Advances in Experimental Medicine and Biology 1089
Cell Biology and Translational Medicine

Kursad Turksen *Editor*

Cell Biology and Translational Medicine, Volume 2

Approaches for Diverse Diseases and Conditions

Springer

Advances in Experimental Medicine and Biology

Cell Biology and Translational Medicine

Volume 1089

Subseries Editor
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Approaches for Diverse Diseases and Conditions

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Editor
Kursad Turksen (Retired)
Ottawa Hospital Research Institute
Ottawa, ON, Canada

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISSN 2522-090X ISSN 2522-0918 (electronic)
Cell Biology and Translational Medicine
ISBN 978-3-030-04169-4 ISBN 978-3-030-04170-0 (eBook)
https://doi.org/10.1007/978-3-030-04170-0

Library of Congress Control Number: 2018953050

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

While there is a great interest in understanding fundamental aspects of the cell and molecular biology of stem cells, a key goal is to be able to harness this understanding in tissue repair, regeneration, and restoration of function in disease states. Realizing the potential of stem cells for therapeutic benefits in specific diseases and conditions will require detailed disease-specific approaches and trials, but lessons can be learned by comparisons of results and advances being made across diverse tissues, organs, and disease states.

With the latter goals in mind, I am very pleased to introduce the second volume in the new series titled Cell Biology and Translational Medicine (CBTMED), part of Springer Nature's long-standing and very successful Advances in Experimental Medicine and Biology book series. Each volume of the CBTMED series will cover emerging areas of regenerative medicine and translational aspects of stem cells. For this second volume, I have recruited outstanding researchers to highlight developments in both the basic research and clinical arenas for a variety of diseases and conditions.

I remain very grateful to Peter Butler, Editorial Director, and Meran Lloyd-Owen, Senior Editor, for their ongoing support of this series that we have embarked upon.

I would also like to acknowledge and thank Sara Germans-Huisman, Assistant Editor, for her outstanding 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 readers will find their contributions interesting and helpful.

Ottawa, ON, Canada — Kursad Turksen

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 1–22
https://doi.org/10.1007/5584_2018_227

Published online: 7 June 2018

Stem Cell and Obesity: Current State and Future Perspective

Moloud Payab, Parisa Goodarzi, Najmeh Foroughi Heravani, Mahdieh Hadavandkhani, Zeinab Zarei, Khadijeh Falahzadeh, Bagher Larijani, Fakher Rahim, and Babak Arjmand

Abstract

Obesity as a worldwide growing challenge is determined by abnormal fat deposition, which may damage general health. Weight loss and control of related risk factors like type2 diabetes, dyslipidemia, hypertension, cardiovascular diseases, and metabolic syndrome is an important concern in obesity management. Different therapeutic approaches such as lifestyle change, medications, and surgery are introduced for obesity treatment. Despite of gaining partially desirable results, the problem is remained unsolved. Therefore, finding a new approach that can overcome previous limitations is very attractive for both researchers and clinicians. Cell-based therapy using adipose-derived stromal cells seems to be a promising strategy to control obesity and related syndromes. To attain this aim, understanding of different type of adipose tissues, main signaling pathways, and different factors involved in development of adipocyte is essential. Recently, several cell-based methods like stem cell administration, brown adipose tissue

M. Payab
Obesity and Eating Habits Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

P. Goodarzi
Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran

N. Foroughi Heravani and M. Hadavandkhani
Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

Z. Zarei
Department of Tissue Engineering and Applied Cell Science, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

K. Falahzadeh
Metabolomics and Genomics Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

B. Larijani
Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

F. Rahim
Health Research Institute, Thalassemia and Hemoglobinopathy Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

B. Arjmand (✉)
Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

Metabolomics and Genomics Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
e-mail: barjmand@sina.tums.ac.ir

transplantation, cell lysates and exosomes have been examined on obese mouse models to manage obesity and related disorders. Successful outcome of such preclinical studies can encourage the cell-based clinical trials in the near future.

Keywords

Adipocyte · Adipose tissue · Animal models · Cell therapy · Mesenchymal stem/stromal cells · Obesity

Abbreviations

ADAMTS5	A disintegrin and metalloproteinase with thrombospondin motif 5
ADSC	Adipose derived mesenchymal stem cells
AKR1B10	Aldo-keto reductase family 1 member B10
aP2	Adipocyte protein 2
ASCs	Adipose derived-stem cells
BAT	Brown adipose tissue
BMI	Body mass index
BM-MSCs	Bone marrow mesenchymal stem cells
BMP4	Bone morphogenic protein 4
C/EBP α (A)/β/δ	CCAAT/enhancer-binding protein α/β/δ
CB-MSC	Umbilical cord blood-mesenchymal stem cell
CB-plasma	Cord blood plasma
CD24	Cluster of differentiation 24
Cidea	Cell death-inducing DFFA-like effector a
CIT	Cold induced thermogenesis
Cox2	Cyclooxygenase 2
CRE	cAMP response element
CXCL3	Chemokine(C-X-C motif) ligand3
DIO	Diet-induced obese
DIT	Diet-induced thermogenesis
Dlk1	Delta like non-canonical notch ligand 1
EBF2	Early B cell factor 2
EHMT1	Euchromatic histone lysine methyltransferase 1
ENG	Endoglin (protein)
ERK	Extra cellular receptor kinase
ES	Embryonic stem cell
EVs	Extracellular vesicles
FDA	Food and drug administration
FGF10	Fibroblast growth factor 10
FPG	Fasting plasma glucose
FTO	Fat mass and obesity associated (gene)
H3K9	Histone H3 lysine 9
HDL	High-density lipoprotein
HFD	High-fat diet
Hh	Hedgehog
HOXC8	HomeoboxC8
IDF	International diabetes federation
IGF1	Insulin-like growth factor1
IL6	Interlukine 6
LOX	lysyl oxidase
M-BA	MSC-derived BAT
Mef2	Myocyte enhancer factor 2
miR-196a	MicroRNA 196a
miRNAs	MicroRNAs
MSC	Mesenchymal stem cells
Myf5	Myogenic factor 5
NICD	Notch intracellular domain
NRs	Number of nuclear receptors
Pax7	Paired box 7
PDGF	Platelet-derived growth factor
PDGPR α / b	Platelet derived growth factor receptor α/β
PGCα	Peroxisome proliferator-activated receptor-gamma coactivator α
PLIN	Perilipin
PPAR-γ/G	Peroxisome proliferator-activated receptor-γ
PPRE	PPAR response element
PRb/Rb	Retinoblastoma protein/ retinoblastoma
PRDM16	PR domain containing 16
PREF1	Preadipocyte factor 1
RARE	Retinoic acid response element
RIP	Receptor interacting protein
SAT	Subcutaneus white adipose tissue
SMA	Spinal muscular atrophy
STAT3	Signal transducers and activators of transcription 3
TC1	Immune response regulator

TNFα	Tumor necrosis factor α
TRE	Thyroid response element
TGFβ	Transforming growth factor beta
UCP1	Uncoupling protein 1
VAT	Visceral white adipose tissue
VEAT	Visceral endothelial adipose tissue
WAT	White adipose tissue
WHO	World health organization
Wisp2	Inducible signaling path-way protein 2
WNT1	Wingless-type MMTV integration family member 1
Zfp516	Zinc finger protein 516
P107	Retinoblastoma-like 1

1 Introduction

Obesity is now a global problem and it is called "Globesity" which means many people around the world suffer from this disease (Pietrabissa et al. 2012). Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health [WHO]. The rate of obesity has risen in recent decades and it is predicted to rise even more because of changing lifestyle and demography. In 2016, more than 650 million adults were obese [WHO]. A review study has estimated the range of overweight and obesity among Iranian adults as 27%–38.5% and 12.6%–25.9% respectively (Jafari-Adli et al. 2014).

Obesity is an important risk factor for type2 diabetes, dyslipidemia, hypertension, cardiovascular disease, and some types of cancers (Matsushita and Dzau 2017; Narayanaswami and Dwoskin 2017; Payab et al. 2014; Payab et al. 2017b). Another associated disorder is Metabolic Syndrome which is defined by IDF as central obesity plus any 2 of these 4 parameters: raised triglycerides, reduced HDL cholesterol, raised blood pressure, raised FPG. Thus, obesity can increase the risk of metabolic syndrome (Pi-Sunyer 2009). The life-threatening increase in obesity evoked some main strategies to control it: lifestyle modification, taking medication, and undergoing surgery(Petroni et al. 2017). These conventional methods have some considerable advantages for treatment and management of obesity for instance an average weight loss of 7 to 10Kg in 6 months by lifestyle modification, providing additional weight loss and positive effects on several metabolic parameters such as systolic blood pressure and total cholesterol by taking medication and a dramatic weight loss along with the rapid remission of type 2 diabetes mellitus by surgery (Yanovski and Yanovski 2014). Nonetheless, there are a number of limitations in the long-term efficacy and safety of these types of treatments such as return of lost weight,adverse effects and invasiveness respectively (Pories 2008).

Despite all the significant achievements of the mentioned methods, the obesity is still a major health problem which is needed to develop a novel treatment to enhance the effectiveness of obesity treatment. Nowadays, cell-based products propose promising advances in treatment of several disorders. Accordingly, clinical application of different types of stem cells can help scientists and clinicians to treat diseases include diabetes, disc degeneration, neurodegenerative disorders and obesity (Aghayan et al. 2017). Mesenchymal stem cells (MSCs) are multipotent cells which are considered to be common applicable type of stem cells. These cells can be derived from various sources like bone marrow, blood, umblical cord tissue and adipose tissue and can differentiate into several cell types like chondrocytes, osteoblasts, adipocytes and myoblasts (Augello and De Bari 2010; Tarte et al. 2010). Adipose derived mesenchymal stem cells (ASCs) are considered to be more beneficial, compared to bone marrow derived mesenchymal stem cells: human subcutaneous adipose tissue can be accessed easily and repetitively, the isolation procedure is rather simple, minimally invasive and it provides a large number of isolated cell (Aghayan et al. 2015; Thirumala et al. 2009).

