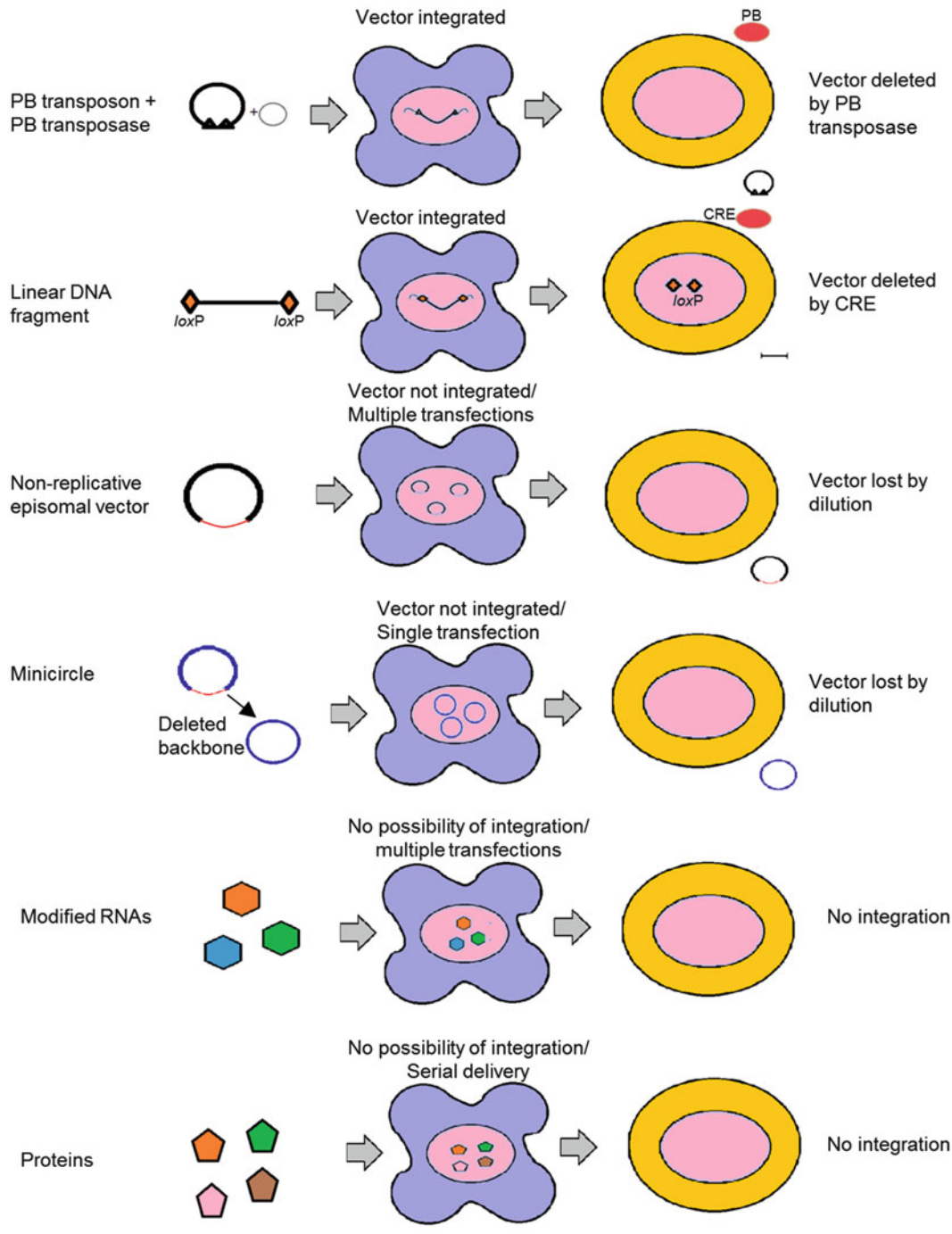


Fig. 2 iPSCs induction with non-viral delivery method. Schematic representation of major non-viral delivery methods used to transfer pluripotency factors

(Masui et al. 2007). On the other hand, SOX-2 overexpressing ES cells are biased towards neural differentiation (Kopp et al. 2008; Zhao et al. 2004). Thus like Oct3/4, Sox2 levels are essential for the maintenance of self-renewal and pluripotency of ES cells (Rizzino 2009).

Moreover, the regulation of SOX-2 during the early differentiation was detected indicating the potential importance of this transcription factor for self-renewal of hES cells (Kallas et al. 2014).

2.3 c-MYC

Myc proteins were known play an important function in stem cell maintenance. The myc protein family comprises of basic helix-loop-helixzipper (bHLHZ) transcription factors (c-, N-, and L-Myc) that can form obligate heterodimers with the small bHLHZ protein. In addition, Myc has been implicated in transcriptional repression of genes that limit cell-cycle progression (Adhikary and Eilers 2005; Cole and Nikiforov 2006; Kleine-Kohlbrecher et al. 2006). Induced expression of c-Myc showed upregulation of six miRNAs which plays an important role in stem cells self-renewal and pluripotency. Genetic studies in mice indicated that c-Myc was involved in the growth, proliferation, and differentiation of epidermal, neural and lung stem/progenitor cells, and haematopoietic stem cells (Arnold and Watt 2001; Dubois et al. 2008; Knoepfler et al. 2002; Okubo 2005). In embryonic stem (ES) cells c-Myc has been shown to be required for the maintenance of self-renewal and its downregulation caused by withdrawal of leukaemia inhibitory factor (LIF) is critical for differentiation (Cartwright 2005). Furthermore, myc family genes along with Oct4, Klf4 and Sox2 act to reprogramme differentiated cells into induced pluripotent stem (iPS) cells with ES cell properties, suggesting that c-Myc is a driver of pluripotency (Takahashi et al. 2007a, b; Wernig et al. 2007). Recent iPSC conversion could occur without introduction of myc, but the overall yield of converted cells is dramatically decreased (Nakagawa et al. 2007).

2.4 KLF-4

Kruppel-like factor (KLF-4) is a transcription factor that regulates diverse cellular processes including cell growth, proliferation and differentiation. KLF-4 belongs to SP/KLF factor family that is characterized by three zinc finger motifs, transactivation domain (TAD) and a repression domain all of which together regulates the transcriptional activity (Dang et al. 2000; Geiman et al. 2000; Yet et al. 1998). It functions as a transcriptional activator in a context-dependent activity by switching its role from anti-apoptotic to pro-apoptotic (Li et al. 2010; Wang et al. 2015; Whitlock 2012). The expression of KLF-4 is known to be regulated by multiple signaling pathways such as WNT (Wingless-type MMTV integration site family member), TGF-β1 (Transforming growth factor beta 1), NF-kB (nuclear factor –kappa B), mTOR (mechanistic target of rapamycin) and factors such as NOTCH 1, p53 and NGF (Nerve growth factor) in transcriptional and post-transcriptional levels. At post-transcriptional level, the activity of KLF-4 has been negatively regulated by ERK1 and ERK2 phosphorylation, resulting inembryonic stem cell differentiation (Kim et al. 2012). Overexpression of KLF-4 induces cell cycle arrest at both G1/S and G2/M transition points in many cell lines (Chen et al. 2003; Shie et al. 2000; Yoon and Yang 2004). The importance of KLF-4 as a stemness factor was reported from the work of Takahashi and Yamanaka (2006) when the fibroblasts were induced into pluripotent stem cells with the combination of four transcription factors. However, Klf-4 is not indispensible for pluripotency development as researchers have generated iPSC lines in the absence of Klf-4 (Yu et al. 2007).

2.5 LIN 28

Unlike other reprogramming factors, LIN 28 is not a transcription factor, but is a RNA binding protein with two unique RNA binding domains, a cold-shock domain (CSD) and two retroviral type CCHC (CysCysHisCys) zinc fingers with predominant cytoplasmic localization andreduced nucleolar localization (Balzer and Moss 2007; Moss et al. 1997). LIN 28 is present in processing bodies or the P-bodies which are the sites of mRNA degradation and microRNA regulation.

Lin28 accelerates the efficiency of iPS cell generation in a cell cycle-dependent manner (Hanna et al. 2009). However, LIN 28 also facilitates the expression of OCT 3/4 at the post-transcriptional level by direct binding to its mRNA (Qiu et al. 2009). In mouse and human, Lin28 is widely expressed in early stage embryos, with expression decreasing dramatically as embryonic development proceeds (Darr and Benvenisty 2009; Richards et al. 2004; Yang and Moss 2003). LIN 28 has been shown to block the production of a group of microRNAs, including LET-7 (lethal-7) which is involved in the regulation of cell proliferation and differentiation (Büssing et al. 2008; Heo et al. 2008; Trabucchi et al. 2009). LIN 28 is also known to reprogram human somatic cells to iPSCs in combination with NANOG, OCT-4, SOX-2.

3 Methods Employed for Inducing Pluripotency

3.1 Integrative Gene Delivery Methods

Integrative delivery methods can efficiently generate iPSCs by the transgene insertions in the chromosomal DNA. It can be achieved using the following approaches.

Viral Delivery System The delivery of OSKM transcription factors can be mediated through viral transfection methods. Murine Leukemia virus (MuLV) derived retrovirus (Takahashi et al. 2007a, b), and Lentivirus have been employed as the viral-mediated delivery system. A single polycistronic retrovirus constructed by using package cell lines can be used for transfection on target cells for generation of iPSCs. This retroviral method integrates the transgene construct which under inducible (Tet or DOX) constitutive promoter expresses the OSKM factors, and can be silenced by removing the induction factors subsequently. However, the viral delivery system has disadvantages like genome integration, insertional mutagenesis, lack of silencing in the pluripotent state, leaky expression, low expression levels and requirement of repeated infection (Maherali and Hochedlinger 2008). Few other methods which are discussed below are available to overcome these shortcomings.

Direct Transfection of Linear DNA There are standard direct DNA transfer methods available which employs liposomes and DNA electroporation for the transfection of target DNA as an alternative for viral vector methods. In this method, the polycistronic constructs were used to express different cDNA under the control of same constitutive promoter (González et al. 2016). This strategy was successfully tested on Embryonic stem cells (Takahashi et al. 2007a, b).

Piggy Bac Transposons Transposons have been used for mammalian transfections in multiple studies, which includes transposons like hAT-like Tol2, Tcl-like transposon and piggy bac (PB) transposon. Compared to other transposons PB has advantages like high transposition activity in mammalian cells, host factor independent reaction, large cargo, steady long-term expression, high safety and was easy to generate *in vitro*. The PB can complete both excision and insertion in the target chromosome by itself in TTAA target sites (Zhao et al. 2016).

3.2 Non-Integrative Methods

As permanent modification by insertion of transgene has been considered as a major limitationin clinical usage of induced pluripotent stem cells, there are other non-integrative approaches available which are described below

Viral Delivery with Defective Integration Mechanism In this method, replication defective adenovirus is used to deliver the OSKM factors for the generation of iPSCs. The resulting iPSCs are devoid of any transgene traces. However, this strategy comes with a limitation of low infection efficiency (Matthias et al. 2008).

Direct Delivery of Synthetic mRNA The mRNA of the OSKM factors are supplied exogenously by using the cationic approach. The mRNAs are modified by treating with phosphatase and incorporating a modified ribonucleoside where uridine and cytidine are replaced with pseudouridine and 5 methylcytidine respectively. These modificationsprotect the mRNA from RNAses of the host machinery. The modified mRNA are then transfected serially to obtain iPSCs without integration of genetic material (González et al. 2016; Warren et al. 2010).

Transient Episomal Delivery A single episomal plasmid with polycistronic OSKM factor genes is used to generate iPSCs without integration of transgenes. The episomal plasmids are replicative and non-integrative in nature which can be transfected by standard transfection approaches into mammalian cells. These transfected episomal gene constructs can be eliminated by culturing the cells without drug selection. (Jia et al. 2010; Okita et al. 2008).

Peptide Mediated Protein Delivery The reprogramming proteins are delivered directly into the cells by avoiding the introduction of exogenous genetic materials with the aid of peptides associated with viral transduction like HIV transactivator of transcription and polyarginine. These recombinant protein-peptides are expressed in *E.coli*in inclusion bodies and are purified for further use. However, this approach has limitations like low efficiency and is laborious. (González et al. 2016).

4 Application in Cellular Regeneration

4.1 Cardiovascular Regeneration

Myocardial infarction (MI) is an ischemic heart disease where the tissues lost is replaced with a non-contractile scar tissue which ultimately leads to congestive heart failure (Pawani and Bhartiya 2013). Conventional management and treatment options do not replace the lost myocardial mass or scar tissue with functional contractile cells. A recent approach is regeneration or repair of ischemic cardiac tissue by cell replacement. iPSCs are viewed as an effective seeding cell type which can be used for autologous transfer while limiting the complexities arising from immune response and cell rejection.

Cardiomyocytes are derived from pluripotent stem cells by using three major methodologies.

(i) *Embryoid body formation:* Cardiomyocytes are one of the primary cell types favoured after formation of embryoid bodies (EB). To induce the formation of embryonic bodies, the cardiomyocytes are grown in a low adherent environment like suspension culture or hanging drop culture along with media components like thefoetal bovine serum. These embryonic bodies spontaneously differentiate into contracting cardiomyocytes once they are seeded into gelatine coated dishes (Kehat and Kenyagin-Karsenti 2001; Segev et al. 2005).

(ii) *Co-culture with END2 cells:* During embryonic development, the anterior endoderm is known to secrete factors and molecular signals that induce the formation of cardiogenic mesoderm. *In vitro,* this can be simulated by co-culturing the pluripotent stem cells with mouse visceral endodermal like cell line END-2. A contracting cardiomyocyte phenotype was observed as early as 12 days in culture (Mummery et al. 2002).