MSCs play an essential role in adipogenesis which is a fundamental part in obesity and this makes them a potential target for therapeutic use (Baptista et al. 2015). In addition, these cells can be used to find a new way of controlling obesity *in vitro* and *in vivo* (Lee et al. 2017; Matsushita

2016). There are complex signaling pathways of adipogenesis from MSCs and many studies have determined the pathways governing MSC adipogenesis and realize therapeutic strategies for obesity (James 2013). Much researches have been carried out into the heterogeneity and properties of different white adipose tissue depots and ASCs to find suitable potentials for treatment of obesity (Cleal et al. 2017). Another research area is MSC differentiation into brown adipocyte which is reported to improve obesity hence this sounds promising to identify therapeutic strategies (Vargas-Castillo et al. 2017). However, our knowledge in these fields is not enough as there are contrasts in many results of related studies. MSCs can hopefully be a therapeautic alternative for obesity and further studies are expected to shed some light on all the complexities and open possibilities for a novel treatment for obesity. The authors in this review are trying to show that what are the ultimate results of the related studies and provide a future direction for more researches leading to clinical application of a safe and efficient type of stem cells for obesity treatment.

2 Adipose Tissue as a Secretory Organ

Adipose tissue can be found in mammals and especially in humans. There are two types of adipose tissue that differ in function, structure, color and position:1) white adipose tissue (WAT) and itself includes visceral white adipose tissue (VAT) and subcutaneus white adipose tissue (SAT), 2) brown adipose tissue (BAT). Moreover, investigators have disovered another type of adipose tissue, the color of which is between BAT and WAT and it is called beige adipose tissue (brite adipose tissue) (Illouz et al. 2011). All of them are differentiated from an identical origin, MSC. First of all, MSC which can differentiate into adipocyte, osteoblasts, myoblasts, and connective tissue is isolated from various tissues like bone marrow and adipose tissue. Secondly, MSC results in adipoblast that differentiates into brown and white preadipocyte in the presence of specific stimuli. After that, preadipocyte differentiates into brown or white adipocyte (Fig. 1 ;Esteve Rafols 2014).

Some of the main duties of the adipose tissue are energy storage, shock absorption, thermal insulation. Additionally, adipose tissue acts as a secretory organ. In fact, due to the link between obesity and metabolic syndrome, attention is also drawn to the adipose tissue system. Adipose tissue secretes proteins which are generally called adipokine include leptin, adiponektine, interlukine 6 (IL6) (Anderson et al. 2003), tumor necrosis factor α (TNFα) and resistine (Gao et al. 2018). They can be made by adipocytes and skeletal muscle cells. Adipokines can perform different physiological activities. In fact, they can be transmitted through the paracrine system to various organs such as the lungs, heart, the skeletal muscle, muscle of vessels and influence activities of these organs.

2.1 Different Types of Adipose Tissue

2.1.1 White Adipose Tissue

In WAT fatty particles are stored in the form of triglycerides molecules and triglycerides consist of two parts: glycerol and fatty acids WAT mass increases with obesity. Moreover, obese people should lose weight and WAT mass by changing lifestyle, excercise training, taking medicine such as dietary supplement, including polyphenols with physician prescription but, because of the pharmacological approachs side effects researchers focus on excercise training and changing lifestyle (Sakurai et al. 2012).

Treating white adipocytes with irisin, a hormone secreted by skeletal muscle, and FGF21 induces browning. It was reported that “the beneficial effects of exercise, reduction of diet induced obesity and decrease of insulin resistance in mice” is related to irisin. Stimulating the conversion of white fat to brown fat by irisin in humans was also proposed. FGF21 increases the expression of uncoupling protein 1 (UCP1) and other

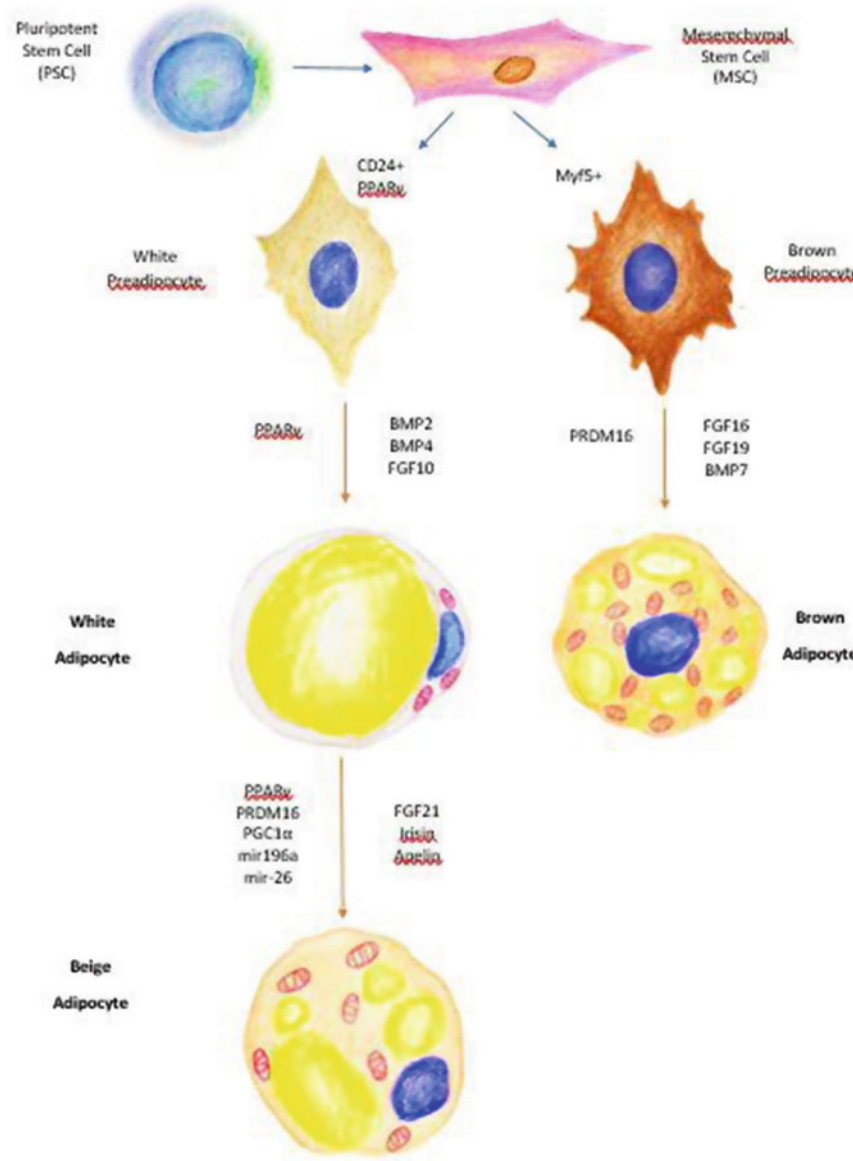

Fig. 1 The development of brown and beige adipocyte (drawn by Rasta Arjmand). Initially, pluripotent stem cell modifies into MSC which can get cluster of differentiation 24 (CD24) and peroxisome proliferator- activated receptor-γ (PPAR-γ) + white preadipocyte and myogenic factor 5 (Myf5) + brown preadypocyte. Secondly, in the face of white preadipocyte and some factors including PPAR-γ, bone morphogenic protein 4 (BMP-4), BMP-2 and fibroblast growth factor 10 (FGF10). Moreover, WAT can come into beige adipocyte throughout exposure to PR domain containing 16 (PRDM16), FGF21, PPAR-γ, Peroxisome proliferator-activated receptor-γ coactivator α (PGCα), irisin, apelin, Cyclooxygenase 2 (Cox2), microRNA 196 (MIR196a) and mir28. Brown preadipocyte is induced to be matured into BAT by way of ministration with some factors, for instance: PRDM16, FGF19, FGF16 and BMP-7 (Unser et al. 2015)

brown–fat-related genes in perirenal and inguinal WAT. Adipocytic FGF21 triggers the browning of WAT and activate BAT in response to cold (Lee et al. 2014; Poher et al. 2015).

2.1.2 Brown Adipose Tissue

The role of BAT is fat burning and producing heat in the body. Although the activity of BAT is reduced with age, it does not lose its activity completely and cold exposure can stimulate reactivation of BAT. BAT color is due to differences in the number of mitochondria and nerve fibers in the brown to yellow color range (Vargas-Castillo et al. 2017). The main location of this tissue are sternocleidomastoid muscles, the supraclavicular region, the armpits, the groin muscles, the adrenal glands, between the subscapularis and pectoralis muscles, the para aortic adipose tissue, and around the viscera in the omentum tissue (Aldiss et al. 2017; Harms and Seale 2013; Lidell et al. 2013). The formation of BAT via brown adipogenesis is an important process due to its ability to expend energy as heat with implication in the treatment of metabolic disorders and obesity (Unser et al. 2015).

Adaptive thermogenesis by BAT activation have been described in two ways: 1) cold induced thermogenesis (CIT), 2) diet-induced thermogenesis (DIT) (Silva and Bianco 2008).

2.1.3 Beige Adipose Tissue

UCP1-positive cells have been demonstrated in WAT as the counterpart of BAT cells (Boucher et al. 2016; Brand et al. 2005; Wu et al. 2012). Nowadays, inducible adipose tissue has been intriguing as an alternative therapy for obesity. Beige adipose tissue can be found in diverse zones as supraclavicular, shoulder-blades, axillary, mediastinal, paravertebral, perirenal and peri-aortic regions (Rogers 2015; Wu et al. 2012). There are different methods for development of beige adipocyte, three of which are more significant: “De novo beige adipogenesis, white-to-beige adipocyte trans differentiation, activation of dormant beige adipocytes” (Rui 2017). In these three types of method, inducing inactive beige adipocytes is motivated by cold exposure, β adregenic’s etc. (Barbatelli et al. 2010; Berkowitz et al. 1998; Rosenwald et al. 2013; Wang et al. 2013; Wu et al. 2012). Beige adipose tissue has offered a new approach in treatment of metabolic diseases. It is similar to both WAT and BAT in rather different ways. Researches have been performed on animal and human subjects investigating to demonstrate the features of this type of adipose tissue.

BAT and brite adipose tissue are distinguished from WAT by their high levels of metabolic rates and thermogenic capacity (Bartelt et al. 2011; Stanford et al. 2013). Mitochondrial energetics, lipid droplet dynamics and metabolic fuel mobilization all influence BAT and beige adipocyte thermogenesis. Also UCP1-mediated thermogenesis is a hallmark of BAT and beige adiocyte. Recent findings illustrate that both of these tissues also do thermogenesis by additional UCP1-independent mechanisms (Rui 2017).

BAT has a common origin with muscles. Both of them are derived from dermomyotom of mesodermal layer. Early B cell factor 2 (EBF2) and BMP7 induce dermomyotom to form brown preadipocyte and PRDM16, CCAAT/enhancer-binding protein β (C/EBPβ), EBF2,PPAR-γ, zinc finger protein 516 (Zfp516) play significant role in differentiation brown preadipocyte to brown adiposyte tissue. There are multitude UCP1 in the mitochondria of BAT, that contributes to thermogenesis (Zhang et al. 2016). “UCP1 of BAT dissipates poroton gradient generated and catalizes proton leak throughout the inner mitochondrial membrane” (Vargas-Castillo et al. 2017). This molecular marker is useful to recognize BAT and Beige adipocyte (Rui 2017). UCP1 locates in the inner mitochondrial membrane. Although, there is no UCP1 in brown and beige progenitor cells, but during adipogenesis its expression enhances under the control of genetic program (Rabelo et al. 1996).

Besides the similarity of these three types of adipose tissue they also have some differences which are depicted in Table 1 (Ikeda et al. 2018; Rui 2017).

Table 1 Characteristics of Brown, White and Beige Adipocyte

	Brown adipose tissue	Beige adipose tissue	White adipose tissue
Location	Infants (dedicated ddepots)	Adults (mammels)	Infants and adults
Function	Methabolicaly active and heat productive	Methabolicaly active and heat productive	As an energy store
Endocrine function	Neurgulin4, IGF-1[(1)], FGF21[(2)], interleukin 6	Neurgulin4, IGF-1, FFGF21, interleukin6	Adipokines (leptin-adiponectin)
	Leptin, adiponectin (alittle)	Leptin, adiponectin (alittle)	
Thermogenic process	UCP1-dependent	UCP1-independent	____
Lineage	Myogenic lineage (ENG^{+}, $Myf5^{+(3)}$, $Pax7^{+(4)}$)	Multiple lineages (SMA^{+}, $PDGFRa^{+(5)}$, $PDGFRb^{+(6)}$)	_____
Continuance	Constitutive	Transient	Constitutive
		(direct transition)	
Heterogeneity	Homogeneous	Heterogeneous	Heterogeneous

+ 1:insulin like growth factor 1. 2:fibroblast growth factor 21. 3: myogenic factor 5. 4: Paired box 7. 5: platelet derived growth factor receptor α. 6: platelet derived growth factor receptor β.

2.2 Factors Secreted from Adipose Tissue

Adipokines influence inflammation within adipose tissue and visceral endothelial adipose tissue (VEAT)(Lehr et al. 2012). As an adipokine, Leptin, was discovered almost simultaneously with the most important adipokine called adiponectin. Leptin is produced by adipocytes and plays an important role in regulating body weight. Accordingly, damaging hypothalamic methabolic circuits is because of the lack of leptin receptor in myeloid cell which cause increasing body weight, enlarging proinflammatory genes to modify 3 T3-L1 adipocyte as another consequence of leptin in adipose tissue (Gao et al. 2018). The studies of leptin till now have been performed on animals especially on mouse but researches on the physiological effects of leptin such as leptin resistance in the brain have not been discovered. Adiponectin has several effects including insulin-sensitizing,anti-inflammatory, anti-atherogenic and anti-carcinogenic activity (especially in breast cancer) (Feve 2013; Payab et al. 2017a). Adipose tissue in obese individuals acts as endocrine and secretory organ and the result is increasing the rate of secretion of pro inflammatory adepokines including TNFα and IL6 (Anderson et al. 2003). IL6 is produced and secreted by adipocytes and muscle cells which is increased in the plasma following obesity (Carey and Febbraio 2004). TNFα was the first adepokine, the activity of which was being studied and its performance in the human body is still unclear (Halse et al. 2001) It is secreted by the innate immune cells (macrophage) and expressed in adipocyte (Lehr et al. 2012). In the context of the relationship between inflammation and obesity it should be said that obesity is a type of low-grade inflammation (Cao et al. 2008).

2.3 Gene Expression and Adipogenesis

The role of genetics and mechanisms which affect gene function in the process of adipocyte differentiation is absolutely essential and it is considered as an interesting research area to find a novel treatment for obesity. There has been a great effort to identify genetic variants affecting obesity traits. The first gene associated to non syndromic obesity is fat mass and obesity associated (FTO) (Herrera et al. 2011). The association of this gene region with obesity explains 1% of BMI heritability. Also, FTO is reported to be involved in decreased lipolytic effect in adipocyte. Further, more gene loci related to obesity and BMI associated variants have been identified (Speliotes et al. 2010). The pattern of fat

distribution, the factors affecting it and the potential risks caused by central and peripheral obesity, need to be fully understood. Accordingly, it was demonstrated that the development or maintenance of specific regional fat depots can be affected by DNA variants. Among 17 novel common obesity loci, 14 loci are related to body fat distribution and some of them are connected with sex in women. In other words, the fat distribution in men and women is influenced by sex-specific genes (Herrera et al. 2011).

There are several genes that regulate the adipogenesis (Fig. 2). BMP family control multiple steps of differentiation processes like adipogenesis. BMP-2 and BMP-4 are adipogenic factor for white fat and direct white adipocyte progenitor cells to white preadipocyte. In contrast, BMP-7 triggers commitment of MSC to brown adipocyte lineage (Chen and Tong 2013; Tseng et al. 2008). In addition to this family, one of the most powerful transcriptional regulators which control the fate of brown fat cells is PRDM16. Over expression of PRDM16 converts skeletal muscle progenitor cells to brown adipocytes (Chen and Tong 2013). Further, it restricts skeletal muscle gene expression in brown fat precursors by its interaction with PGC-1α and PGC-1β, as other transcriptional co-regulators. Among many nuclear factors regulating adipocyte differentiation, PPARγ2 is a key regulator that triggers differentiation to adipocyte. PRDM16 binds to PPAR-γ and coactivates its function (Seale et al. 2008). In addition to this factor, C/EBPs, a transcription factor family, has some members that induce adipogenesis: C/EBP α, β, δ. C/EBPβ and C/EBP δ "are expressed early and transiently" in the adipogenesis process and are induced by cAMP and glucocorticoids respectively(Ming Shi et al. 2000). It was found that ectopic expression of PPAR-γ and C/EBPα alters the program of differentiation of myoblasts and convert them to adipocytes upon hormonal stimulation. This suggests that the mentioned factors have the "dominant role in adipocyte determination and differentiation processes" (Hu et al. 1995).

In addition to the mentioned genes, there are some other genes, which intervene in this process. A gene of vertebrate which has regulatory effect on adipogenesis is called transcriptional and immune response regulator (TC1). It down-regulates PPAR-γ and C/EBPα while it up-regulates the wingless-type MMTV integration family member 1(WNT1), inducible signaling path-way protein 2 (Wisp2) and delta like non-canonical notch ligand 1(Dlk1)(Jang et al. 2016). Wisp2 inhibits adipogenesis in both mesenchymal stem cells and preadipocytes (Hammarstedt et al. 2013).

Another gene which can promote expression of PPARγ2, c/ebpb and c/ebpd is chemokine (C-X-C motif) ligand3 (CXCL3), a chemokine produced by different types of cells including adipocytes (Kusuyama et al. 2016).

One of the most compelling topics in this field is about BAT, as the thermogenesis was found intriguing in this type of adipose tissue. The precursor cells of BAT can also differentiate into muscle cell in the presence of some special transcription factors like PPAR-γ, PRDMI6 (which is of great importance in differentiation of adipocytes and is highly expressed in Brown adipocytes) and Euchromatic histone lysine methyltransferase 1 (EHMT1). As it was mentioned before, UCP1 is the hallmark protein for promoting thermogenesis which its transcriptional process is initiated by a number of transcription factors like: thyrioid response element (TRE), PPAR response element(PPRE), retinoic acid response element(RARE) and cAMP response element(CRE). All of these factors influence the expression of UCP1 as it was mentioned before that can be summarized: TRE which is activated by triiodothyronine (T3) is a positive regulator of UCP1, the binding of PPRE to PPAR controls UCP1 gene expression related to brown adipose differentiation, RARE triggers UCP1 expression in BAT and CRE seems vital as a mutation in its sites diminishes the UCP1 expression. RecentlyZfp516 has been added in the list of transcription factors that binds to UCP1 promoter leading to UCP1 expression (Vargas-Castillo et al. 2017).

2.4 Hypertrophy and Hyperplasia

Extensive adipose tissue growth, which can potentially lead to obesity, generally has two mechanisms: hyperplasia or increasing in fat cell number and hypertrophy or enhancement of fat cell size. In the development of obesity, hyperplasia occurs only in the first stages and it is triggered by hypertrophy. Hypertrophy is used for storing additional fat in the progression and is prior to hyperplasia. A study on C57BL/6 mice fed a high-fat diet explained that hypertrophy is the major cause of increased VAT while hyperplasia due to the presence of higher number of adipose progenitor cells in SAT mostly occurs in this type of adipose tissue (Joe et al. 2009). In an animal study on mice, it was suggested that hyperplasia does not contribute to fat mass increase because it occurs in small cells that have small volume of stored fat, whereas hypertrophy is the main contributor to the increase of fat mass. Studies on of genetic and diet effects on these mechanisms in mice has revealed that increase in number of fat cells is affected by genes while enlargement of fat cells is a diet variable (Jo et al. 2009). In regard to the role of obesity in the development of non-insulin-dependent diabetes and relatively, glucose intolerance and insulin resistance, the size of adipocytes might be important as it is positively correlated with glucose intolerance and hyperinsulinemia (Ferrannini and Camastra 1998). Moreover, as the adipocytes get larger, they become more susceptible to inflammation and cell death (Pellegrinelli et al. 2016).

3 Adipose Tissue-Derived Stem Cells and Adipogenesis

3.1 Mesenchymal Stem Cells (MSCs)

MSCs are spindle shape, multipotent cells with self-renewal capacity that can expand thousand folds and differentiate into different cell lineages including adipocyte, osteocyte and chondrocyte (Bianco 2014; Short et al. 2003). MSCs can be isolated from different tissues like bone marrow (Penfornis and Pochampally 2011), adipose tissue (Gimble and Guilak 2003), umbilical cord(Han et al. 2013), Wharton's jelly (Chatzistamatiou et al. 2014), placenta (Vellasamy et al. 2012), dental pulp (Alkhalil et al. 2015) etc**.** Regarding to the multipotent capacity, MSCs seem to be substuted injured cells, although more findings are need to confirm this claim (Baksh et al. 2004). Moreover, MSCs secrete various type of growth factors and cytokines that execute significant role in repair, regeneration and immunogenic balance (Caplan and Dennis 2006). Low immunogenicity, as the main characteristic of MSCs, allow the allogenic use of cell products that is very important in cell-based therapies and regenerative medicine (Aggarwal and Pittenger 2005). ASCs are more considerable source in regenerative medicine research and clinical trials.