(iii) *Directed differentiation:* Directed differentiation involves the addition of media additives into the culture media to facilitate the differentiation of pluripotent stem cells into a particular phenotype. For cardiomyocyte differentiation, a high-density monolayer of pluripotent stem cells is treated with activin A, BMP2 and BMP4 (bone morphogenetic proteins 2&4), members of WNT pathway and FGF (fibroblast growth factor) in a stage specific manner (Laflamme et al. 2007)

Multiple studies as early as 2009 demonstrated the use of iPSC in animal models. Nelson et al; reported that iPSCs derived by transfecting human OSKM factors in mouse embryonic fibroblasts were able to produce stable engraftment into the myocardial infarcted immunocompetent mouse without any tumour formation. They also observed that these cells were able to restore sinus rhythm in a 4 week follow up experiments (Nelson et al. 2009). FlK1 (Foetal liver kinase 1) positive cardiac progenitor cells (MCPs) derived from iPSCs showed increased functional benefits like vascularization and larger re-aggregates which may increase its efficiency in treating larger myocardial infarcts (Mauritz et al. 2011). Recovery from myocardial infarction not only relies on replacement of cardiomyocytes, but the replaced tissues should be able to grow supporting components like vascularization. To enable this, hiPSCs were differentiated into three lineages *viz.,* cardiomyocytes (CM), endothelial cells (EC) and smooth muscle cells (SMC) and injected through a pericardial fibrin patch releasing IGF-1 (insulin like growth factor-1) to increase grafting efficiency. This strategy increased grafting efficiency with relatively low number of cell dosage and it eliminated arrhythmias which are usually associated with these treatments (Ye et al. 2014). A similar study employed the technique of growing iPSC induced CM cells in a temperature-responsive polymer, poly (N-isopropylacrylamide) (PIPAAm) which enabled the collection of transplantable tissue-like cell sheet without the need for enzymatic digestion of the polymer. In early phase 3-day time point, cells positive for HNA (human nuclear antigen) and cTnT (cardiac troponin) were observed in distinct clusters and prevailed after the 28-day chronic period. These grafted cells also initiated paracrine factors which significantly increased capillary density within the infarcted area (Masumoto et al. 2014). Although several safety concerns and issues still are yet to be resolved, we believe that hiPSC-mediated cardiomyocytes prove to be imminent source forcardiac regeneration.

4.2 Neuronal Regeneration

Neurodegeneration is a collective term used for many conditions encompassing but not limited to chronic, progressive degenerative diseases like Alzheimer's (AD) and Parkinson's (PD). Early diagnosis and treatment of these diseases are challenging mainly due to the fact that neurons involved are already largely dysfunctional or dead. Most neurons do not replicate in*in vivo* conditions, so therapy cannot ensure full recuperation of the patient (Walker 2009). Thus, cell therapy in neurodegenerative disease involves introducing functional cells to restore damaged neural tissues (Yong-Wook et al. 2012). As in Parkinson's disease (PD) which is known to result from loss of dopamine neurons in the substantia nigra and is characterized by severe motor symptoms of tremor, rigidity, bradykinesia and postural instability, allogeneic cell replacement is hugely hindered by difficulty in securing donor dopamine neurons and immuno-rejection of these neural transplants. In this scenario, induced pluripotent stem cells (iPS cells) derived from somatic cells of the same patient may provide a source for replacement therapies in this neurodegenerative disease (Chen et al. 2011).

Neuronal progenitor cells are characterized by the formation of neurospheres whichare generated from pluripotent stem cells by using three major approaches. These progenitor cells can be terminally differentiated into glial, neural or neural crest cells.

(i) *Noggin treatment:* Noggin is a protein involved in the development of neuronal tissues. It is known to antagonise the action ofTGF-β, BMP-2 and BMP-4 proteins (McMahon et al. 1998). The somatic cells are cultured in media substituted by recombinant protein for a time period of 14 days with media change everyday. Then the fragments of the iPSC colonies are transferred into separate culture wells for neurosphere formation (Walker 2009).

(ii) *Co-culturing on PA6 cells:* Undifferentiated hiPSC colonies are mechanically dissected

and seeded on PA6 mouse stromal cells along with differentiation media constituted with Glasgow's-MEM (GMEM) + 10% knock out serum replacer (KO-SR). The stromal feeder cells and KO-SR are replaced with N2 neuronal supplement after 4 days. Eventually, neural rosette-like structures are observed in 11–13 days. These colonies are mechanically transferred into a low adherence culture vessel and neurosphere formation is observed within 7 days (Chang et al. 2013).

(iii) *Media supplementation:* iPScsare seeded and maintained in suspension culture for 8 days to form embryonic bodies (EB). EBs are then cultured in a neural media constituting of DMEM/F12, NEAA (Non-essential amino acids), N2 supplement and FGF2 and seeded on a high attachment culture vessel. Neural rosettes containing neural stem cells are observed in 2–3 days (Swistowski et al. 2010).

Preliminary studies conducted in mouse model proved that fibroblasts transduced with OSKM factors could be differentiated to neural progenitor cells (NPCs) and these cells migrated and differentiated to glia and neurons when injected into the mouse embryonic cerebral cortex (Wernig et al. 2008). iPSCs derived from PD murine model (PDiPSCs) were able to form stable grafts for at least 12 weeks without any tumour formation for at least 16 weeks post-transplant. These grafts also reduced motor asymmetry in the PD mice (Hargus et al. 2010). hiPSCs were reported to restore spatial memory in PDGF promoter driven amyloid precursor protein (PDAPP) transfected mouse (Fujiwara et al. 2013). MGE (Medial ganglionic eminence) -like progenitors derived from both hiPSCs could be induced to differentiate into GABAergic interneurons and displayed mature physiological properties in mouse brain up to 7 months post-transplantation (Nicholas et al. 2013). Similarly, iPSCs derived from SOD1 (Superoxide dismutase) mutated fibroblast from ALS (Amyotrophic lateral sclerosis) patients proved to have the ability to differentiate into motor neurons and glial cells (Dimos et al. 2008). A recent study conducted in 2016 revealed that hiPSCs and murine iPSCs differentiated into micro-glia through a hematopoietic progenitor-like intermediate. Apart from both of them demonstrating the *in vitro* and *in vivo* functional properties of native microglia, the murine iPSC derived microglia increased the survival of intracranial malignant glioma-bearing mice (Pandya et al. 2017). This increasing evidence of iPSC derived neuronal cell's ability to migrate and integrate into specific regions of the brain and non-integrating induction techniques to avoid tumorigenesis might pave way for cellular regenerative treatment to combat neurodegenerative diseases.

4.3 Bone Regeneration

Bone disorders can be loosely classified as varied acute and chronic traumatic, degenerative, malignant or congenital conditions that affect the skeletal system (Csobonyeiova et al. 2017). Adult mesenchymal stem cells were predominantly used for cell replacement and regenerative therapies for these diseases as it is comparatively easier to obtain and could differentiate without any genetic reprogramming. But this source of stem cells becomes less effective in aging patients where these problems are more magnified. In such cases, iPSCs may present a solution to this problem. A recent study has evidenced that though the iPSCs from donor cells may have some epigenetic effect associated with aging it does not affect the reprogramming efficiency of these cells (Lo Sardo et al. 2016). The most common method of differentiating iPSCs is directed differentiation through media additives such as dexamethasone and/or BMP4. Another promising approach was to differentiate iPSCs into iMSCs by inducing the formation of EBs and then treating with TGFβ1. These iMSCs expressed mesenchymal markers CD44, CD90, and CD105 similarly to BM-MSCs. The results concluded that early outgrowing iMSCs were able to form ectopic bone *in vivo* similar to MSCs (Sheyn et al. 2016). Remarkably, these

iMSCs were also able to attenuate tissue ischemia in a murine model more effectively than BM-MSCs. This might be due to the fact that iMSCs have longer survivability after transplantation and enhance vascular and muscle regeneration through paracrine mechanisms (Lian et al. 2010). Osteoblasts and osteoclasts derived from hiPSCs was reported to promote bone regeneration in a hydroxyapatite-based 3D scaffold *in vivo* through OPG/RANKL pathway (Jeon et al. 2016). There are numerous other studies that feature iPSCs or iMSCs used along with an inductive scaffold that could be effectively grafted into a mammalian model, but recent reports also suggest an alternative method where an osteogenic "construct" can be developed in a low adherence scaffold-free environment. Mouse gingiva derived iPSCs (GF-iPSCs) were reported to form anosteoinductive cellular construct which developed a white core which could be stained by von Kossa staining (Okawa et al. 2016). This growing evidence that iPSCs can surpass adult stem cells in post grafting survival and could secrete secondary factors that induce angiogenesis and muscular regeneration indicate that iPSCs and iMSCs might be a contending replacement for adult stem cells in cell replacement therapies of musculoskeletal defects.

5 Disease Modelling

The discovery of OSKM factors and the concept of converting any somatic cells revolutionized the field of disease modelling. Studies as early as 2008 reported a collection of experiments which were focused on creating immortalized iPSCs from cells of diseased patients which retainthe disease phenotype (Park et al. 2008). These models proved to be greatly advantageous for modelling diseases of cells with limited proliferative capacitiessuch ascardiomyocytes and neurons. Disease modelling of iPSCs makes possible the studying of sporadic disease forms with unknown pathogenesis.

(i) *Alzheimer's Disease (AD):*Alzheimer's disease (AD) is a neurodegenerative disease which is pathologicallycharacterized by oligomerization, aggregation and accumulation of amyloid β peptide. AD derived iPSCs were first isolated by Yagi et al., from familial patients with a mutation inpresenilin genes PS1 (A246E) and PS2 (N141I) genes and differentiated into neurons. They observed that these neurons differentiated from patient fibroblasts had overexpression of amyloid β42 recapitulating the molecular pathogenesis in patients (Yagi et al. 2011). APPV717I was the first mutation linked to AD and one of the most common APP mutation associated with familial AD. iPSCs generated from skin fibroblasts (APPV717I) phenotype AD patients were also found to overexpress amyloid β42 (Muratore et al. 2014).iPSCs derived from Alzheimer's patients with amyloid-β precursor protein gene duplication (APP^{DP}) and sporadic Alzheimer's disease (sAD) were able to retain disease phenotype with over expression of amyloid-β, p-tau and aGSK-3β proteins which are known to drive familial Alzheimer's disease (Israel et al. 2012). Similar iPSCs derived from AD patients with mutation in APP gene AD (APP-E693Δ) also showed amyloid-β oligomer accumulation. Gene ontology studies of these iPSCs revealed upregulation of oxidative stress-related genes which suggested that ER and Golgi function might be perturbed in AD neural cells (Kondo et al. 2013).

(ii) *Cardiomyopathy:*Inherited cardiomyopathy is one of the major causes of heart failure and is characterized byventricular dilatation, systolic dysfunction, and progressive heart failure. Cardiomyopathies can be loosely classified into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) (Ioannis Karakikes and Vittavat Termglinchan 2014). iPSCs were generated from familial DCM patients with a point mutationin exon 12 of the TNNT2 (Troponin T2) gene. These iPSC-CMs demonstrated a

consistent increased heterogeneous sarcomeric organization at early stage post differentiation similar to the disease phenotype (Sun et al. 2012). Barth syndrome (BTHS) is a monogenic mitochondrial cardiomyopathy which is linked to the mutation of single TAZ gene. A study comparing the BTHS iPSC-derived cardiomyocytes (iPSC-CMs) to the genetically induced iPSCs reported that the BTHS iPSC-CMs assembled sparse and irregular sarcomeres and generated lesser contractile force in engineered 'heart-on-chip' tissues. The outcomes of their experiments demonstrated that *TAZ* mutation is necessary and sufficient for these phenotypes (Wang et al. 2014)

6 Challenges in Using iPSCs in Regenerative Therapy

The primary roadblock in using iPSCs in regenerative medicine is the variation exhibited by the cells with respect todifferences in gene expression, DNA methylation, potential and teratoma-forming propensity. The gene set OCT-4, SOX-2, KLF-4 and c-MYC itself might possess a problem since many of them were known to be expressed in aggressive cancer cells (Ben-porath et al. 2008). The current techniques, both integrating and non-integrating have a very low efficiency which means that the donor cells need to have a considerably higher proliferative capacity which characterises relatively narrow spectrum of the cultured cells (Medvedev et al. 2010). A major concern with disease modelling of long-latency diseases such as Alzheimer's or Parkinson's is that the dynamics of disease progression is widely different in patients from the iPSC model (Saha and Jaenisch 2010). Also, cellular functions and disease phenotype are influenced by microenvironmental stimuli, which include neighbouring cells, extracellular matrix, soluble factors and physical forces which cannot be recapitulated in iPSCs (Guilak et al. 2009).

7 Concluding Remarks

The technology to endow pluripotency in otherwise multipotent or committed adult somatic cells has definitely opened up multiple new avenues in disease therapy such as regenerative therapy, disease modelling and cellular replacement. The pluripotent stem cells thus derived have characteristics resembling ESCs such as differentiation capacity and expression of ESC specific molecular markers while retaining lineage and disease-specific properties like genetic mutations. Primarily iPSCs are still considered as a source of cells for cellular replacement therapy especially in diseases such as myocardial infarctions where the cells have limited proliferation capacities and larger defects required a larger number of implantable cells. Further, this perspective has been enhanced by added advantages like homing of the induced cells into the defected region which was reported in many neuronal diseases where direct implantation was difficult. Similarly, these cells were also able to secrete paracrine factors which facilitates vascularization. Though there are multiple reports which present convincing results in animal models, the usage of iPSCs or iPSCs derived cells in cellular replacement in humans is complicated by certain limitations. The primary concern is the idea of implanting a genetically modified cell into a human system needs much more comprehensive research. These studies are further complicated by the fact that the source cell inherently presents variations within patients and may carry the disease-specific traits. Though many concerns such as teratoma formation are addressed by advancements in transfection technology such as non-integrative methods, the actual clinical application seems farfetched.