One of the most significant concern in cell therapy is providing cells from less or non-invasive sources. ASCs can be isolated in large numbers from abandon and waste adipose tissue that obtained by liposuction as less invasive method (Yarak and Okamoto 2010). On the other hand, the superior potential of ASCs is determined in basic and clinical researches (Aghayan et al. 2015).

3.2 Potential Pathways

Adipogenesis is a multi-step process involving expression of some transcription factors resulted in differentiation of fibroblast-like preadipocytes into mature adipocytes(Ali et al. 2013). According to literature, adipogenesis includes two main phases: 1) the determination phase underlying commitment of MSCs into preadipocyte and 2) the terminal differentiation phase that leads to maturate adipocyte (Matsushita and Dzau 2017).

The role of MSCs in adipogenesis is complicated and requires cross talking between major signaling pathways. Several different pathways are studied that involved in different phases of adipogenesis. Here, we described some important signaling pathways that present positive or

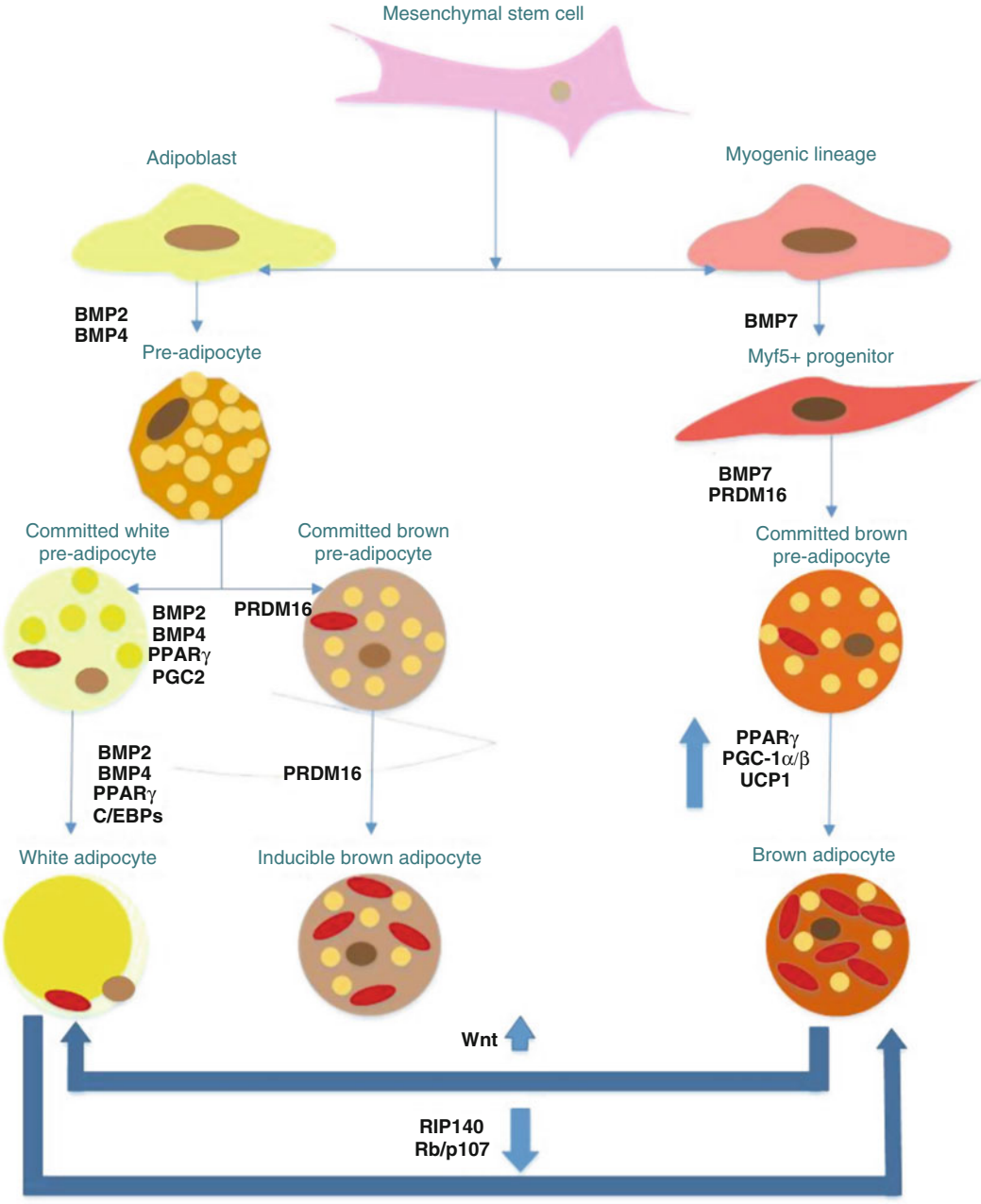

Fig. 2 An Overview of potential mechanisms/pathways underlying MSC white, beige and brown adipogenesis. Brown adipocyte is known to be arised from myogenic lineage which is a different origin to white and beige adipocyte lineage (Chen and Tong 2013). In the developmental pathway leading to the differentiations of white and beige adipocyte, BMP-2 and BMP-4 direct adipoblasts or white adipocyte progenitor cells to pre-adipocyte. Then, in the presence of some factors including PPAR-γ, C/EBPs and PGC-2, pre adipocyte forms committed white pre-adipocyte and then white adipocyte. Whereas, PRDM16 activates brown adipogenesis and differentiation of pre-adipocyte to committed brown pre-adipocyte. It can also promote inducible brown adipocyte or beige adipocyte. On the other hand, BMP-7 drives the brown-fat cell fate and it induces PRDM16, which represses skeletal muscle differentiation. Hence, $Myf5^{+}$ progenitors are driven to committed brown adipocytes. PRDM16 acts as a key regulator and co-activates PPAR-γ, which results in subsequent induction of PGC-1α, PGC-1β and UCP1 that lead to brown adipocyte (Fruhbeck et al. 2009;

negative regulatory effect on adipogenic differentiation (Fig. 3).

3.2.1 Classic Pathways and Adipogenesis

BMPs are the members of Transforming growth factor beta 1 (TGF-β1) family performing different roles in the adipogenic differentiation of MSCs (Chen et al. 2016). BMP-2 and BMP-4 can persuade commitment of C3H10T1/2 stem cells as mouse MSC model into adipocyte. BMPs affect adipogenesis mediated by canonical Smad and p38 MAPK pathways, which lead to overexpression of lysyl oxidase (LOX) as a target gene of adipocyte lineage commitment (Huang et al. 2009). BMP-7 stimulates adipogenesis in human MSCs while, BMP-2 shows inhibitory effects on differentiation of human MSCs to adipocytes (Gori et al. 1999).

Wnt signaling pathway is very important in cell proliferation and differentiation. Up to date, 19 molecules of Wnt are recognized that trigger one of the canonical and noncanonical Wnt/calcium pathways by binding to Frizeld family receptors. The outcome of Wnt pathway on commitment of MSC to adipogenic lineage is well studied and the inhibitory effect of both pathways is determined. The canonical pathway suppresses expression of PPAR-γ mRNA, whereas the noncanonical pathway stimulates histone methyltransferases that prevent PPAR-γ activation via histone H3 lysine 9 (H3K9) methylation (Yuan et al. 2016).

Wnt 1 and Wnt 10b inhibit the expression of PPAR-γ in 3 T3-L1 preadipocytes, which resulted in retaining undifferentiated state (Liu and Farmer 2004). The existence of Wnt 3a in 3 T3-L1 cell culture medium can suppress the expression of adipogenic genes via prevention of PPAR-γ induction (Kawai et al. 2007). Similarly, neutralization of Wnt 5a promote determination of human MSC to preadipocytes by inactivation of PPAR-γ (Bilkovski et al. 2010) and reduction in middle stage adipogenesis was observed after treatment of rat ASCs by 50 ng/mL Wnt5a in the anti-β-catenin and MAPK pathway independent manner (Tang et al. 2018).

Consequently, Wnt signaling pathways act as an adipogenesis blocker, hence Wnt antagonist like secreted frizzled-related protein 1 (SFRP1) can induce invitro adipogenesis and invivo accumulation of adipose tissue (Lagathu et al. 2010). Hedgehog (Hh) signaling pathway controls several biological process during embryogenesis, development, organ patterning, cellular proliferation and differentiation. Treatment of C3H10T1/2 cells whit Hh reduces the amount of adipogenic transcription factors, (Spinella-Jaegle et al. 2001) this effect was emerged via induction of aniadipogenic factors like Gata 2 upstream of Hh (Suh et al. 2006). In the case of human ASCs, Hh pathway does not alter the entire number of adipocytes, while adipogenesis has been impaired, with declined lipid accumulation, a reduction in adipocyte specific markers, and appearance of an insulin-resistant phenotype. It seems that Hh signaling affects the late events of human MSC adipogenesis nor the commitment stage. In spite of evidences related to inhibitory effects of Hh on adipogenic differentiation, elimination of this pathway is essential but not adequate to promote adipogenesis in both human and mouse MSCs (Fontaine et al. 2008).

Notch signaling is a highly conserved pathway that regulates cell proliferation, differentiation, cell death and cell fate determination in several cell types. After binding to ligand, two proteolytic cleavages has been occurred and the emerged

Fig. 2 (continued) Yao et al. 2011). Brown adipocyte may change its phenotype into white by up regulation of Wnt which suppresses the characters of brown adipocyte . In the same way, white adipocyte may transform into brown adipocyte by changing the expression of RIP140, Rb, and p107 (Yao et al. 2011). Receptor interacting ptotein140 (RIP140)is suggested to repress UCP1 enhancer in brown adipocytes (Rosell et al. 2011). Pocket proteins like retinoblastoma protein (pRb) and Retinoblastoma-like 1(p107) have been shown to alter the adipocyte differentiation and evoke white fat phenotype. Therefore, down regulation of these genes may result in trans-differentiation of white adipocytes to brown adipocytes (Yao et al. 2011)

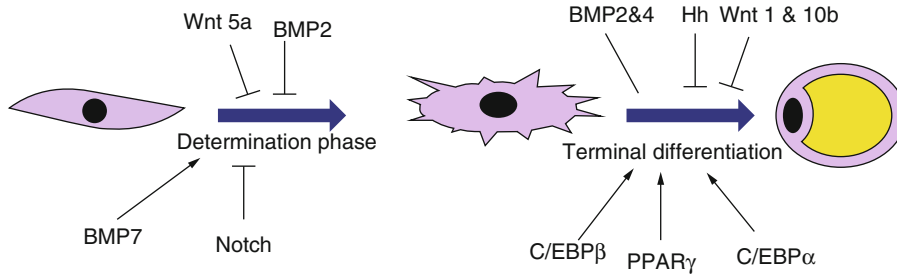

Fig. 3 An overview of positive and negative regulators of MSCs adipogenesis. Adipogenesis pathway can be divided into two main phases: determination phase that characterized by differentiation of MSCs into preadipocytes and terminal differentiation phase that resulted in developing adipocyte phenotype. Several factors are involved in these two different phases of adipogenic differentiation. Among these, Wnt signaling pathway has been showed negative role in both phases of MSC adipogenic development. On the other hand, PPAR-γ and C/EBPα are two main adipogenic transcription factors that stimulate second phase of adipocyte differentiation. More researches are required to reveal all aspects of MSCs differentiation to adipocytes and knowledge of signaling pathway network improve our comprehension to find new strategies for treatment of obesity

notch intracellular domain (NICD) moves into nucleus to stimulate transcription of target genes (Kopan and Ilagan 2009). The expression of notch gene decreased during adipogenic differentiation of human MSCs and inhibition of notch signaling by γ-Secretase inhibitors enhanced MSCs adipogenesis mediated by autophagy activation involving PTEN-PI3K/Akt/mTOR pathway (Song et al. 2015). However, the role of Notch signaling in adipose progenitor cells proliferation is conversional since the inhibition of Notch leads to decrease in human MSC expansion but increase in mouse 3 T3-L1 preadipocyte cell counting (Shan et al. 2017).