Using iPSCs as *in vitro* models for studying complex disease with a strong genetic factor was viewed as a more plausible application. The concerns such as uninhibited proliferation and recapitulation of disease factors are considered advantageous in disease modelling. This paradigm shift was evident in clinical trials conducted

Table 1 A list of clinical trials that have been conducted using iPSCs reveal that the trials have been largely focused on generating iPSCs from various disease conditions and developing disease models that can serve as research tools that facilitate the development of treatment modalities in the future. The data has been compiled from the website *clinicaltrials.gov*

Sl. No.	Purpose of the study	Condition or study	Clinical trial No.	Status
1.	**Generation of iPSCs that are patient-specific (or) condition-specific**	DM1	NCT02084407	**Completed**
		Hepatic disorders, eye disorders	NCT00953693	
		Genetics and drug addiction	NCT01534624	
		Normal and diseased	NCT02056613	**On-going**
		Retinoblastoma	NCT02193724	
		Leukemia, lymphoma	NCT02564484	
		Amyotrophic lateral sclerosis (ALS)	NCT00801333	
		Pregnant, healthy females, healthy male newborns	NCT00801372	
		COPD	NCT01860898	
		Neurodegenerative disorders	NCT00874783	
		Electrophysiology of iPS-derived Cardiomyocytes	NCT01865981	
		Heritable cardiac arrhythmias	NCT02413450	
		Breast cancer	NCT02772367	
		Retinal disease, AMD, retinal degeneration	NCT01432847	
		Lymphohistiocytosis, Hemophagocytic, common variable immunodeficiency, severe combined immunodeficiency	NCT00895271	
		Peripheral nervous system diseases, testicular neoplasms	NCT02492360	
		Sickle cell anemia	NCT00840567	**Terminated**
		Age Related Macular Degeneration (AMD)		**Unknown**
		Generation of haploid stem cells from human germ cells	NCT01454765	
		Amyotrophic lateral sclerosis	NCT01639391	
2.	**Patient-specific characterization/modeling of a disease condition**	Fibromuscular dysplasia, early onset CAD	NCT01808729	**Completed**
		Coronary artery disease	NCT01517425	**On-going**
		Intellectual deficiency, asymptomatic carrier of the mutation of the gene MYT1L, healthy volunteers	NCT02980302	
		Ataxia-telangiectasia	NCT02246491	
		Autism spectrum disorder	NCT02720939,	
		Marfan's syndrome, Fontan cardiovascular models	NCT02815072	
		Retinal disease, Bestrophinopathy, best Vitelliform macular dystrophy	NCT02162953	
		ALS, primary lateral sclerosis, flail arm ALS	NCT02574390	
		Graft rejection, transplantation, diabetes mellitus	NCT02469207	
		Pulmonary disease, chronic obstructive, smoking	NCT03181204	
		CADASIL	NCT02032225	
		Endothelial dysfunction, inflammation in cardiopulmonary and vascular disease states, healthy volunteers	NCT03253705	

(continued)

Table 1 (continued)

Sl. No.	Purpose of the study	Condition or study	Clinical trial No.	Status
		Obesity, morbid	NCT03263390	
		C9ORF72 Amyotrophic Lateral Sclerosis (ALS)	NCT02686268	
		Craniosynostosis	NCT03025763	
		Cystic fibrosis	NCT03161808	
		Spinal muscular atrophy	NCT02831296	
		Alpha-1 Antitrypsin deficiency	NCT02014415	
		Erythromelalgia, pain insensitivity, congenital, hereditary sensory and autonomic neuropathies, chronic pain	NCT02696746	
		Malignant hyperthermia	NCT02964481	
		Healthy volunteer, Sjorgren's syndrome, salivary gland disease	NCT02327884	
		Uncommon cardiac diseases	NCT01143454	
		Inherited arrhythmias and Valvulopathies	NCT01734356	**Terminated**
		Chronic granulomatous disease	NCT02926963	
		Autism, tuberous sclerosis	NCT01092208	
3.	**Treatment**	Ataxia-Telangiectasia	NCT02246491	**On-going**
		Graft rejection, transplantation, diabetes mellitus	NCT02469207	
		Beta-Thalassemia	NCT03222453	
		Graft vs. host disease	NCT02923375	
		Cardiomyopathies, sepsis, septic shock	NCT03252613	
		Antihypertensive effects	NCT01943383	
		Pulmonary hypertension	NCT01839110	
4.	**Secondary outcome**	Histamine intolerance	NCT02418221	**Completed**
		Toxicity due to chemotherapy, cardiovascular morbidity, cancer, treatment-related,	NCT03199300	**On-going**
		Ectodermal dysplasia	NCT02896387	
		Lung neoplasms, carcinoma, non-small-cell lung	NCT01717482	
		Depression, anxiety, eating disorders, drug use disorders	NCT02450240	
5.	**Biomarkers**	Female urinary incontinence and pelvic organ prolapse	NCT02836145	**On-going**
		C9ORF72 Amyotrophic Lateral Sclerosis (ALS)	NCT02686268	
		Traumatic spinal cord injury, neuropathic pain	NCT00913471	
		ALS	NCT02559869	
6.	**Risk assessment**	Cardiomyopathy	NCT02417311	**On-going**
		Autism spectrum disorders	NCT02628808	

Table 2 Human Induced Pluripotent stem cells (hiPSCs) derived from various sources that are available for procurement for research. Specialized research centers have patient-specific or condition-specific cell lines that can be procured for research. The table depicts a list of iPSC cell lines from commercial institutions and patient-specific ones from research foundations

Sl. No.	Disease	Tissue origin		Bank/Company	Reprogramming method	Cell line no./ Catalog no.
1.	Normal	Fibroblast	Foreskin	ATCC	Sendai virus	ACS-1019
					Retroviral	ACS-1011,
				ALSTEM cell advancements	Retroviral	iPS01
			Dermal	ATCC	Retroviral	ACS-1023 (Yamanaka)
				Takara (Clonetech)		ChiPSC7, ChiPSC12, ChiPSC18, ChiPSC21, ChiPSC22
				ALSTEM cell advancements	Episomal	iPS11
				iX Cells Biotechnologies	Episomal	30HU-002
				Allele Biotechnology	mRNA	ABP-SC-HDFAIPS
			Hepatic	ATCC	Sendai virus	ACS-1020
					Retroviral	ACS-1007
			Unspecified	Applied Stem Cell	Retroviral	ASE-9101
					Episomal	ASE-9203
				Cedars-Sinai Medical Center	Unkown	CS00iCTR-nxx
				Allele Biotechnology	mRNA	ABP-SC-YFPIPS (reporter line)
		Peripheral blood		Cedars-Sinai Medical Center	Unkown	CS01iCNL-nxx, CS02iCTR-Tnxx
		Human peripheral blood mononuclear cells (PBMCs)		ALSTEM cell advancements	Episomal	iPS15
				iX Cells Biotechnologies	Episomal	30HU-002
		Bone marrow CD34+ cells		ATCC	Sendai virus	ACS-1030, ACS-1027, ACS-1028, ACS-1026, ACS-1031, ACS-1025, ACS-1024, ACS-1029
				ALSTEM cell advancements	Episomal	iPS16
				ThermoFisher Scientific	Episomal	A18945
				Applied Stem Cell	Episomal	ASE-9109, ASE-9110
		Human mesenchymal stem cells		ALSTEM cell advancements	Episomal	iPS12
		Adipose stem cells		Cedars-Sinai Medical Center	Unkown	CS68iASC-nxx
				iX Cells Biotechnologies	Episomal	30HU-002
		Not specified		Sigma-Aldrich	Retroviral	IPSC0028, IPSC1028 (reported line), IPSC1030
					mRNA	ASE-9104

(continued)

Table 2 (continued)

Sl. No.	Disease	Tissue origin		Bank/Company	Reprogramming method	Cell line no./ Catalog no.	
				Applied Stem Cell			
2.	Parkinson's disease, asthma, depression	Fibroblast	Dermal	ATCC	Sendai virus	ACS-1013	
					Retroviral	ACS-1012	
					Plasmid	ACS-1014	
				iX Cells Biotechnologies	Episomal	30HU-003	
		Human peripheral blood mononuclear cells (PBMCs)		iX Cells Biotechnologies	Episomal	30HU-003	
		Adipose stem cells		iX Cells Biotechnologies	Episomal	30HU-003	
3.	Cystic fibrosis	Fibroblast	Foreskin	ATCC	Episomal	ACS-1004	
4.	Down's syndrome	Fibroblast	Foreskin	ATCC	Episomal	ACS-1003	
5.	Progeria	Fibroblast	Dermal	Progeria Research Foundation	Retroviral	Clinically affected	HGADFN003 iPS1B, HGADFN003 iPS1C, HGADFN003 iPS1D, HGADFN167 iPS1J, HGADFN167 iPS1Q
						Unaffected	HGMDFN090 iPS1B, HGMDFN090 iPS1C, HGFDFN168 iPS1D2, HGFDFN168 iPS1P
6.	Diabetes	Corneal epithelial cells		Cedars-Sinai Medical Center	Unknown	CS01iCNL-nxx, CS21iCNL-nxx, CS26iCNL-nxx, CS38iCNL-nxx, CS03iDCNL-nxx, CS04iDCNL-nxx, CS15iDCNL-nxx	
		Fibroblast	Dermal	iX Cells Biotechnologies	Episomal	30HU-005	
7.	Breast cancer	Mammary epithelial cells		Cedars-Sinai Medical Center	Unknown	CS01iMEC-nxx	
		EB-B cells		Cedars-Sinai Medical Center	Unknown	CS08iBRCA-nxx, CS70iBRCA-nxx, CS79iBRCA-nxx	
8.	Familial Alzheimer's Disease (FAD)	Fibroblast		Cedars-Sinai Medical Center	Unknown	Clinically affected	CS06iCTR-nxx, CS88iCTR-nxx, CS08iFAD-nxx, CS40iFAD-nxx
						Clinically normal	CS06iCTR-nxx, CS88iCTR-nxx

(continued)

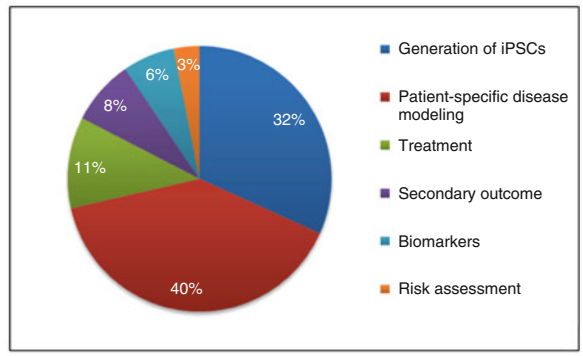
Table 2 (continued)

Sl. No.	Disease	Tissue origin		Bank/Company	Reprogramming method	Cell line no./ Catalog no.
9.	Spinal Muscular Atrophy (SMA)	Fibroblast	Not specified	Cedars-Sinai Medical Center	Unknown	CS14iCTR-nxx, CS15iCTR-nxx, CS13iSMA1-nxx, CS32iSMA1-nxx, CS77iSMA1-nxx, CS92iSMA2-nxx
			Fetal			CS83iSMA-nxx
		EB B-cells				CS87iCTR-nxx, CS688iCTR-nxx, CS84iSMA1-nxx, CS55iSMA3-nxx
10.	Huntington's Disease (HD)	Fibroblast		Cedars-Sinai Medical Center	Unknown	CS14iCTR-21nxx, CS25iCTR-18nxx, CS71iCTR-20nxx, CS83iCTR-33nxx, CS13iHD-43nxx, S04iHD-46nxx, CS87iHD-50nxx, CS03iHD-53nxx, CS92iHD-57nxx, CS21iHD-60nxx, CS02iHD-66nxx, CS81iHD-71nxx, CS77iHD-77nxx, CS09iHD-109nxx, S97iHD-180nxx
11.	Keratoconus	Activated fibroblast from primary keratocytes		Cedars-Sinai Medical Center	Unknown	CS17iCTR-nx, CS67iKC-nxx, CS88iKC-nxx
12.	Amyotrophic Lateral Sclerosis (ALS)	Fibroblast	Not specified	Cedars-Sinai Medical Center	Unknown	CS28iALS-C9nxx, CS29iALS-C9nxx, CS30iALS-C9nxx, CS52iALS-C9nxx, CS37iALSFUSnxx, CS47iALSTDPnxx,
				Applied Stem Cell		Clinically unaffected: ASE-9042
			Dermal	iX Cells Biotechnologies	Episomal	30HU-004, 30HU-A4V
		Human peripheral blood mononuclear cells (PBMCs)		iX Cells Biotechnologies	Episomal	30HU-004, 30HU-A4V
		Adipose stem cells		iX Cells Biotechnologies	Episomal	30HU-004, 30HU-A4V
13.	Charcot-Marie-Tooth Disease Type 1A (CMT1A)	Fibroblast		Cedars-Sinai Medical Center	Unknown	CS41iCMT1A-nxx, CS42iCMT1A-nxx, CS50iCMT1A-nxx, CS67iCMT1A-nxx (currently unavailable)
14.	IncontinentiaPigmenti (IP)	Fibroblast		Cedars-Sinai Medical Center	Unknown	CS93iIP-nxx

(continued)

Table 2 (continued)

Sl. No.	Disease	Tissue origin	Bank/Company	Reprogramming method	Cell line no./ Catalog no.	
15.	Monocarboxylate Transporter 8 (MCT8) deficiency	Fibroblast	Cedars-Sinai Medical Center	Unknown	Clinically normal	CS03iCTR-nxx
					Mutations	CS01iMCT8-nxx, CS58iMCT8-nxx
16.	Neurofibromatosis type 1 (NF1)	Fibroblast	Cedars-Sinai Medical Center	Unknown	CS22iNF1-nxx	
17.	Skeletal Dysplasia (SKD)	Fibroblast	Cedars-Sinai Medical Center	Unknown	CS01iSKD-nxx, CS23iSKD-nxx, CS41iSKD-nxx, CS64iSKD-nxx, CS84iSKD-nxx	



Generation of iPSCs	Patient-specific disease modeling	Treatment	Secondary outcome	Biomarkers	Risk assessment
20	25	7	5	4	2

Fig. 3 Out of the total number of clinical trials conducted including those that have been aborted, 39.68% form the class of trials carried out to generate patient-specific disease models, 31.75% were intended to generate iPSCs of different conditions, 11.11% formed treatment modalities, 6% focused on biomarkers, 3.2% were studies assessing the genetic risks involved in certain conditions and 7.9% were only a secondary outcome of the study. The table represents the number of studies in each of these categories in a total of 63 clinical trials. The data has been compiled from the website *clinicaltrials.gov*

using iPSCs (Table 1). A large percentage of the studies were focused on generation patient-specific iPSCs and their applications (Fig. 3). Owing to the high volume of successful research using iPSCs in disease modelling, iPSCs from normal and diseased sources can be procured for research purpose from commercial and research institutions. A comprehensive list of procurable cell lines along with information such as tissue of origin and reprogramming method is depicted in Table 2. Based on our observations we think that with proper research initiatives that highlight the underlying molecular mechanisms, iPSCs would prove to be a valuable tool for therapeutic interventions.

Conflict of Interest The authors declare no conflict of interest.

References

Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F et al (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 26(11):1276–1284. https://doi.org/10.1038/nbt.1503

Adhikary S, Eilers M (2005) Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 6(8):635–645. https://doi.org/10.1038/nrm1703

Arnold I, Watt FM (2001) c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. Curr Biol 11 (8):558–568. https://doi.org/10.1016/S0960-9822(01)00154-3

Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R (2003) Multipotent cell lineages in early mouse development on SOX2 function. Genes Dev 17:126–140. https://doi.org/10.1101/gad.224503

Balzer E, Moss EG (2007) Localization of the developmental timing regulator Lin28 to mRNP complexes, P-bodies and stress granules. RNA Biol 4(1):16–25. https://doi.org/10.4161/rna.4.1.4364

Ben-porath I, Thomson MW, Carey VJ, Ge R, George W, Regev A, Weinberg R a (2008) An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet 40 (5):499–507. https://doi.org/10.1038/ng.127.An

Boyer LA, Tong IL, Cole MF, Johnstone SE, Levine SS, Zucker JP et al (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122 (6):947–956. https://doi.org/10.1016/j.cell.2005.08.020

Büssing I, Slack FJ, Großhans H (2008) let-7 microRNAs in development, stem cells and cancer. Trends Mol Med 14(9):400–409. https://doi.org/10.1016/j.molmed.2008.07.001

Cartwright P (2005) LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development 132(5):885–896. https://doi.org/10.1242/dev.01670

Chang D, Lee N, Park I, Choi C, Jeon I, Kwon J et al (2013) Therapeutic potential of human induced pluripotent stem cells in experimental stroke. Cell Transplant 22(8):1427–1440. https://doi.org/10.3727/096368912X657314

Chen J, Bao JC, Cai CX (2003) Fabrication, characterization and electrocatalysis of an ordered carbon nanotube electrode. Chin J Chem 21(6):665–669. https://doi.org/10.1016/S0022-2836(02)01449-3

Chen LW, Kuang F, Wei LC, Ding YX, Yung KKL, Chan YS (2011) Potential application of induced pluripotent stem cells in cell replacement therapy for Parkinson' s disease. CNS & Neurol Disord 10:449–458

Cole MD, Nikiforov MA (2006) Transcriptional activation by the Myc oncoprotein. Curr Top Microbiol Immunol 302:33–50. https://doi.org/10.1007/3-540-32952-8_2

Cowan CA (2005) Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. Science 309(5739):1369–1373. https://doi.org/10.1126/science.1116447

Csobonyeiova M, Polak S, Zamborsky R, Danisovic L (2017) iPS cell technologies and their prospect for bone regeneration and disease modeling: a mini review. J Adv Res 8(4):321–327. https://doi.org/10.1016/j.jare.2017.02.004

Dang DT, Pevsner J, Yang VW (2000) The biology of the mammalian Krüppel-like family of transcription factors. Int J Biochem Cell Biol 32 (11–12):1103–1121. https://doi.org/10.1016/S1357-2725(00)00059-5

Darr H, Benvenisty N (2009) Genetic analysis of the role of the reprogramming gene LIN-28 in human embryonic stem cells. Stem Cells (Dayton, Ohio) 27 (2):352–362. https://doi.org/10.1634/stemcells.2008-0720

Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W et al (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321 (5893):1218–1221. https://doi.org/10.1126/science.1158799

Dubois NC, Adolphe C, Ehninger A, Wang RA, Robertson EJ, Trumpp A (2008) Placental rescue reveals a sole requirement for c-Myc in embryonic erythroblast survival and hematopoietic stem cell function. Development 135(14):2455–2465. https://doi.org/10.1242/dev.022707

Ebert AD, Yu J, Rose FFR Jr, Mattis VB, Christian L, Thomson JA, Svendsen CN (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. NIH Public Access 457(7227):277–280. https://doi.org/10.1038/nature07677.Induced

Fong H, Hohenstein KA, Donovan PJ (2008) Regulation of self-renewal and Pluripotency by Sox2 in human embryonic stem cells. Stem Cells 26(8):1931–1938. https://doi.org/10.1634/stemcells.2007-1002

Fujiwara N, Shimizu J, Takai K, Arimitsu N, Saito A, Kono T et al (2013) Restoration of spatial memory dysfunction of human APP transgenic mice by transplantation of neuronal precursors derived from human iPS cells. Neurosci Lett 557(PB):129–134. https://doi.org/10.1016/j.neulet.2013.10.043

Geiman DE, Ton-That H, Johnson JM, Yang VW (2000) Transactivation and growth suppression by the gut-enriched Krüppel-like factor (Krüppel-like factor 4) are dependent on acidic amino acid residues and protein-protein interaction. Nucleic Acids Res 28 (5):1106–1113. https://doi.org/10.1093/nar/28.5.1106

González F, Boué S, Belmonte JCI (2016) Methods for making induced pluripotent stem cells: reprogramming à la carte. Nat Rev Genet 17(2):122. https://doi.org/10.1038/nrg2937

Guilak F, Cohen D, Estes B, Gimble J, Liedtke W, Chen C (2009) Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem

Cell 5(1):17–26. https://doi.org/10.1016/j.stem.2009.06.016.Control

Haase A, Olmer R, Schwanke K, Wunderlich S, Merkert S, Hess C et al (2009) Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 5(4):434–441. https://doi.org/10.1016/j.stem.2009.08.021

Hanna J, Saha K, Pando B, van Zon J, Lengner CJ, Creyghton MP et al (2009) Direct cell reprogramming is a stochastic process amenable to acceleration. Nature 462(7273):595–601. https://doi.org/10.1038/nature08592

Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E et al (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc Natl Acad Sci 107(36):15921–15926. https://doi.org/10.1073/pnas.1010209107

Hay DC, Sutherland L, Clark J, Burdon T (2004) Oct-4 knockdown induces similar patterns of endoderm and trophoblast differentiation markers in human and mouse embryonic stem cells. Stem Cells 22 (2):225–235. https://doi.org/10.1634/stemcells.22-2-225

Heo I, Joo C, Cho J, Ha M, Han J, Kim VN (2008) Lin28 mediates the terminal Uridylation of let-7 precursor MicroRNA. Mol Cell 32(2):276–284. https://doi.org/10.1016/j.molcel.2008.09.014

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C et al (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482(7384):216–220. https://doi.org/10.1038/nature10821

Jeon OH, Panicker LM, Lu Q, Chae JJ, Feldman RA, Elisseeff JH (2016) Human iPSC-derived osteoblasts and osteoclasts together promote bone regeneration in 3D biomaterials. Sci Rep 6(May):26761. https://doi.org/10.1038/srep26761

Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Robbins RC et al (2010) A nonviral Minicircle vector for deriving human iPS cells. Nat Methods 7(3):197–199. https://doi.org/10.1038/nmeth.1426.A

Kallas A, Pook M, Trei A, Maimets T (2014) SOX2 is regulated differently from NANOG and OCT4 in human embryonic stem cells during early differentiation initiated with sodium butyrate. Stem Cells Int 2014(2):1–12. https://doi.org/10.1155/2014/298163

Karakikes I, Vittavat Termglinchan JCW (2014) Human induced pluripotent stem cell models of inherited cardiomyopathies Ioannis. Curr Opin Cardiol 29 (3):214–219. https://doi.org/10.1002/ana.22528.Toll-like

Kehat I, Kenyagin-Karsenti D (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Investig 108(3):407–414. https://doi.org/10.1172/JCI200112131.Introduction

Kim JB, Sebastiano V, Wu G, Araúzo-Bravo MJ, Sasse P, Gentile L et al (2009) Oct4-induced Pluripotency in adult neural stem cells. Cell 136(3):411–419. https://doi.org/10.1016/j.cell.2009.01.023

Kim MO, Kim S-H, Cho Y-Y, Nadas J, Jeong C-H, Yao K et al (2012) ERK1 and ERK2 regulate embryonic stem cell self-renewal through phosphorylation of Klf4. Nat Struct Mol Biol 19(3):283–290. https://doi.org/10.1038/nsmb.2217

Kleine-Kohlbrecher D, Adhikary S, Eilers M (2006) Mechanisms of transcriptional repression by Myc. Curr Top Microbiol Immunol 302:51–62. https://doi.org/10.1007/3-540-32952-8_3

Knoepfler PS, Cheng PF, Eisenman RN (2002) N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. Genes Dev 16 (20):2699–2712. https://doi.org/10.1101/gad.1021202

Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y et al (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. Cell Stem Cell 12(4):487–496. https://doi.org/10.1016/j.stem.2013.01.009

Kopp JL, Ormsbee BD, Desler M, Rizzino A (2008) Small increases in the level of Sox2 trigger the differentiation of mouse embryonic stem cells. Stem Cells 26 (4):903–911. https://doi.org/10.1634/stemcells.2007-0951

Ku S, Soragni E, Campau E, Thomas EA, Altun G, Laurent LC et al (2010) Friedreich's ataxia induced pluripotent stem cells model intergenerational GAA TTC triplet repeat instability. Cell Stem Cell 7 (5):631–637. https://doi.org/10.1016/j.stem.2010.09.014.Friedreich

Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK et al (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol 25(9):1015–1024. https://doi.org/10.1038/nbt1327

Li Z, Zhao J, Li Q, Yang W, Song Q, Li W, Liu J (2010) KLF4 promotes hydrogen-peroxide-induced apoptosis of chronic myeloid leukemia cells involving the bcl-2/bax pathway. Cell Stress Chaperones 15(6):905–912. https://doi.org/10.1007/s12192-010-0199-5

Lian Q, Zhang Y, Zhang J, Zhang HK, Wu X, Zhang Y et al (2010) Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation 121 (9):1113–1123. https://doi.org/10.1161/CIRCULATIONAHA.109.898312

Liu H, Ye Z, Kim Y, Sharkis S, Jang Y-Y (2010) Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. Hepatology (Baltimore, Md) 51(5):1810–1819. https://doi.org/10.1002/hep.23626

Lo Sardo V, Ferguson W, Erikson GA, Topol EJ, Baldwin KK, Torkamani A (2016) Influence of donor age on induced pluripotent stem cells. Nat Biotechnol 35 (1):69–74. https://doi.org/10.1038/nbt.3749

Loh YH, Wu Q, Chew JL, Vega VB, Zhang W, Chen X et al (2006) The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet 38(4):431–440. https://doi.org/10.1038/ng1760

Loh Y, Agarwal S, Park I, Urbach A, Huo H, Heffner GC et al (2009) Generation of induced pluripotent stem cells from human blood. Hematopoiesis Stem Cells 113(22):1–3. https://doi.org/10.1182/blood-2009-02-204800.The

Maherali N, Hochedlinger K (2008) Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell Elsevier Inc 3:595. https://doi.org/10.1016/j.stem.2008.11.008

Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K et al (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1 (1):55–70. https://doi.org/10.1016/j.stem.2007.05.014

Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K et al (2007) Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol 9(6):625–635. https://doi.org/10.1038/ncb1589

Masumoto H, Ikuno T, Takeda M, Fukushima H, Marui A, Katayama S et al (2014) Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. Sci Rep 4(1):6716. https://doi.org/10.1038/srep06716

Matthias S, Nagaya M, Utikal J, Weir G, K. H. (2008) Induced pluripotent stem cells generated without viral integration. Science 322(5903):945–949. https://doi.org/10.1126/science.1162494.Induced

Mauritz C, Martens A, Rojas SV, Schnick T, Rathert C, Schecker N et al (2011) Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction. Eur Heart J 32 (21):2634–2641. https://doi.org/10.1093/eurheartj/ehr166

McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP (1998) Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes Dev 12(10):1438–1452. https://doi.org/10.1101/gad.12.10.1438

Medvedev SP, Shevchenko AI, Zakian SM (2010) Induced pluripotent stem cells: problems and advantages when applying them in regenerative medicine. Acta Nat 2(2):18–28. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22649638%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3347549

Moss E, Lee R, Ambros V (1997) Control of developmental timing by the cold shock domain protein Lin-28 and its regulation by the lin-4 RNA. Cell 88(5):637–646

Mummery C, Ward D, Van Den Brink CE, Bird SD, Doevendans PA, Opthof T et al (2002) Cardiomyocyte differentiation of mouse and human embryonic stem cells. J Anat 200(3):233–242. https://doi.org/10.1046/j.1469-7580.2002.00031.x

Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LNP et al (2014) The familial alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. Hum Mol Genet 23(13):3523–3536. https://doi.org/10.1093/hmg/ddu064

Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T et al (2007) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 26(1):101–106

Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, Ikeda Y, Terzic A (2009) Repair of acute myocardial infarction with iPS induced by human stemness factors. Circulation 120(5):408. https://doi.org/10.1161/CIRCULATIONAHA.109.865154.Repair

Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D et al (2013) Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. Cell Stem Cell 12(5):573–586. https://doi.org/10.1016/j.stem.2013.04.005

Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I et al (1998) Formation of pluripotent stem cells in the mammalian embryo dependes on the POU transcription factor Oct4. Cell 95(3):379–391. https://doi.org/10.1016/S0092-8674(00)81769-9

Niwa H (2001) Molecular mechanism to maintain stem cell renewal of ES cells. Cell Struct Funct 26 (3):137–148. https://doi.org/10.1247/csf.26.137

Niwa H, Miyazaki J, Smith AG (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24 (4):372–376. https://doi.org/10.1038/74199

Okawa H, Kayashima H, Sasaki JI, Miura J, Kamano Y, Kosaka Y et al (2016) Scaffold-free fabrication of Osteoinductive cellular constructs using mouse gingiva-derived induced pluripotent stem cells. Stem Cells Int 2016(9):1–11. https://doi.org/10.1155/2016/6240794

Okita MN, Hyenjong H, Tomoko Ichisaka SY (2008) Generation of mouse induced pluripotent stem cells without viral vectors. Science 322(1998):949–953

Okubo T (2005) Nmyc plays an essential role during lung development as a dosage-sensitive regulator of progenitor cell proliferation and differentiation. Development 132(6):1363–1374. https://doi.org/10.1242/dev.01678

Ota K, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA (1990) T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 346(6280):183–187. https://doi.org/10.1038/346183a0

Pandya H, Shen MJ, Ichikawa DM, Sedlock AB, Choi Y, Johnson KR et al (2017) Differentiation of human and murine induced pluripotent stem cells to microglia-like cells. Nat Neurosci 20(5):753–759. https://doi.org/10.1038/nn.4534

Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A et al (2008) Disease-specific induced pluripotent stem cells. Cell 134(5):877–886. https://doi.org/10.1016/j.cell.2008.07.041

Pawani H, Bhartiya D (2013) Pluripotent stem cells for cardiac regeneration: overview of recent advances & emerging trends. Indian J Med Res 137(2):270–282. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23563370%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3657850

Pesce M, Schöler HR (2000) Oct-4: control of totipotency and germline determination. Mol Reprod Dev 55 (4):452–457. https://doi.org/10.1002/(SICI)1098-2795(200004)55:4<452::AID-MRD14>3.0.CO;2-S

Qiu C, Ma Y, Wang J, Peng S, Huang Y (2009) Lin28-mediated post-transcriptional regulation of Oct4 expression in human embryonic stem cells. Nucleic Acids Res 38(4):1240–1248. https://doi.org/10.1093/nar/gkp1071

Richards M, Tan S, Tan J, Chan W, Bongso A (2004) The transcriptome profile of human embryonic stem cells as defined by SAGE. Stem Cells 22(1):51–64. https://doi.org/10.1634/stemcells.22-1-51

Rizzino A (2009) Sox2 and Oct-3/4: a versatile pair of master regulators that orchestrate the self-renewal and pluripotency of embryonic stem cells. Wiley Interdiscip Rev Syst Biol Med 1(2):228–236. https://doi.org/10.1002/wsbm.12

Saha K, Jaenisch R (2010) Technical challenges in using human induced pluripotent stem cells to model disease. Cell Stem Cell 5(6):584–595. https://doi.org/10.1016/j.stem.2009.11.009.Technical

Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P (1990) New type of POU domain in germ line-specific protein Oct-4. Nature 344(6265):435–439. https://doi.org/10.1038/344435a0

Segev H, Kenyagin Karsenti D, Fishman B, Gerecht Nir S, Ziskind A, Amit M et al (2005) Molecular analysis of cardiomyocytes derived from human embryonic stem cells. Develop Growth Differ 47(5):295–306. https://doi.org/10.1111/j.1440-169X.2005.00803.x

Sheyn D, Ben-David S, Shapiro G, Demel S, Bez M, Ornelas L et al (2016) Human induced pluripotent stem cells differentiate into functional mesenchymal stem cells and repair bone defects. Stem Cells Transl Med 5:1447–1460. https://doi.org/10.1002/stem.1607

Shie JL, Chen ZY, Fu M, Pestell RG, Tseng CC (2000) Gut-enriched Krüppel-like factor represses cyclin D1 promoter activity through Sp1 motif. Nucleic Acids Res 28(15):2969–2976. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=102679&tool=pmcentrez&rendertype=abstract

Sugii S, Kida Y, Kawamura T, Suzuki J, Vassena R, Yin Y-Q et al (2010) Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. Proc Natl Acad Sci 107(8):3558–3563. https://doi.org/10.1073/pnas.0910172106

Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez OJ, Navarrete EG, Hu S, Wang L, Lee A, Pavlovic A, Lin S, Chen R, Hajjar RJ (2012) Patient-specific induced pluripotent stem cells as model for familial dilated Cardiomyopthy. Sci Transl Med 4 (130):130ra47. https://doi.org/10.1126/scitranslmed.3003552.Patient-Specific

Swistowski A, Peng J, Liu Q, Mali P, Rao MS, Cheng L, Zeng X (2010) Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. Stem Cells 28 (10):1893–1904. https://doi.org/10.1002/stem.499

Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T (2001) Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. Curr Biol 11 (19):1553–1558. https://doi.org/10.1016/S0960-9822(01)00459-6

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. https://doi.org/10.1016/j.cell.2006.07.024

Takahashi K, Okita K, Nakagawa M, Yamanaka S (2007a) Induction of pluripotent stem cells from fibroblast cultures. Nat Protoc 2(12):3081–3089. https://doi.org/10.1038/nprot.2007.418

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007b) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872. https://doi.org/10.1016/j.cell.2007.11.019

Thomson JA, Itskovitz-eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Sci Rep 282:1145–1147. https://doi.org/10.1126/science.282.5391.1145

Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A et al (2009) The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. Nature 459(7249):1010–1014. https://doi.org/10.1038/nature08025

Walker JM (2009) Neurodegeneration. Life Sci 531. https://doi.org/10.1007/978-1-62703-239-1_1

Wang G, McCain ML, Yang L, He A, Pasqualini FS, Agarwal A et al (2014) Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat Med 20(6):616–623. https://doi.org/10.1038/nm.3545

Wang B, Zhao M-Z, Cui N-P, Lin D-D, Zhang A-Y, Qin Y et al (2015) Krüppel-like factor 4 induces apoptosis and inhibits tumorigenic progression in SK-BR-3 breast cancer cells. FEBS Open Bio 5:147–154. https://doi.org/10.1016/j.fob.2015.02.003

Warren L, Manos PD, Ahfeldt T, Loh Y, Li H, Daley Q et al (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells using synthetic modified mRNA Luigi. Cell Stem Cell 7(5):618–630. https://doi.org/10.1016/j.stem.2010.08.012.Highly

Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K et al (2007) In vitro reprogramming of

fibroblasts into a pluripotent ES-cell-like state. Nature 448 (7151):318–324. https://doi.org/10.1038/nature05944

Wernig M, Zhao J-P, Pruszak J, Hedlund E, Fu D, Soldner F et al (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci 105(15):5856–5861. https://doi.org/10.1073/pnas.0801677105

Whitlock NC (2012) Resveratrol-induced apoptosis is mediated by early growth Response-1, Krüppel-like factor 4, and activating transcription factor 3. Cancer Prev Res 4(1):116–127. https://doi.org/10.1158/1940-6207.CAPR-10-0218.Resveratrol-induced

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS (1997) Viable offspring derived from fetal and adult mammalian cells. Cloning Stem Cells 9(1):3–7. https://doi.org/10.1089/clo.2006.0002

Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T et al (2011) Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum Mol Genet 20(23):4530–4539. https://doi.org/10.1093/hmg/ddr394

Yang DH, Moss EG (2003) Temporally regulated expression of Lin-28 in diverse tissues of the developing mouse. Gene Expr Patterns 3(6):719–726. https://doi.org/10.1016/S1567-133X(03)00140-6

Ye L, Chang YH, Xiong Q, Zhang P, Zhang L, Somasundaram P et al (2014) Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell 15(6):750–761. https://doi.org/10.1016/j.stem.2014.11.009

Yet S, Nulty MMM, Folta SC, Yen H, Yoshizumi M, Hsieh C et al (1998) Human EZF, a Krüppel-like zinc finger protein, is expressed in vascular endothelial cells and contains transcriptional activation and repression domains. J Biol Chem 273(2):1026–1031

Yong-Wook J, Hysolli E, Kim K-Y, Tanaka Y, Park I-H (2012) Human induced pluripotent stem cells and neurodegenerative disease: prospects for novel therapies. Curr Opin Neurol 25(2):125–130. https://doi.org/10.1097/WCO.0b013e3283518226.Human

Yoon HS, Yang VW (2004) Requirement of Krüppel-like factor 4 in preventing entry into mitosis following DNA damage. J Biol Chem 279(6):5035–5041. https://doi.org/10.1074/jbc.M307631200

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920. https://doi.org/10.1126/science.1151526

Zafarana G, Avery SR, Avery K, Moore HD, Andrews PW (2009) Specific knockdown of OCT4 in human embryonic stem cells by inducible short hairpin RNA interference. Stem Cells 27(4):776–782. https://doi.org/10.1002/stem.5

Zhao S, Nichols J, Smith AG, Li M (2004) SoxB transcription factors specify neuroectodermal lineage choice in ES cells. Mol Cell Neurosci 27(3):332–342. https://doi.org/10.1016/j.mcn.2004.08.002

Zhao S, Jiang E, Chen S, Gu Y, Shangguan AJ, Lv T et al (2016) PiggyBac transposon vectors: the tools of the human gene encoding. Translational Lung Cancer Res 5(1):120–125. https://doi.org/10.3978/j.issn.2218-6751.2016.01.05

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Safety and Efficacy of Epigenetically Converted Human Fibroblasts Into Insulin-Secreting Cells: A Preclinical Study

Tiziana A.L. Brevini, Georgia Pennarossa, Elena F.M. Manzoni, and Fulvio Gandolfi

Abstract

Type 1 Diabetes Mellitus (T1DM) is a chronic disease that leads to loss of insulin secreting β-cells, causing high levels of blood glucose. Exogenous insulin administration is not sufficient to mimic the normal function of β-cells and, consequently, diabetes mellitus often progresses and can lead to major chronic complications and morbidity. The physiological control of glucose levels can only be restored by replacing the β-cell mass.

We recently developed a new strategy that allows for epigenetic conversion of dermal fibroblasts into insulin-secreting cells (EpiCC), using a brief exposure to the demethylating agent 5-aza-cytidine (5-aza-CR), followed by a pancreatic induction protocol. This method has notable advantages compared to the alternative available procedures and may represent a promising tool for clinical translation as a therapy for T1DM. However, a thought evaluation of its therapeutic safety and efficacy is mandatory to support preclinical studies based on EpiCC treatment.

We here report the data obtained using human fibroblasts isolated from diabetic and healthy individuals, belonging the two genders. EpiCC were injected into 650 diabetic severe combined immunodeficiency (SCID) mice and demonstrated to be able to restore and maintain glycemic levels within the physiological range. Cells had the ability to self-regulate and not to cause hypoglycemia, when transplanted in healthy animals. Efficacy tests showed that EpiCC successfully re-established normoglycemia in diabetic mice, using a dose range that appeared clinically relevant to the concentration 0.6×10^6 EpiCC. Necropsy and histopathological investigations demonstrated the absence of malignant transformation and cell migration to organs and lymph nodes.

The present preclinical study demonstrates safety and efficacy of human EpiCC in diabetic mice and supports the use of epigenetic converted cells for regenerative medicine of diabetes mellitus.

Keywords

Diabetes · Efficacy · Epigenetically converted cells · Glycaemia · Preclinical study · Safety

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1 Introduction

In Type 1 Diabetes Mellitus (T1DM) patients, the onset of overt disease is assumed to occur when the β-cell mass falls below 20% of the normal range (Gepts 1965; Pipeleers and Ling 1992; Butler et al. 2007). Exogenous insulin administration is not sufficient to mimic the normal function of β-cells. Consequently, diabetes mellitus often progresses and can lead to major chronic complications and morbidity (Nathan et al. 1993). The physiological control of blood glucose levels can only be restored by replacing the β-cell mass. In patients with T1DM, transplantation of pancreatic islet cells has proven successful for functional replenishment of damaged islets (Keymeulen et al. 1998; Shapiro et al. 2000; Ryan et al. 2001). However, to achieve sustained metabolic control for 1 year, at least 2 million β-cells per kg body weight need to be transplanted (Keymeulen et al. 2006), which usually requires 2–3 donor pancreata. Indeed, many β-cells are lost during the isolation procedures, during the early period of post-engraftment neo vascularization and possibly also as a result of inflammation at the transplantation site. Furthermore, the functional β-cell mass of the graft seems to decline after 1 year, and after 5 years only 20% of transplanted patients retain a functioning graft (Ryan et al. 2005). This, together with the limited availability of donor organs, severely limit the widespread application of transplantation therapy in patients with T1DM.

In line with this, during the last years several studies have been carried out in order to identify an alternative source of viable insulin-producing cells for regenerative medicine.