3.2.2 Adipogenesis and Master Transcription Factors

PPAR-γ and C/EBPα are two key transcription factors that regulate adipogenic differentiation. PPAR-γ is a nuclear hormone receptors with two distinct isoforms, PPARγ1 and PPARγ2, are detected. Overexpression of PPAR-γ in fibroblast cell line by retroviral vectors caused to appearance of preadipocyte features. The mentioned ability indicates the role of PPARγ2 in early phase of adipogesis and adipocyte determination (Tontonoz et al. 1994). Accordingly, embryonic stem cells are successfully generated by homologues recombination and showed that the null clones cannot undergo adipogenic differentiation. Moreover, creation chimeric mice of PPAR-γ null ES cells demonstrate no distribution of null cells in adipose tissue that prove the *in vivio* role of PPAR-γ in adipogenesis and fat formation (Rosen et al. 1999).

C/EBPs are basic region-leucine zipper proteins comprised of six isoforms: C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPε and C/EBPζ. C/EBPα and C/EBPβ are highly expressed transcription factors in liver, lung and adipose tissue (Nerlov 2007). C/EBPβ plays an essential role in adipocyte differentiation of 3 T3-L1 cells via inducing the expression of C/EBPα and PPAR-γ genes (Guo et al. 2015).

C/EBPα is vital for adipogenic differentiation, since the expression of C/EBPα antisense RNA in 3 T3-L1 cells interrupts regular differentiation and gene knock out of C/EBPα in mice cause to failure in normal development of adipose tissue (Lin and Lane 1994).

More evidences shows that PPARγ can induce adipogenesis in the C/EBPα null cells but the ability of C/EBPα to trigger similar condition in the absence of PPARγ was not proven (Rosen et al. 2002).

3.2.3 Hyperplasia Involved Signaling Pathways

Adipocyte number increasing (hyperplasia) is related to signaling pathways that involved in cell proliferation. Adipogenic differentiation is arrested and cell growth triggered while

hyperplasia occurring in adipose tissue (White and Tchoukalova 2014). *In vivo* studies via *Tc1* $^{-/-}$ mice showed that hyperplasia is regulated in stem cell levels and ASCs revealed more proliferative capacity and adipogenesis activity (Jang et al. 2016).

Abdesselem et al. suggested that reduction of sirtuin 1 expression in preadipocytes followed by hyperacetylation of c-Myc leads to uncontrolled fat hyperplasia so, Sirt1/c-Myc signaling pathway might be more studied as a potential therapeutic pathway for obesity (Abdesselem et al. 2016). Moreover, Wnt-β catenin may be the master pathway regulating adipose hyperplasia, and the induction of this pathway beside cross-talking with glucocorticoid-related signalings alter the activity of preadipocytes. Such alteration is appeared in disruption of adipocyte differentiation and increasing cell proliferation (Wong et al. 2016). Furthermore, activation of the protein kinase C which stimulated by endothelin-1 can improve *invitro* preadipocyte expansion and *invivo* hyperplasia. On the other hand, Extracellular receptor kinase(ERK)-dependent pathway inhibits the hypertrophy in 3 T3-L1 adipocytes after endothelin-1 treatment (Lien et al. 2016).

The study of transcription factors and signaling pathways involved in adipogenesis can introduce negative or positive regulators in adipogenic differentiation. Such knowledge might paved the path to plan a new approach in controlling obesity.

4 Therapeutic Use of Adipose Tissue-Derived Stem Cells (Adscs) in Obesity

4.1 Experimental Background

Obesity, which is the excess accumulation of adipose tissue, can be concluded from adipogenic differentiation of MSCs. Therefore, this pandemic disease can be managed by inhibiting this process. In this regard, different factors were investigated to promote an alternative therapy of obesity such as IGF-1, glucorticoids and prostaglandins which have stimulatory effects on adipogenesis and androgens like testosteron and dihydrotestosterone, growth hormone and inflammatory cytokines as adipogenesis inhibitors (Ali et al. 2013; Matsushita and Dzau 2017; Zerradi et al. 2014).

4.1.1 *Invitro* Experiments

Due to the importance of BAT and thermogenesis, several studies have focused on the BAT and induction of preadipocytes to brown adipocytes. Generally, two main approaches could be used to increase BAT mass and activity: 1) *invivo* infusion of small molecules and growth factors to spark BAT growth, 2) *exvivo* cell based approach in which progenitor cells are differentiated into brown adipocytes more and then the brown adipocytes will be implanted in patients (Cypess and Kahn 2010). According to several studies, some genes especially BMP7, persuade preadipocytes differentiation to form BAT. BMP-7 treatment in the brown adipocyte cell line arouses the expression of genes including PGC-1α and PGC-1β which are involved in mitochondrial biogenesis and function. Also, treating multipotent C3H10T1/2 cells with BMP-7 shows an increased expression of UCP-1, C/EBPα, β and δ, PPAR-γ and adipocyte protein 2(aP2) (Tseng et al. 2008). In a similar way, when PRDM16 is expressed in white fat cell progenitors, it provokes PGC-1α, UCP-1, and type2 deiodinase expression, then, the brown fat phenotype is activated (Seale et al. 2007).

Interestingly, umbilical cord blood-MSC (CB-MSC) has indicated a low potential for adipogenic differentiation, while the adipose tissue and bone marrow-MSCs are able to develop adipogenic phenotype. Gene expression analysis verified a higher average expression of preadipocyte factor 1 (PREF1), which has been demonstrated to inhibit adipocyte formation, in CB-MSC compared to the other MSC sources. On the other hand, PPARG, perilipin (PLIN), adiponectin (ADIPOQ) and C/EBPA were upregulated in adipose tissue-and BM-MSCs, resulted in adipogenic differentiation. However, the mRNA levels of these genes were remained unchanged in CB-MSC. Although, treating ASCs with umbilical cord blood plasma (CB-plasma)

caused adipogenesis inhibition due to high concentration of Pref-1, but siRNA knock down of PREF1 did not induce adipogenesis. Indeed, endogeneous PREF1 expression has not an essential function in impaired adipogenesis in CB-MSCs whereas, Pref-1 in plasma seems to mediate inhibition of adipogenesis (Karagianni et al. 2013).

Additionally, the adipogenic differentiation of MSCs cultured in the presence of TGF-β and cAMP-enhancing agents revealed that this cytokine reduces expression of adipogenic genes like PPAR γ, a disintegrin and metalloproteinase with thrombospondin motif 5 (ADAMTS5) and aldo-keto reductase family 1 member B10 (AKR1B10). In more detail, MSCs were cultured in adipogenic differentiation medium and it was depicted that TGF-β blocked adipocyte transformation of MSCs in a dose-dependent manner. Despite of the significant effect of TGF-β in adipogenesis, systematic treatment with this cytokine is not a realistic option as it has strong inhibitory effect on the immune system. Also, It may cause skin fibrosis and toxicity which were indicated in animals. Since FDA has approved some drugs for the mentioned genes, they are potential targets for treatment of obesity though further studies are required (van Zoelen et al. 2016).

Recently, MicroRNAs (miRNAs) have also shown the regulatory potential and they are involved in cell fate decision. For example, miR-17-5p and miR-106a target BMP-2 and increase adipogenic CEPAα and PPAR-γ to promote adipogenesis of ASCs (Li et al. 2013). In addition, miRNAs can regulate brown adipogenesis as miR-193b-365 and miR-196a regulate brown adipogenesis positively. The miR-193b-365 is called a key regulator of brown fat development since blocking miR-193b and/or miR-365 impaires brown adipogenesis in primary brown preadipocytes. Also, miR-193b is able to induce differentiation of C2C12 myoblasts to brown adipocytes in adipogenic condition (Sun et al. 2011). Likewise, HomeoboxC8 (HOXC8), a white- fat gene that represses the brown adipogenesis, is down regulated by miR-196a in brown adipogenesis of WAT-Progenitor cells. It has been demonstrated that the transgenic miR-196a mice have a lower blood glucose level in the glucose tolerance test and a lower insulin level than wild type mice. Also, transgenic mice exhibite a resistance to obesity despite the increased food intake compared to wild types (Mori et al. 2012). On the other hand, miR-27 is down regulated during brown differentiation of WAT. MiR-27a and b are decreased in the beige differentiation of SAT preadipocytes. What is more, inhibition of miR-27 increases the expression levels of Ucp1, Prdm16,Pparγ, Pparα, cell death-inducing DFFA-like effector a (Cidea), Pgc1α and aP2 in SAT precursors and the brown adipogenic markers in VAT precursors (Sun and Trajkovski 2014). Similarly, miR-133 negatively regulates PRDM16, hence inhibition of miR-133 or its transcriptional regulator Myocyte enhancer factor 2 (Mef2) leads to differentiation of precursors of BAT and SAT to mature adipocytes (Trajkovski et al. 2012; Unser et al. 2015).

Adipokines and adipokine receptors which are expressed in several type of cells have the capacity to affect the adipogenic differentiation. Analyzing the expression of chemokine and chemokine receptor genes in 3 T3-L1 and ST2 cell lines revealed that some mRNAs are highly increased during the differentiation. Among these mRNAs, Cxcl3,which has the greatest effect in adipogenesis, is increased in both 3 T3-L1 preadipocyte differentiation and ST2 MSC in the inducing condition of adipogenesis (Kusuyama et al. 2016).

4.1.2 *In-vivo* Experiments

There are several preclinical studies in the field of obesity treatment which used obese mouse models *(*Fig. 4*)*. Here, we are reviewing some attempts to develop therapeutic way for obesity with more focus on cell-based therapies.

Rieusset et al. reported that decrease in the activity of PPAR-γ by using its antagonists, protects mice from high-fat diet-induced adipocyte hypertrophy and insulin resistance. *In vitro* inhibition of PPAR-γ prevents adipocyte differentiation and *in vivo* inhibition suppresses full development of WAT and BAT (Rieusset et al. 2002). Additionally, PPAR-γ

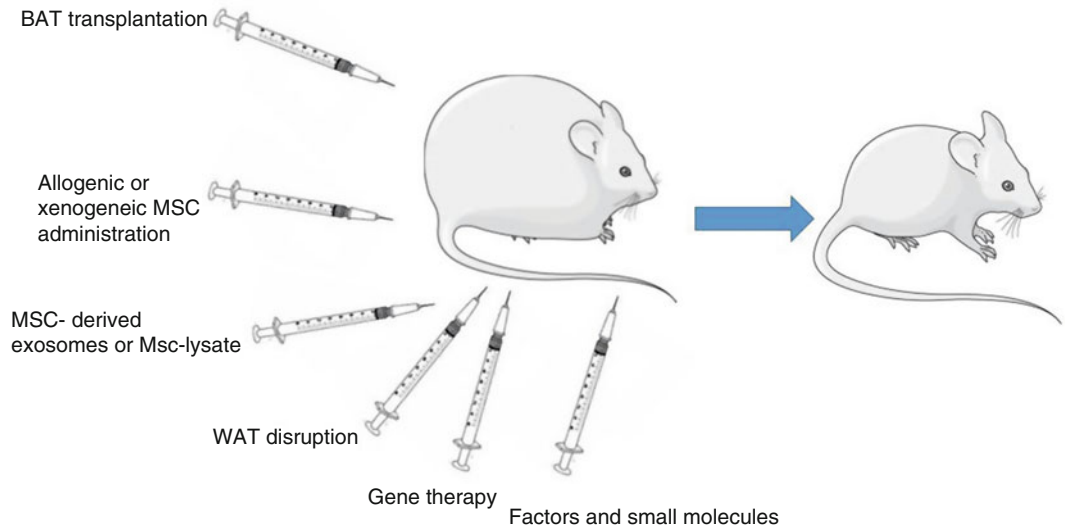

Fig. 4 Several useful methods for obesity treatment in preclinical phase. Different strategies have been investigated to treat feature of obesity in mice model. Administration of cell or derived exosomes, injection of inappropriate factors, transplantation of BAT and disruption of WAT are examples of these strategies. Cell-based approaches are more considered to develop as the new promising therapeutic strategies in human obesity. Infusion of ASCs or secreted exosomes led to significant improvement of obesity and related syndromes in mouse models

and C/EBPα are up-regulated in ASCs from TC1 deleted mice, whereas the inhibitors of adipogenesis, Wisp2 and Dlk1 can be down-regulated. This data and the point that $Tc1^{-/-}$ mice has more capacity for adipogenesis than wild types may introduce TC1 as a new regulator of ASCs (Jang et al. 2016). Directing the WAT or preadipocytes to form BAT is also a valuable area of research. For instance, implanting BMP-7 treated C3H10T1/2cells into athymic nude mice developed a large number of UCP1 positive brown adipocytes and a small portion of white adipocytes (Tseng et al. 2008).

The expansion, metabolism, viability, and regenerative capacities of ASCs is damaged in obese mice and the cell recovery does not happen even after the weight loss (Perez et al. 2016) since ASCs graft seems to be the appropriate therapeutic option for obesity-related disorders.

Cao et al. investigated the anti-obesity influence of allogeneic ASCs in high-fat diet-induced obese (DIO) mouse model. Single dose treatment of ASCs led to the reduction in body weights, decrease of the liver inflammation and level of blood glucose and also triglycerides while, the levels of HDL and expression of PPAR-γ was increased (Cao et al. 2015).

In another study, the anti- obesity effects of ASCs but not umbilical cord-derived MSCs have been proved in both dyslipidemia and obese mouse model. This study showed that administration of ASCs could activate AMPK HSL/ACC1 signaling cascades in adipose tissue. The final outcome of such pathways is determined by lipolysis and WAT browning functions (Liu et al. 2016).

Interestingly, the anti-obesity effects of human ASCs treatment as a xenogeneic source has been studied in obese mouse model. Furthermore, ASCs, ASC-lysate and brown adipocyte differentiated from MSCs (M-BA) are compared to analyze therapeutic their effects. Significantly, M-BA due to 60% expression of Ucp1, exhibited the strongest effect on reduction of body weight, triglycerides, cholesterol and increasing the HDL/LDL ratio after injection into high-fat diet (HFD) mice(Lee et al. 2017).

Apart from the agents which were mentioned above, there are other factors affecting adipogenesis including: stem cell microenvironment, surface biochemistry, cell adhesion,

geometric and mechanical characteristics and also co-culture. Cell shape influences adipogenesis, as spheroidal MSCs are more capable for adipogenesis than protruded ones. Dynamic loading like cyclic stretching inhibits adipogenesis while static stretching accelerates adipogenic differentiation. Furthermore, adipogenesis is influenced by neighboring cells and the paracrine interactions between them. As a result, mature adipocytes promote adipogenic differentiation as an example (Unser et al. 2015).

Besides the mentioned approaches, BAT transplantation is another strategy in the cell-based therapy. Two different research groups determined that transplantation of BAT into DIO and HFD mice model enhances glucose tolerance, energy balance, insulin sensitivity and reduces fat mass (Liu et al. 2015; Stanford et al. 2013). Moreover, transplantation of BAT into dorsal subcutaneous region of leptin-deficient Ob/Ob mice as a genetically obese model showed similar effects. Improvement of obesity symptoms like reduction of body weight, upregulation of BAT activity, increasing in insulin sensitivity and thermogenesis was observed. Gaining promising results from preclinical studies could introduce BAT transplantation as a novel option for treatment of obesity and diabetes (Liu et al. 2015).

Beside BAT transpalntation strategy, destruction of WAT tissue can be a useful approach in obesity treatment. In this specific case, Anti-angiogenic strategies can be used as a supporting agent since adipogenesis and angiogenesis are very closed and occurred in cell clusters near adipose tissue neovascularisation region (Nishimura et al. 2007). Kolonin et al. designed attractive gene construct by fusion of WAT vasculature receptor and cytotoxic genes. By delivery of such construct to obese mice, WAT tissue is targeted and disrupted and the obesity development reversed via oblation the WAT growth (Kolonin et al. 2004).

Other than all the strategies mentioned above, recently, exosomes have received lots of attention both in basic science and clinic-wise for finding treatment of many diseases. Exosomes are nano vesicles that are secreted from the cells and can act as a key transporter of paracrine factors in angiogenesis, immune regulation and tissue regeneration (Zhao et al. 2018). Extracellular vesicle produced by adipocytes has been studied and it was found that under hypoxic condition, which can be a result of adipocyte hypertrophy, exosomes were enriched in enzymes and were able to stimulate lipid accumulation in target cells (Sano et al. 2014). Brown and Beige adipocyte exosome production, specifically, is enhanced by cAMP treatment in the mouse (Gao et al. 2017).

The immunomodulatory function of MSC-derived exosome was assessed in a study on C57BL/6 male mice. Exosomes secreted from ASCs of WAT (WAT-derived ASCs) polarized M2 macrophages with highly expressing of Arg-1 (due to transferred Signal transducers and activators of transcription 3 (STAT3) from exosomes) and IL-10 thus reduced the inflammatory ability of macrophages. The macrophages then promoted beiging of WAT.and this is why in obese (HFD-fed) mice treated with ADSC-derived exosomes, WAT inflammation and obesity progression were reduced and metabolic hemostasis was improved (Shang et al. 2015; Zhao et al. 2017).

The angiogenic potential of extracellular vesicles (EVs) was also studied. The data suggested that EVs from ASCs of obese individual have lesser pro angiogenic capacity,indicating that circulating fatty acids in obesity alter the function of ASCs. However, platelet-derived growth factor(PDGF) evokes ADSC EV secretion and enhances angiogenic potential (Lopatina et al. 2014).

5 Future Perspective

The pathway of Adipogenesis is divided into two main phases: the determination phase that is characterized by the differentiation of MSCs into the preadipocytes and the phase of terminal differentiation, which leads to the developing adipocyte phenotype. Several factors in these two different phases are involved in the adipogenic differentiation, such as transcription factors, molecular signals, epigenetics, and etc. Among these, a number of factors play an inhibitory role

and other categories have stimulatory role. The challenge for future studies is the insight into obtaining the key to identifying these factors and their mechanisms in adipogenesis with the potential of providing new therapeutic goals for treatment of obesity and its comorbidities. In-vitro and in-vivo studies currently support stem cells therapies in the treatment of obesity. The results of studies conducted in this review revealed that the use of stem cells (infusion or injection) can significantly suppress obesity and related diseases such as cardiovascular and diabetes and improves dyslipidemia and insulin resistance.

Since autologous stem cells have been used in previous studies, this treatment does not have the risk of rejection and can be ideal for the treatment of obesity.

Also, future research on molecular control of brown or beige adipogenesis may result in new and novel achievements. Intervention studies such as controlling the adipogenicity from MSC to brown adipocyte or from white to brown adipogenesis can be used as a therapeutic strategy for obesity.

The potential effective and safety of stem cell therapy on obesity and its comorbidities must be further studied.

6 Conclusion

In conclusion, stem cell therapy is a therapeutic option for obesity in the future. However, long term studies are required to evaluate the efficacy and safety of stem cells therapy to find new and novel strategies for the treatment of obesity; and further studies in humans are necessary to investigate the results on animal models. This review showed that current studies are promising tools that can answer many questions in this regard.

In the future, one can hope that stem cell therapy can be used in control adipogenesis along with other obesity treatments and promises a new therapeutic approach in clinical applications.