We recently described a protocol for the epigenetic conversion of human dermal fibroblasts into insulin-secreting cells (EpiCC) (Pennarossa et al. 2013; Brevini et al. 2016). This method is based on a brief exposure (18 h) to the demethylating drug 5-aza-cytidine (5-aza-CR), followed by a three-step induction protocol. This allows cells to transit from the early endodermic and pancreatic differentiation stage to mature endocrine cells. At the end of the epigenetic conversion process, fibroblasts acquire an epithelial morphology and form large three-dimensional spherical structures that tend to detach and float freely in the culture medium, reminiscent of in vitro-cultured pancreatic islets. Most importantly, EpiCC express the main pancreatic hormones and glucose sensor genes, distinctive of mature endocrine cells. Furthermore, 35 ± 8.9% of starting cell population is able to actively release of C-peptide and insulin after exposure to 20 mM glucose, showing a dynamic response similar to pancreatic β-cells, in which changes in ambient glucose represent the primary and physiological stimulus for insulin secretion. Preliminary experiments also demonstrate *in vivo* functionality of EpiCC, after their injection in streptozotocin (STZ)-induced diabetic mice. Indeed, cell transplantation lead to a restoring of physiological glycemic levels, that are stably maintained for a long period in diabetic animals.

The epigenetic conversion method has notable advantages compared to the alternative available procedures: it is highly efficient, it does not require any transgenic modification, and it does not induce a stable pluripotent stage that may be cause malignant transformations. Based on these observations, EpiCC look very promising candidates for their clinical translation as a therapy for diabetes including type I diabetes mellitus (T1DM).

In this perspective, preclinical studies in animal models are necessary in order to ensure the safety of cell treatment and to establish the adequate dose that enables patient adherence to treatment.

2 Approaches

2.1 Ethical Statement

Cells were isolated from adult patients, after written informed consent and approved by the Ethical Committee of the Department of Internal Medicine of the University of Genoa and by the Ethical Committee of the University of Milan. All the methods in our study were carried out in accordance with the approved guidelines. Animals were handled and treated in agreement with the

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

2.2 Epigenetic Cell Conversion into Insulin-Secreting Cells

In this study, four adult human dermal fibroblast primary lines were used. Two lines were isolated from diabetic patients and kindly donated by Gianpaolo Zerbini (Scientific Institute San Raffaele, University of Milan, Milan, Italy). One line was obtained from male healthy individual, and one from female healthy individual.

All dermal fibroblasts were treated as previously described (Pennarossa et al. 2013; Brevini et al. 2016). Briefly, cells were plated on 0.1% gelatin pre-coated dishes (Sarstedt) at concentration of 7.8×10^4 fibroblasts/cm^2. After 24 h, they were epigenetically erased by exposure to 1 μM 5-aza-CR (Sigma) for 18 h, and, subsequently, incubated for 3 h with ESC culture medium (14) (Brevini et al. 2009). Pancreatic differentiation was induced culturing cell in in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 1% B27, 1% N2, 0.1 mM β-mercaptoetanol (Sigma), 2 mM glutamine (Sigma), 1 mM MEM Non-essential amino acids and 0.05% bovine serum albumin (BSA, Sigma). During the first 6 days, medium was enriched with 30 ng/ml activin A. The following 2 days, 10 μM retinoic acid (Sigma) was added. From day 9 onward, a medium containing 1% B27, 20 ng/ml basic fibroblast growth factor and 1% insulin–transferrin–selenium was used to further encourage differentiation. Medium was refreshed daily. Cells were in vitro differentiated for a total of 36 days.

2.3 Experimental Design

EpiCC Safety Assessment To exclude the possible risk of EpiCC tumorigenicity, 300 eight-week-old male SCID mice (Harlan) were xenogratfed with 5×10^6 EpiCC. Experimental diabetes was induced in 200 mice, by a single intraperitoneal injection of streptozotocin (STZ; Sigma, 150 mg/kg of body weight) freshly dissolved in 0.1 M of citrate buffer, pH 4.6 (Lumelsky et al. 2001). STZ-induced diabetic animals were randomly divided in 4 groups of 50 individuals. Group 1 received diabetic female EpiCC (DF), group 2 was injected with EpiCC obtained from diabetic male (DM), group 3 was xenografted with healthy female cells (HF), and group 4 was transplanted with healthy male EpiCC (HM). Furthermore, in order to rule out tumorigenic potential also in the presence of an autologous source of insulin, the scheme described above was applied on 100 SCID mice that were not treated with STZ and animals received 5×10^6 EpiCC obtained to the four different cell lines: diabetic female (DF, $n = 25$), diabetic male (DM, $n = 25$), healthy female (HF, $n = 25$), and healthy male (HM, $n = 25$).

Six days after STZ treatment, all animals were anesthetized by isoflurane (3.5% gas in oxygen, Vet-Merial). EpiCC were injected subcutaneously in the shoulder area through a 19-gauge hypodermic needle. Mice were macroscopically monitored daily for signs of tumor formation/growth or visible morbidity. Blood glucose levels were measured using Accu-Chek glucometer (Roche) at 1 week intervals. Mice were euthanized 50 weeks after transplantation. At the end of the experiment, randomly selected mice from each group were subjected to a complete necropsy and histopathological evaluation for any sign of tumorigenic transformation and/or cell migration from the injection site.

EpiCC Efficacy Assessment 210 STZ-induced diabetic SCID mice were xenografted, as described above, with different doses of EpiCC obtained from the four different cell lines: diabetic female (DF, $n = 50$), diabetic male (DM, $n = 50$), healthy female (HF, $n = 50$), healthy male (HM, $n = 50$). The following doses were tested: 10×10^6 ($n = 40$), 5×10^6 ($n = 40$),

2.5×10^6 (n = 40), 1.25×10^6 (n = 40), 0.6×10^6 (n = 40), and 0 (n = 10) cells.

40 additional animals received STZ treatment and 5×10^6 untreated control fibroblasts belonging to the four cell lines: DF, n = 10; DM, n = 10; HF, n = 10; and HM, n = 10. Mice were carefully monitored as described above and euthanized 4 and 30 weeks after fibroblasts and EpiCC transplantation, respectively. At the end of the experiment, randomly selected mice from each group were subjected to a complete necropsy and histopathological evaluation.

Statistical Analysis Statistical analysis was performed using ANOVA test (SPSS 19.1; IBM). Data were presented as mean ± standard deviation (SD). Differences of $p \leq 0.05$ were considered significant and were indicated with different superscripts.

3 Observations

3.1 EpiCC Safety Assessment

EpiCC obtained from the four different human cell lines described above were able to quickly restore and maintain blood glucose physiological levels in all STZ-induced diabetic mice (Table 1 and Fig. 1A). Interestingly enough, EpiCC ability to release insulin was comparable and completely independent from the health state of the patient of origin. Furthermore, when cells were xenografted in healthy animals (no STZ treatment), glycemic values remained normal and were not affected by the injection, demonstrating EpiCC ability to self-regulate and to secrete insulin in a controlled physiological manner.

Necropsy and histopathological evaluations, carried out 50 weeks after transplantation, demonstrated the complete absence of malignant transformation and no traces of cell migration to organs and lymph nodes of all subjects analyzed. Furthermore, no major advice effects were observed.

3.2 EpiCC Efficacy Assessment

EpiCC efficacy experiments demonstrated re-establishment of normoglycemia in STZ-induced diabetic SCID mice with all the different doses tested (Table 2 and Fig. 1B). More in detail, the normal and physiological glucose concentrations were restored 2 weeks after transplantation in a cell concentration ranging from 10×10^6 to the lower dose of 0.6×10^6. Furthermore, all dose range were able to steady maintain glycemic levels for the entire length of the experiments (30 weeks).

In contrast, injection of vehicle only (no EpiCC) or untreated control fibroblasts obtained from DF, DM, HF and HM patients, resulted in not reduction of hyperglycemic values in diabetic animals.

Necropsy and histopathological evaluations, carried out 30 weeks after injection, demonstrated the complete absence of malignant transformation and/or no traces of cell migration to organs and lymph nodes in all experimental groups. Furthermore, no major advice effects were observed.

4 Discussion

According to the World Health Organization (WHO), more than 400 million individuals suffer from diabetes and it is estimated that this disease will represents the seventh leading cause of death in 2030 (Mathers and Loncar 2006). Similar to other autoimmune diseases, caused by a combination of genetic and environmental factors, the incidence of T1DM is on the increase at an alarming rate in industrialized countries, leading, in the first place, to high levels of blood glucose, and, subsequently, acute and late complications, such as ketoacidosis, and atherosclerosis, retinopathy, kidney failure, neuropathy, and infection, respectively.

Exogenous insulin administration is a life-saving treatment rather than a cure, and individuals suffering from T1DM and receiving

Table 1 EpiCC safety assessment

Weeks	−1	−0.5	0	1	2	3	4
STZ + DF	123.1 ± 6.4	423.2 ± 26.1*	459.5 ± 7.6*	235.4 ± 31.3**	162.7 ± 11.0	153.8 ± 16.1	147.3 ± 13.4
STZ + DM	122.2 ± 10.1	411.0 ± 31.2*	443.8 ± 9.2*	236.3 ± 25.5**	148.7 ± 14.2	156.5 ± 8.9	147.0 ± 17.1
STZ + HF	130.6 ± 9.2	407.5 ± 21.8*	443.8 ± 11.4*	237.6 ± 22.6**	151.8 ± 16.1	158.4 ± 12.3	157.2 ± 16.2
STZ + HM	123.1 ± 12.3	400.0 ± 21.9*	441.0 ± 10.4*	228.1 ± 23.2**	176.1 ± 9.7	166.8 ± 10.8	144.2 ± 11.2
No-STZ + DF	129.6 ± 8.5	146.9 ± 11.3	122.7 ± 8.4	134.8 ± 13.4	156.5 ± 11.6	128.4 ± 8.7	138.3 ± 9.9
No-STZ + DM	126.7 ± 7.6	132.5 ± 12.1	131.2 ± 12.3	127.6 ± 8.7	138.3 ± 12.3	131.4 ± 9.1	141.1 ± 10.4
No-STZ + HF	131.1 ± 10.2	101.0 ± 9.5	125.6 ± 11.7	133.8 ± 8.1	140.8 ± 9.8	127.4 ± 12.1	144.6 ± 15.5
No-STZ + HM	123.0 ± 9.1	104.4 ± 9.7	146.4 ± 10.9	133.2 ± 9.8	138.6 ± 8.8	131.6 ± 10.7	145.2 ± 9.1

Weeks	8	12	16	20	30	40	50
STZ + DF	136.7 ± 14.5	153.0 ± 14.7	136.8 ± 9.8	136.1 ± 15.4	134.1 ± 13.1	124.6 ± 12.1	127.5 ± 16.4
STZ + DM	145.1 ± 14.6	128.4 ± 8.8	132.2 ± 15.0	111.3 ± 13.2	126.8 ± 15.6	122.1 ± 9.9	134.0 ± 13.8
STZ + HF	141.6 ± 8.9	135.0 ± 11.0	134.2 ± 8.7	134.6 ± 8.8	136.8 ± 17.2	143.2 ± 11.3	138.6 ± 10.7
STZ + HM	141.8 ± 9.2	140.2 ± 9.8	128.3 ± 14.2	129.6 ± 9.7	140.0 ± 7.9	144.6 ± 12.3	135.0 ± 9.2
No-STZ + DF	142.3 ± 11.9	128.5 ± 12.7	134.3 ± 10.7	140.2 ± 14.7	127.1 ± 12.1	132.8 ± 9.9	129.0 ± 12.7
No-STZ + DM	135.4 ± 9.3	137.6 ± 10.8	127.8 ± 11.4	145.0 ± 8.5	124.2 ± 9.9	137.6 ± 8.9	136.3 ± 8.6
No-STZ + HF	135.2 ± 11.1	133.4 ± 7.8	128.4 ± 13.6	171.0 ± 10.0	137.2 ± 8.9	105.2 ± 11.1	166.0 ± 9.9
No-STZ + HM	147.6 ± 10.8	147.2 ± 9.2	133.8 ± 7.9	111.8 ± 9.3	167.4 ± 11.2	194.4 ± 13.4	129.8 ± 11.1

Blood glucose values (means ± SD) in STZ-treated (STZ) and healthy (no-STZ) mice transplanted with 5×10^6 EpiCC obtained from the four different cell lines (*DF* diabetic female, *DM* diabetic male, *HF* healthy female, and *HM* healthy male). Different superscripts denote significant differences between groups ($P < 0.05$)

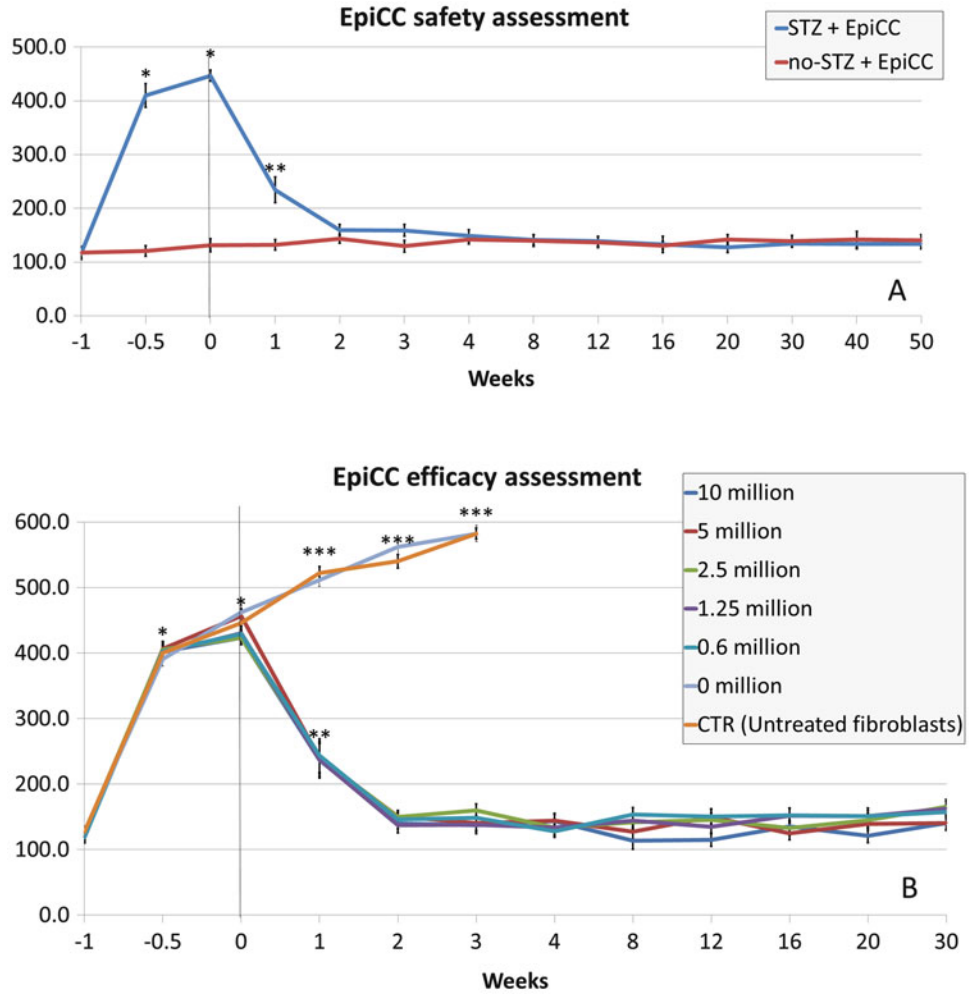

Fig. 1 (**a**) Blood glucose levels (means±SD) in STZ-treated (STZ) and healthy (no-STZ) mice transplanted with 5×10^6 human EpiCC. (**b**) Blood glucose values (means±SD) in STZ-treated (STZ) mice transplanted with different doses of human EpiCC. Different superscripts denote significant differences between groups ($P < 0.05$)

insulin still show an unacceptable 15 year reduction in life expectancy and complications of the disease, according to the Juvenile Diabetes Research Foundation (JDRF, http://www.jdrf.org.uk/page.asp?section=163&sectionTitle=FAQs+about+type+1+diabetes). Indeed, this treatment is not sufficient to mimic the normal function of β-cells and the physiological control of blood glucose levels can only be maintained or restored by preventing the evolution of the disease or by the replacement/regeneration of β-cells mass. In line with this, during the last years, several studies have been carried out to find novel treatments to cure T1DM. Much investigation has been directed at interrupting its process both during the stage of evolution or at the time of disease onset, using immune-therapeutic approaches. The goal of this intervention is to arrest the immune destruction and thus delay or prevent clinical disease. However, to effectively accomplish this requires identification of individuals at risk of T1DM (Ziegler and Nepom 2010). This represents a relevant limitation, since the major symptoms of the disease

Table 2 EpiCC efficacy assessment

Weeks	−1	−0.5	0	1	2	3
DF (10×10^6)	123.2 ± 8.9	399.3 ± 13.0*	411.5 ± 9.7*	225.1 ± 25.1**	116.8 ± 13.2	122.2 ± 12.5
DM (10×10^6)	131.6 ± 7.6	410.7 ± 9.9*	423.0 ± 11.3*	245.5 ± 24.2**	131.1 ± 11.2	126.1 ± 16.5
HF (10×10^6)	142.4 ± 8.2	401.9 ± 11.9*	408.6 ± 12.0*	238.2 ± 26.2**	144.4 ± 10.4	149.4 ± 9.0
HM (10×10^6)	109.3 ± 7.1	399.8 ± 13.8*	451.3 ± 10.7*	238.2 ± 24.1**	168.5 ± 9.1	151.5 ± 14.1
DF (5×10^6)	126.7 ± 9.9	421.9 ± 11.0*	432.6 ± 11.1*	244.2 ± 28.7**	169.2 ± 9.4	145.6 ± 13.2
DM (5×10^6)	131.1 ± 7.6	387.5 ± 12.1*	461.4 ± 9.8*	240.2 ± 21.6**	156.1 ± 8.7	141.9 ± 9.6
HF (5×10^6)	112.7 ± 6.6	417.4 ± 13.2*	481.1 ± 12.5*	243.4 ± 22.4**	125.3 ± 9.9	127.8 ± 11.2
HM (5×10^6)	108.4 ± 5.9	401.1 ± 8.9*	452.4 ± 11.9*	231.8 ± 24.6**	151.2 ± 13.5	147.6 ± 10.2
DF (2.5×10^6)	118.3 ± 11.9	409.0 ± 11.1*	461.7 ± 14.0*	234.6 ± 27.0**	157.5 ± 11.7	177.5 ± 11.7
DM (2.5×10^6)	127.0 ± 8.5	387.6 ± 10.1*	416.8 ± 8.2*	247.4 ± 26.1**	148.1 ± 12.2	172.7 ± 8.3
HF (2.5×10^6)	131.1 ± 6.7	420.1 ± 9.8*	407.2 ± 10.8*	242.3 ± 24.2**	140.4 ± 9.8	128.1 ± 9.6
HM (2.5×10^6)	125.2 ± 7.4	406.7 ± 8.8*	409.2 ± 9.1*	237.4 ± 23.4**	153.7 ± 7.8	161.8 ± 11.2
DF (1.25×10^6)	116.8 ± 10.1	403.4 ± 9.6*	413.3 ± 14.2*	231.1 ± 23.1**	122.5 ± 11.2	121.9 ± 8.6
DM (1.25×10^6)	124.4 ± 6.8	379.9 ± 11.2*	437.1 ± 9.8*	220.7 ± 29.9**	149.4 ± 8.5	151.0 ± 10.9
HF (1.25×10^6)	131.5 ± 7.3	419.3 ± 12.1*	448.8 ± 9.6*	241.6 ± 28.9**	137.5 ± 15.5	133.5 ± 9.4
HM (1.25×10^6)	119.8 ± 8.8	402.1 ± 8.9*	422.2 ± 11.1*	255.8 ± 29.4**	140.4 ± 13.5	147.6 ± 14.2
DF (0.6×10^6)	131.9 ± 9.5	412.3 ± 12.0*	417.3 ± 12.0*	229.0 ± 22.3**	135.3 ± 11.3	130.3 ± 15.1
DM (0.6×10^6)	124.7 ± 9.8	399.4 ± 11.6*	412.4 ± 13.3*	253.1 ± 28.9**	169.8 ± 10.8	172.1 ± 8.1
HF (0.6×10^6)	117.3 ± 7.6	388.6 ± 8.9*	435.6 ± 8.4*	243.4 ± 27.4**	129.0 ± 9.6	147.5 ± 8.6
HM (0.6×10^6)	104.0 ± 11.0	411.7 ± 9.9*	452.4 ± 11.3*	249.4 ± 23.1**	152.2 ± 9.5	146.6 ± 12.4
VEHICLE	127.8 ± 8.1	391.2 ± 11.5*	462.3 ± 9.6*	511.3 ± 9.7***	562.1 ± 12.3***	582.1 ± 12.5***
DF (CTR)	139.1 ± 8.5	386.3 ± 13.1*	477.5 ± 10.8*	502.3 ± 12.8***	506.1 ± 11.1***	577.7 ± 8.5***
DM (CTR)	129.6 ± 8.3	415.3 ± 7.5*	454.4 ± 7.9*	502.2 ± 7.9***	507.8 ± 13.2***	584.6 ± 10.0***
HF (CTR)	127.6 ± 7.5	389.8 ± 10.1*	436.8 ± 10.2*	524.1 ± 8.6***	553.6 ± 9.7***	569.7 ± 7.4***
HM (CTR)	113.5 ± 9.8	410.1 ± 9.8*	416.9 ± 11.2*	561.4 ± 11.3***	594.9 ± 7.8***	598.8 ± 7.9***

Weeks	4	8	12	16	20	30
DF (10×10^6)	142.4 ± 13.2	121.4 ± 15.9	108.9 ± 9.8	131.3 ± 8.7	112.0 ± 9.9	114.2 ± 12.1
DM (10×10^6)	145.0 ± 9.3	110.4 ± 9.1	123.8 ± 10.5	142.3 ± 11.1	134.0 ± 10.4	138.6 ± 14.1
HF (10×10^6)	148.3 ± 7.4	103.8 ± 10.9	100.4 ± 11.2	128.6 ± 7.9	108.6 ± 11.1	155.6 ± 9.7
HM (10×10^6)	139.1 ± 9.9	119.7 ± 15.3	129.5 ± 9.5	139.5 ± 9.8	130.2 ± 12.1	154.5 ± 7.8
DF (5×10^6)	143.4 ± 15.1	120.1 ± 11.4	141.7 ± 11.1	109.6 ± 12.2	121.6 ± 9.8	136.5 ± 7.9

(continued)

Table 2 (continued)

Weeks	4	8	12	16	20	30
DM (5×10^6)	173.5 ± 11.6	118.4 ± 9.7	181.2 ± 14.3	122.1 ± 9.7	154.1 ± 6.9	146.3 ± 13.1
HF (5×10^6)	137.0 ± 9.7	124.2 ± 10.7	131.2 ± 15.9	125.4 ± 9.9	137.8 ± 12.4	130.7 ± 9.8
HM (5×10^6)	123.1 ± 8.7	146.3 ± 12.1	146.2 ± 9.8	142.6 ± 8.9	144.8 ± 11.0	149.0 ± 9.9
DF (2.5×10^6)	134.5 ± 14.4	156.7 ± 12.3	139.5 ± 12.3	129.4 ± 16.1	122.7 ± 11.5	168.1 ± 8.9
DM (2.5×10^6)	137.6 ± 11.3	135.5 ± 9.7	157.0 ± 9.8	135.6 ± 9.4	162.5 ± 12.1	172.0 ± 9.9
HF (2.5×10^6)	115.3 ± 16.4	146.4 ± 8.6	160.1 ± 7.9	151.6 ± 11.3	171.6 ± 9.8	138.4 ± 12.5
HM (2.5×10^6)	148.6 ± 9.8	129.2 ± 9.4	125.2 ± 14.5	117.4 ± 9.1	123.6 ± 11.4	182.4 ± 11.9
DF (1.25×10^6)	131.6 ± 15.3	149.1 ± 11.0	145.7 ± 8.9	159.8 ± 10.9	149.4 ± 11.2	188.3 ± 11.2
DM (1.25×10^6)	128.7 ± 8.2	151.3 ± 13.2	130.5 ± 12.1	137.1 ± 14.5	170.2 ± 15.1	134.8 ± 12.1
HF (1.25×10^6)	119.1 ± 10.9	157.9 ± 7.9	121.5 ± 9.2	177.3 ± 12.3	145.9 ± 13.2	140.4 ± 9.8
HM (1.25×10^6)	155.3 ± 12.3	119.8 ± 8.9	144.6 ± 14.2	134.5 ± 9.8	141.6 ± 9.9	186.8 ± 13.2
DF (0.6×10^6)	126.2 ± 12.5	134.3 ± 9.7	131.7 ± 12.3	146.1 ± 12.1	184.1 ± 8.7	157.7 ± 11.2
DM (0.6×10^6)	124.0 ± 9.9	189.1 ± 8.4	174.2 ± 9.9	142.0 ± 10.7	136.7 ± 6.9	124.2 ± 13.4
HF (0.6×10^6)	134.1 ± 8.6	143.3 ± 10.9	142.1 ± 9.1	172.3 ± 9.1	139.4 ± 7.9	165.1 ± 9.4
HM (0.6×10^6)	128.6 ± 7.9	148.4 ± 12.4	154.3 ± 15.1	148.6 ± 11.1	144.8 ± 11.6	184.1 ± 7.9
VEHICLE	–	–	–	–	–	–
DF (CTR)	–	–	–	–	–	–
DM (CTR)	–	–	–	–	–	–
HF (CTR)	–	–	–	–	–	–
HM (CTR)	–	–	–	–	–	–

Blood glucose values (means ± SD) in STZ-treated mice transplanted with different doses of EpiCC, obtained from the four different cell lines (DF: diabetic female, DM: diabetic male, HF: healthy female, and HM: healthy male), vehicle only (VEHICLE) or untreated control fibroblasts (CTR) isolated from the four patients (*DF* diabetic female, *DM* diabetic male, *HF* healthy female, and *HM* healthy male). Different superscripts denote significant differences between groups ($P < 0.05$)

usually become manifest only once the number of β-cells mass has become inadequate (Weir and Bonner-Weir 2013). Transplantation of pancreas or pancreatic islets, with the obligatory immunosuppression, has been the most commonly used alternative, applied for over two decades in patients suffering from hypoglycemia (Alejandro et al. 2008). Even this procedure does not represent a definitive cure, since the pre-existing autoimmunity cause ~85% of islet transplants to fail 10 years post-transplantation (Ryan et al. 2005). Furthermore, the limited availability of an adequate source of transplantable β-cells and the low number of donors still represent a critical and primary obstacle for this therapy (Halban et al. 2010).