Acknowledgement The authors would like to acknowledge Rasta Arjmand for her assistance in figure design. We also thank Dr. Mohsen khorshidi, and Maryam Afshari for their kind support.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 23–36
https://doi.org/10.1007/5584_2018_205

Published online: 4 May 2018

A Comprehensive Review of Stem Cells for Cartilage Regeneration in Osteoarthritis

Gauthaman Kalamegam, Adnan Memic, Emma Budd, Mohammed Abbas, and Ali Mobasheri

Abstract

Osteoarthritis (OA) is an age related joint disease associated with degeneration and loss of articular cartilage. Consequently, OA patients suffer from chronic joint pain and disability. Weight bearing joints and joints that undergo repetitive stress and excessive 'wear and tear' are particularly prone to developing OA. Cartilage has a poor regenerative capacity and current pharmacological agents only provide symptomatic pain relief. OA patients that respond poorly to conventional therapies are ultimately treated with surgical procedures to promote cartilage repair by implantation of artificial joint structures (arthroplasty) or total joint replacement (TJR). In the last two decades, stem cells derived from various tissues with varying differentiation and tissue regeneration potential have been used for the treatment of OA either alone or in combination with natural or synthetic scaffolds to aid cartilage repair. Although stem cells can be differentiated into chondrocytes *in vitro* or aid cartilage regeneration *in vivo*, their

G. Kalamegam
Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia

Sheikh Salem Bin Mahfouz Scientific Chair for Treatment of Osteoarthritis with Stem Cells, King Abdulaziz University, Jeddah, Saudi Arabia
e-mail: kgauthaman@kau.edu.sa

A. Memic
Center of Nanotechnology, King AbdulAziz University, Jeddah, Saudi Arabia
e-mail: amemic@kau.edu.sa

E. Budd
Department of Veterinary Pre-Clinical Sciences, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
e-mail: e.budd@surrey.ac.uk

M. Abbas
Sheikh Salem Bin Mahfouz Scientific Chair for Treatment of Osteoarthritis with Stem Cells, King Abdulaziz University, Jeddah, Saudi Arabia

Department of Orthopaedic Surgery, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
e-mail: mmabbas@kau.edu.sa

A. Mobasheri (✉)
Sheikh Salem Bin Mahfouz Scientific Chair for Treatment of Osteoarthritis with Stem Cells, King Abdulaziz University, Jeddah, Saudi Arabia

Department of Veterinary Pre-Clinical Sciences, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis, Queen's Medical Centre, Nottingham, UK

Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Republic of Lithuania
e-mail: a.mobasheri@surrey.ac.uk

potential for OA management remains limited as cartilage regenerated by stem cells fails to fully recapitulate the structural and biomechanical properties of the native tissue. Efficient tissue regeneration remains elusive despite the simple design of cartilage, which unlike most other tissues is avascular and aneural, consisting of a single cell type. In this article, we have comprehensively reviewed the types of stem cells that have been proposed or tested for the management of OA, their potential efficacy as well as their limitations. We also touch on the role of biomaterials in cartilage tissue engineering and examine the prospects for their use in cell-based therapies.

Keywords
Chondrocytes · *In vitro* · *In vivo* · Osteoarthritis · Regenerative medicine · Stem cells

Abbreviations

2D	Two dimensional
3D	Three dimensional
ACI	Autologous chondrocyte implantation
ACT	Autologous chondrocyte transplantation
BM	Bone marrow
BMAC	Bone marrow aspirate concentrate
BMP	Bone morphogenetic protein
CD	Cluster of Differentiation
COX-2	Cyclooxygenase-2
CP	Cartilage pellet
EBs	Embryoid bodies
ESCs	Embryonic stem cells
FDA	Food and drug administration
HA	Hyaluronic acid
HSCs	Haematopoietic stem cells
iPSCs	Induced pluripotent stem cells
ISCT	International Society for Cellular Therapy
MMP-13	Matrix metallo-proteinase-13
MSCs	Mesenchymal stem cells
NSAID	Nonsteroidal anti-inflammatory drug
OA	Osteoarthritis
PRP	Platelet rich plasma
SF	Synovial Fluid
SNRIs	Serotonin-norepinephrine reuptake inhibitors
TGF-β	Transforming growth factor beta
TKA	Total knee arthroplasty

1 Introduction

Ageing is inevitable and so is the decline in function of the various organ systems, particularly connective load-bearing tissues in synovial joints of the musculoskeletal system. Articular cartilage is prone to 'wear and tear' and the articular cartilage undergoes persistent degeneration. The poor regenerative ability of articular cartilage combined with continued cartilage degeneration leads to the development of the most common joint disease, osteoarthritis (OA). OA is a painful multifactorial degenerative joint disease characterized by low-grade inflammation in cartilage and synovium, which is associated with the loss of joint structure and progressive deterioration of cartilage (Fig. 1) (Musumeci et al. 2015). Traumatic injuries and some sequelae of anti-inflammatory events activate immune cells as well as inducing the secretion of pro-inflammatory cytokines which leads to development of subchondral bone lesions (Shabestari et al. 2016; Zhu et al. 2017). Aberrant metabolism of the synovial joint tissue and cell function is also implicated in the onset of OA (Mobasheri et al. 2017). Irrespective of the aetiology, OA is associated with potentially crippling symptoms such as pain, swelling, stiffness and limited mobility. The incidence of OA increases with age, and the associated altered molecular pathways causes progressive deterioration of the biomechanical properties of cartilage (Lotz and Loeser 2012).

In the United states alone, people aged over 60 years (10% men and 13% women) are most commonly afflicted with knee OA (Zhang and Jordan 2010). A cross sectional study that

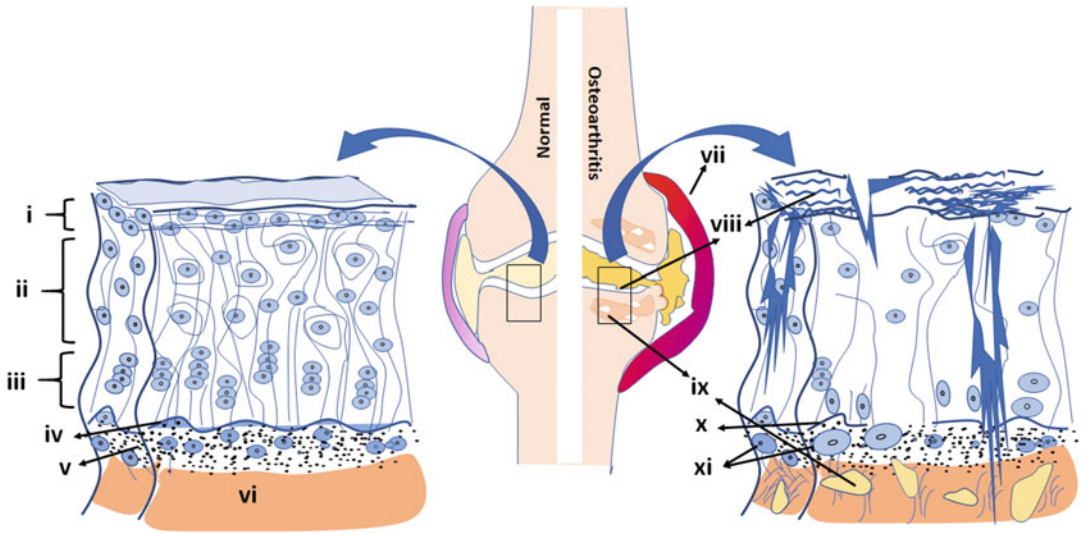

Fig. 1 A normal and osteoarthritic (OA) joint showing the major anatomical structures and the key pathological changes is diagrammatically represented. The enlarged boxed area from the normal and OA knee joint shows cartilage structure and the pattern of cellular arrangement or its derangement respectively. In the normal knee joint the cartilage surface is smooth and clear demarcation exists between the superficial, mid and deep zones. The tidemark, calcified cartilage and the underlying subchondral bone are intact. In the OA knee joint the changes include thickening of the joint capsule, inflammation, cartilage destruction and osteophyte formation. The changes in cartilage include fragmentation (fibrillation), degradation and loss of chondrocytes (hypocellularity). The changes in the subchondral bone include degradation, cystic degeneration and minor fractures. **(i)** – Superficial zone (10%–20%) with tangential arrangement of chondrocytes; **(ii)** – middle zone (40% – 60%) with vertical arrangement of chondrocytes; **(iii)** – deep zone (30%) with compact and longitudinal arrangement of chondrocytes; **(iv)** – intact tide mark; **(v)** – calcified cartilage; **(vi)** – normal subchondral bone; **(vii)** – inflamed joint capsule in OA knee joint; **(viii)** – cartilage degradation and fibrillation; **(ix)** – subchondral bone cysts; **(x)** – damaged/absent tide mark; **(xi)** – hypertrophied chondrocytes

evaluated the prevalence of radiographic OA involving 300 patients from 14 different primary health centres in Saudi Arabia reported that 53.3% men and 60.9% women had clinical evidence of knee OA (Al-Arfaj and Al-Boukai 2002). Another study on 243 patients with radiographic evidence of OA from the Eastern province of Saudi Arabia reported that more than 90% of these patients were obese indicating a strong association between obesity and OA. The incidence of OA was higher in obese female patients (73.09%) than males (41.65%) (Ismail et al. 2006). Other independent risk factors of OA include gender, increased physical activity, trauma, genetic predisposition and lifestyle (Zhang and Jordan 2010). Existing treatment methods fail to cure OA, leading to active research initiatives and integration of multiple disciplines to find a permanent cure. Regenerative medicine is a translational research solution to some of the protracted or incurable diseases which integrates both cells/stem cells and tissue engineering principles to regenerate tissues/organs and help functional restoration. It is hoped that regenerative medicine will provide strategies for effective regeneration of articular cartilage.

2 Current Treatment of Options for OA

The main methods of OA treatment involve non-pharmacological, pharmacological and surgical measures with the aim of reducing pain and improving tolerance for functional activity.

Non-pharmacological management includes moderate exercises to strengthen the muscles, weight loss and massage therapies (Christensen et al. 2005; Ernst and Posadzki 2011).