Accordingly, a series of potentially innovative solutions have been proposed during the last years. In particular, stem-cell research had a progressive improvement in this field, exploring a variety of cells, namely, embryonic, mesenchymal, bone marrow, cord blood, adipocyte-derived, and others, for the derivation of insulin-producing cells. To date, no standard protocol has been established to obtain terminally differentiated and functional β-cells from embryonic stem cells (ESCs) and transplantation of pancreatic endocrine progenitors, which can further differentiate and mature *in vivo*, has been proposed. However, a severe limit may derive from the risk that undifferentiated cells could be potentially included among them, with a tendency to form teratomas (Kroon et al. 2008; Sui et al. 2013), severely limiting their use in human patients. The same issue can be hypothesized for induced pluripotent stem cells (iPSC), where the use of DNA-based reprogramming techniques and the introduction of oncogenic reprogramming factors could lead to insertional mutagenesis and increase risk of tumoural transformation. Multipotent stem cells, obtained from the adult pancreas and capable of β-cell neogenesis are also suggested as an alternative source of cells for pancreatic regeneration. Currently, their identification is under investigation, but the preliminary results are heavily controversial. In contrast, mesenchymal stem cells (MSCs) outside the pancreas are well-characterized and easily accessible. They can be obtained from umbilical cord blood, bone marrow or adipose tissue, highly expandable *in vitro* and can give rise to multiple cell lineages (Nombela-Arrieta et al. 2011). However, it must be noted that the involvement of MSCs in epithelial tissue regeneration and their ability to differentiate in functional pancreatic cells remains uncertain (Mathews et al. 2004).

Transdifferentiation or 'direct reprogramming' (Nicholas and Kriegstein 2010) has also been proposed as a possible option. This method is based on the conversion of differentiated cells into another phenotype without inducing a stable multipotent or pluripotent state and introducing key transcription factors, usually with the help of viral vectors. In the case of the pancreas, the transcription factors successfully used are known to regulate the major transitions during embryonic morphogenesis, and are PDX-1 (pancreatic and duodenal homeobox 1; associated with the primary transition to multipotent pancreatic progenitors) (Jonsson et al. 1994), NGN-3 (neurogenin 3; involved in the secondary transition to endocrine precursors) (Gradwohl et al. 2000) and MafA (MAF bZIP transcription factor A; implicated in the transition from immature to functional β cells) (Matsuoka et al. 2004). This approach is very promising, however, similar to some of the techniques described above, bears the limit related to the risks of insertional mutagenesis and oncogenesis, associated with the insertion of exogenous DNA.

More recently, different studies demonstrated that acinar cells can dedifferentiate in vitro to a state resembling embryonic multipotent progenitors, through the induction of endogenous gene expression, via stimulation with soluble signaling factors (Pinho et al. 2011). Several different lineages can then be derived, including β cells (Lardon et al. 2004). Yet, the general scarcity of donor organs still poses a problem for this approach, as acinar cells (unlike stem cells) have limited expansion potential.

Interestingly, although the approaches discussed above have some limits that hinder their potential use in patients, they paved the way for other possible easier and safer alternatives to translate, based on epigenetic

tools, to the clinical setting. A small-molecule-based method, able to directly convert a terminally differentiated cell into a different cell type, has been recently proposed. This new approach demonstrated that it is possible to dynamically interact with cell genotype and phenotype through the use of epigenetic modifiers, with no toxic effect and without the induction of a stable pluripotent state, that makes cells prone to transformation (Harris et al. 2011; Pennarossa et al. 2013; Brevini et al. 2014; Pennarossa et al. 2014; Mirakhori et al. 2015; Brevini et al. 2016; Chandrakanthan et al. 2016). In particular, a brief exposure to a demethylating agent, able to remove the epigenetic 'blocks' that are responsible for tissue specification (Jones and Taylor 1981; Taylor and Jones 1982; Jones et al. 1983; Jones 1985a, b; Glover et al. 1986), can push cells to a less committed state, increasing their plasticity for a short window of time, though sufficient to re-address cells towards a different phenotype (Harris et al. 2011; Pennarossa et al. 2013, 2014; Brevini et al. 2014, 2016; Mirakhori et al. 2015; Chandrakanthan et al. 2016). In agreement with these findings, we demonstrated that adult skin fibroblasts, derived from different species, namely human (Pennarossa et al. 2013), porcine (Pennarossa et al. 2014), dog (Brevini et al. 2016), cat (Brevini et al. 2018), and mouse (Pennarossa et al. 2018), can be converted towards the pancreatic lineage. This is achieved using a three-step induction protocol that allow cells to transit from the early endodermic and pancreatic differentiation stage to mature endocrine cells. At the end of the epigenetic conversion, over 30% of cells expressed the main pancreatic hormones and were able to actively release C-peptide and insulin. Similar to β-cells, they dynamically responded to 20 mM glucose stimulation as a primary and physiological stimulus for insulin secretion. These results demonstrated that the use of an epigenetic modifier provides notable advantages, compared to the alternative available procedures, and could represent a very promising tool for cell therapy of diabetes. In order to translate these very encouraging experimental findings, it was however mandatory to progress through preclinical in vivo animal studies, in order to evaluate efficacy and toxicity.

The data presented in this manuscript confirm in vivo cell functionality and demonstrate converted cell ability to restore normo-glycaemia, and stably maintain it within the physiological range, in diabetic STZ-induced SCID mice. A very interesting point is related to the results obtained when treatment was carried out in healthy non-diabetic animals. In particular, EpiCC engrafted into non-STZ treated SCID mice did not cause hypoglycemia, but, rather sensed the glucose concentration ambient, and responded in an adequate manner, suggesting cell acquisition of self-regulatory property.

Autoptic investigations and histopathological analysis showed the complete absence of malignant transformation. This finding clearly indicates that epigenetic conversion confers a steady pancreatic phenotype to human fibroblasts, which is independent of the health state and gender of the patient of origin and is stably maintained even after a 50-week engraftment period. Interestingly, no migrated cells were detected in any organ and lymph node, suggesting that cells colonize the area of injection and do not spread elsewhere, making the adoption of a containing safety device unnecessary.

In our understanding, a key aspect was represented by the establishment of the dose range that may restore normal glycemic values in diabetic individuals. Our findings point out that it is possible to gain and steadily maintain physiological levels in a cell concentration ranging from 10×10^6 to the lower dose of 0.6×10^6. The possibility to obtain a therapeutic effect with a low concentration as the latter, represents a great advantage for the overall procedure, since it requires a shorter fibroblast expansion time from patient biopsy.

In conclusion, this preclinical study demonstrates that epigenetic conversion of

dermal fibroblasts into insulin secreting cells may represent a powerful, safe, and promising approach for personalized regenerative medicine of diabetic patients.

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Conflict of Interest The authors declare no conflict of interest in relation to this article.

References

Alejandro R, Barton FB, Hering BJ, Wease S (2008) 2008 update from the collaborative islet transplant registry. Transplantation 86(12):1783–1788

Brevini TA, Pennarossa G, Antonini S, Paffoni A, Tettamanti G, Montemurro T, Radaelli E, Lazzari L, Rebulla P, Scanziani E, de Eguileor M, Benvenisty N, Ragni G, Gandolfi F (2009) Cell lines derived from human parthenogenetic embryos can display aberrant centriole distribution and altered expression levels of mitotic spindle check-point transcripts. Stem Cell Rev 5(4):340–52

Brevini TA, Pennarossa G, Rahman MM, Paffoni A, Antonini S, Ragni G, deEguileor M, Tettamanti G, Gandolfi F (2014) Morphological and molecular changes of human Granulosa cells exposed to 5-Azacytidine and addressed toward muscular differentiation. Stem Cell Rev 10(5):633–642

Brevini TA, Pennarossa G, Maffei S, Zenobi A, Gandolfi F (2016) Epigenetic conversion as a safe and simple method to obtain insulin-secreting cells from adult skin fibroblasts. J Vis Exp: JoVE 109:e53880

Brevini T, Manzoni E, Gandolfi F (2018) Methylation mechanisms and biomechanical effectors controlling cell fate. Reprod Fertil Dev 30:64–72

Butler AE, Galasso R, Meier JJ, Basu R, Rizza RA, Butler PC (2007) Modestly increased beta cell apoptosis but no increased beta cell replication in recent-onset type 1 diabetic patients who died of diabetic ketoacidosis. Diabetologia 50(11):2323–2331

Chandrakanthan V, Yeola A, Kwan JC, Oliver RA, Qiao Q, Kang YC, Zarzour P, Beck D, Boelen L, Unnikrishnan A, Villanueva JE, Nunez AC, Knezevic K, Palu C, Nasrallah R, Carnell M, Macmillan A, Whan R, Yu Y, Hardy P, Grey ST, Gladbach A, Delerue F, Ittner L, Mobbs R, Walkley CR, Purton LE, Ward RL, Wong JW, Hesson LB, Walsh W, Pimanda JE (2016) PDGF-AB and 5-Azacytidine induce conversion of somatic cells into tissue-regenerative multipotent stem cells. Proc Natl Acad Sci U S A 113(16):E2306–E2315

Gepts W (1965) Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes 14(10):619–633

Glover TW, Coyle-Morris J, Pearce-Birge L, Berger C, Gemmill RM (1986) DNA demethylation induced by 5-azacytidine does not affect fragile X expression. Am J Hum Genet 38(3):309–318

Gradwohl G, Dierich A, LeMeur M, Guillemot F (2000) neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci U S A 97(4):1607–1611

Halban PA, German MS, Kahn SE, Weir GC (2010) Current status of islet cell replacement and regeneration therapy. J Clin Endocrinol Metab 95 (3):1034–1043

Harris DM, Hazan-Haley I, Coombes K, Bueso-Ramos C, Liu J, Liu Z, Li P, Ravoori M, Abruzzo L, Han L, Singh S, Sun M, Kundra V, Kurzrock R, Estrov Z (2011) Transformation of human mesenchymal cells and skin fibroblasts into hematopoietic cells. PLoS One 6(6):e21250

Jones PA (1985a) Altering gene expression with 5-azacytidine. Cell 40(3):485–486

Jones PA (1985b) Effects of 5-azacytidine and its 2′-deoxyderivative on cell differentiation and DNA methylation. Pharmacol Ther 28(1):17–27

Jones PA, Taylor SM (1981) Hemimethylated duplex DNAs prepared from 5-azacytidine-treated cells. Nucleic Acids Res 9(12):2933–2947

Jones PA, Taylor SM, Wilson VL (1983) Inhibition of DNA methylation by 5-azacytidine. Recent Results Cancer Res 84:202–211

Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371(6498):606–609

Keymeulen B, Ling Z, Gorus FK, Delvaux G, Bouwens L, Grupping A, Hendrieckx C, Pipeleers-Marichal M, Van Schravendijk C, Salmela K, Pipeleers DG (1998) Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia 41(4):452–459

Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, Ysebaert D, Roep B, Vandemeulebroucke E, Marichal M, In't Veld P, Bogdani M, Hendrieckx C, Gorus F, Ling Z, van Rood J, Pipeleers D (2006) Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. Proc Natl Acad Sci U S A 103(46):17444–17449

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG,

Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26(4):443–452

Lardon J, De Breuck S, Rooman I, Van Lommel L, Kruhoffer M, Orntoft T, Schuit F, Bouwens L (2004) Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture. Hepatology 39(6):1499–1507

Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292(5520):1389–1394

Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3(11):e442

Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA (2004) Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. Diabetes 53(1):91–98

Matsuoka TA, Artner I, Henderson E, Means A, Sander M, Stein R (2004) The MafA transcription factor appears to be responsible for tissue-specific expression of insulin. Proc Natl Acad Sci U S A 101(9):2930–2933

Mirakhori F, Zeynali B, Kiani S, Baharvand H (2015) Brief azacytidine step allows the conversion of suspension human fibroblasts into neural progenitor-like cells. Cell J 17(1):153–158

Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329(14):977–986

Nicholas CR, Kriegstein AR (2010) Regenerative medicine: cell reprogramming gets direct. Nature 463 (7284):1031–1032

Nombela-Arrieta C, Ritz J, Silberstein LE (2011) The elusive nature and function of mesenchymal stem cells. Nat Rev Mol Cell Biol 12(2):126–131

Pennarossa G, Maffei S, Campagnol M, Tarantini L, Gandolfi F, Brevini TA (2013) Brief demethylation step allows the conversion of adult human skin fibroblasts into insulin-secreting cells. Proc Natl Acad Sci U S A 110(22):8948–8953

Pennarossa G, Maffei S, Campagnol M, Rahman MM, Brevini TA, Gandolfi F (2014) Reprogramming of pig dermal fibroblast into insulin secreting cells by a brief exposure to 5-aza-cytidine. Stem Cell Rev 10(1):31–43

Pennarossa G, Santoro R, Manzoni E, Pesce M, Gandolfi F, Brevini T (2018) Epigenetic erasing and pancreatic differentiation of dermal fibroblasts into insulin-producing cells are boosted by the use of low-stiffness substrate. Stem Cell Rev Rep. https://doi.org/10.1007/s12015-017-9799-0

Pinho AV, Rooman I, Reichert M, De Medts N, Bouwens L, Rustgi AK, Real FX (2011) Adult pancreatic acinar cells dedifferentiate to an embryonic progenitor phenotype with concomitant activation of a senescence programme that is present in chronic pancreatitis. Gut 60(7):958–966

Pipeleers D, Ling Z (1992) Pancreatic beta cells in insulin-dependent diabetes. Diabetes Metab Rev 8(3):209–227

Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AM (2001) Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 50 (4):710–719

Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM (2005) Five-year follow-up after clinical islet transplantation. Diabetes 54(7):2060–2069

Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343(4):230–238

Sui L, Mfopou JK, Chen B, Sermon K, Bouwens L (2013) Transplantation of human embryonic stem cell-derived pancreatic endoderm reveals a site-specific survival, growth, and differentiation. Cell Transplant 22 (5):821–830

Taylor SM, Jones PA (1982) Changes in phenotypic expression in embryonic and adult cells treated with 5-azacytidine. J Cell Physiol 111(2):187–194

Weir GC, Bonner-Weir S (2013) Islet beta cell mass in diabetes and how it relates to function, birth, and death. Ann N Y Acad Sci 1281:92–105

Ziegler AG, Nepom GT (2010) Prediction and pathogenesis in type 1 diabetes. Immunity 32(4):468–478

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