2.1 Conventional Pharmacological Agents

Current commonly prescribed pharmacological agents in the management of OA include (i) acetaminophen; (ii) non-steroidal anti-inflammatory agents (NSAIDs); (iii) opioid analgesics; (iv) serotonin-norepinephrine reuptake inhibitors (SNRIs) and (v) intra-articular injections (Zhang et al. 2016). Acetaminophen which is normally used to control fever, headache and muscle aches is also used in the treatment of mild OA. However, acetaminophen cannot be used long term due to its hepatotoxic side-effects. NSAIDs are used in mild to moderate cases of OA and appear to be more effective than acetaminophen as they possess anti-inflammatory effects in addition to analgesic properties. Prolonged treatment with NSAIDs is not desirable due to the associated incidence of gastritis and gastrointestinal bleeding. Selective cyclooxygenase-2 (COX-2) inhibitors such as celecoxib, etoricoxib and polmacoxib are effective and relatively safe than less selective NSAIDs (Lee et al. 2017; Huang and Tso 2018). Opioid agents are used in moderate to severe OA, when acetaminophen and NSAIDs fail to provide symptomatic relief. However, the associated side effects such as nausea, vomiting, drowsiness, constipation and addiction risks limit their potential use (DeLemos et al. 2011). Placebo controlled randomized clinical trials have identified duloxetine an SNRI agent observed to be effective in the management of OA, but studies on its long-term safety and recommendation for use in OA are currently lacking (Chappell et al. 2011). Intra-articular injections of hyaluronic acid (HA) or corticosteroids are used for the treatment of moderate to severe OA, however, they have variable acceptance and efficacy (Zhang et al. 2016).

2.2 New Biological and Pharmacological Agents

Lack of sustained benefits with conventional pharmacological agents have led to the development of novel agents that can limit OA disease progression at the molecular level and broadly these agents are known to stimulate chondrogenesis, inhibit apoptosis, inhibit matrix degradation and reduce inflammation. Biological recombinant agents [bone morphogenetic protein-7 (OP-1); fibroblast growth factor 18 (Sprifermin)] and autologous platelet rich plasma (PRP) act as inducers of chondrogenesis (Zhang et al. 2016). Novel pharmacological agents include the disintegrin and metalloproteinase with thrombospondin motifs 5 - (ADAMTS-5) inhibitor (compound 114,810) and matrix metalloproteinase 13 (MMP-13) inhibitor (compound CL82198) which inhibit apoptosis and matrix degradation respectively. Interleukin-1 beta receptor antibody (AMG108) and ultrafiltrate of human serum albumin (Ampion) are known to exert their effects by modulation of inflammatory cytokines (Zhang et al. 2016).

2.3 Surgical Management of OA

Surgical intervention is indicated in severe OA with full thickness cartilage loss, concomitant to failures with non-pharmacological and pharmacological methods leading to disease progression. Knee realignment or joint replacement surgeries are common in patients with end stage OA, which helps in the relief of arthritic pain and improvement of function. Approximately 700,000 total knee arthroplasties (TKAs) were performed in the united states in the year 2010, of which nearly 50% were reported to be in individuals who were less than 65 years of age (Nguyen et al. 2016). The number of TKA procedures continues to rise irrespective of the aetiology of OA, despite the excessive cost involved. Recent advancements in regenerative medicine using cell-based therapies may provide an alternative and efficient treatment strategy thereby reducing the need for TKA.

Regenerative therapy uses various cell types to enable both structural and functional recovery. Cell therapies using both mature cells and stem cells have been practised for nearly three decades. Cell therapy may alleviate the symptoms associated with OA and offer a long term solution (Jiang et al. 2011).

2.4 Autologous Chondrocyte Implantation (ACI)

In the early 90s, autologous chondrocytes were used for autologous implantation/transplantation (ACI/ACT) which induced hyaline cartilage regeneration. Chondrocytes removed from adjacent healthy cartilage using arthroscopy were expanded in culture and subsequently implanted at the site of lesion and covered with a periosteal flap (First generation ACI) (Brittberg et al. 1994). However, the procedure utilising periosteal flap for ACI was associated with cartilage hypertrophy. Subsequent changes to the procedure were made including the use of a bilayer collagen membrane instead of the periosteal flap (Second generation ACI) (Gooding et al. 2006; Niemeyer et al. 2014) or a composite chondrocyte laden scaffold in which chondrocytes were previously cultured upon biodegradable matrix prior to implantation (Third generation ACI) (Brittberg 2008; Mistry et al. 2017). Unlike the first and second generation ACI methods whereby the membranes need to be sutured, the matrix-induced autologous chondrocyte transplantation/implantation (MACT, third generation ACI) utilized fibrin glue to hold the cell laden matrix in place. Although the clinical outcome with ACI was effective their utilization was limited due to insufficient numbers of chondrocytes as well as the tendency of chondrocytes to dedifferentiate upon *in vitro* culture.

3 Stem Cells for Articular Cartilage Regeneration

Cartilage by nature has poor regeneration capacity. Cartilage tissue being avascular does not incite an immediate inflammatory response when there is an injury/lesion, unlike other tissues with vascular supply (Poulet and Staines 2016). As such the key players of tissue regeneration namely stem cells are not recruited to the site of lesion and hence it becomes a necessity to use stem cells isolated from other sources to aid cartilage regeneration. Stem cells are undifferentiated cells that are capable of self-renewal and differentiation towards a specific cell lineage. Stem cells have variable differentiation potential and accordingly they can be classified as pluripotent (ESCs, iPSCs) as these cells can give rise to almost all the tissues of the human body or multipotent (eg. adult and foetal MSCs) as these cells can be differentiated only into specific cell lineages. Stem cells are therefore an attractive choice for regenerative medicine applications and it is essential to understand the different types of stem cells that have the potential to be used for cartilage regeneration.

3.1 Types of Stem Cells

Embryonic stem cells (ESCs) are derived from day 5 blastocyst stage embryos that have a clearly demarcated inner cell mass, a trophectoderm and a blastocoelic cavity (Bongso 2006; Thomson et al. 1998). To qualify as *bona fide* ESCs, ESCs must be (i) derived from a pluripotent cell population, (ii) maintain normal karyotype, (iii) propagated indefinitely in the embryonic state, (iv) and capable of spontaneous differentiation into cells representing all three embryonic germ layers namely ectoderm, mesoderm and endoderm in teratomas or *in vitro* (Pera et al. 2000). Mesenchymal stem cells refer to hypothetical common progenitors originating from a wide range of mesenchymal (mesodermal, non-epithelial, non-haematopoietic) tissues (Budd et al. 2017) and have been recorded to have been isolated from the niches of various tissues including bone marrow, brain, liver, bone, retina, pancreas, adipose tissue, epidermis, synovial fluid, amniotic fluid, umbilical cord and placenta (Bianco et al. 2008; De Coppi et al. 2007; Kern et al. 2006). Not to be confused with

the heterogeneous population of cultured plastic adherent cells isolated from bone, referred to as bone marrow stromal cells (BMSCs) from which a diminutive population of bone marrow-derived stem cells exist, often also referred to as MSCs. Due to the controversy surrounding the MSC identity and differentiation potential, some research groups utilise the term skeletal stem cell (SSC) or bone marrow-derived stem cell (BMSC) to refer to this stem cell population isolated from bone marrow which possess specific differentiation potential towards skeletal tissues inclusive of cartilage, bone and marrow adipocytes (Bianco and Robey 2015). An alternative to adult tissue as a source for stem cells is foetal tissue, which has been shown to contain stem cell populations with enhanced plasticity, proliferation and expansion potential compared to stem cells derived from adult sources (Guillot et al. 2007). Stem cells with similar potential to that of ESCs are induced pluripotent stem cells (iPSCs) (Takahashi et al. 2007). However, unlike ESCs, iPSCs possess multipotency which has been induced artificially through the introduction of Oct3/4, c-Myc, Kl4 and Sox2, genes which encode transcription factors involved with maintaining pluripotency (Takahashi and Yamanaka 2006). The generation of iPSCs from human somatic cells (Takahashi et al. 2007) was a major step towards tissue personalization, opening up the potential to use autologous stem cells in applications which may be otherwise impeded by cell rejection following transplantation. In recent years the use of stem cells in orthopaedics has gained significant attention as a potential application for cartilage repair. To achieve efficient cartilage regeneration different types of stem cells have been explored using both *in vitro* and *in vivo* studies.

3.2 Pluripotent Stem Cells and Cartilage Repair/ Regeneration

Human ESCs (BresaGen variant cell line, BG01V) were differentiated into chondrocytes *in vitro* using either embryoid bodies (EBs), micromass or pellet cultures with defined xenofree media containing Knockout serum replacement and growth factors including transforming growth factors (TGF-β3, TGF-β1) and bone morphogenetic protein (BMP-2) for up to 21 days (Suchorska et al. 2017). The authors reported that differentiation into chondrocytes was more efficient with EBs followed by pellet and micromass cultures. However, chondrocytic differentiation *via* EBs involved an additional step of EBs generation prior to their differentiation, unlike pellet or micromass cultures which can be directly differentiated (Suchorska et al. 2017). Transplantation of undifferentiated ESCs embedded in fibrin glue into the knee joint in an ovine osteochondral defect model resulted in successful hyaline cartilage regeneration and integration compared to sham controls after 24 months (Manunta et al. 2016). iPSCs derived from cord blood mononuclear cells were also demonstrated to be successfully differentiated into cartilage upon pellet culture for 30 days. Embryoid bodies (EBs) were generated from the iPSCs and the outgrowth cells from EBs were used in a pellet culture system for cartilage differentiation. The differentiated cells expressed cartilage associated genes and extracellular matrix indicating efficient cartilage differentiation (Nam et al. 2017). *In vitro* differentiation of human iPSCs and transplantation into articular joints of immunodeficient mice resulted in hyaline cartilage differentiation at 8 weeks but there was teratoma formation at 16 weeks (Saito et al. 2015). Undifferentiated ESCs produce teratomas upon transplantation to immunocompromised mice, thus making it mandatory to ensure complete differentiation of ESCs into target tissues or removal of any undifferentiated ESCs prior to transplantation, to render them safe for use in cell-based therapies (Aldahmash et al. 2013).

3.3 Multipotent Stem Cells and Cartilage Differentiation

MSCs have long been considered as an attractive choice of stem cell for cartilage repair and regeneration as they are multi-potent and have anti-

inflammatory and immunomodulatory effects. Adult MSCs have been used for cartilage regeneration as early as 1990s, however, there are no *bona fide* cell surface markers to identify or isolate these MSCs. Their identification is mainly dependent upon the minimal essential criteria stipulated by the International Society for Cellular Therapy (ISCT), namely plastic adherence; differentiation into three different mesodermal lineages; osteoblasts, chondrocytes, adipocytes and expression of CD73, CD90, CD105 and lack expression of CD34 as well as being HLA-DR negative (Dominici et al. 2006). MSC progenitors are reported to reside within articular cartilage, however, their contribution to cartilage regeneration following disease/damage appears insufficient (Grogan et al. 2009). Human BM-MSCs cultured as micromass pellets in the presence of transforming growth factor-beta 3 (TGF-β3) and dexamethasone differentiated into chondrocytes *in vitro* and expressed chondrocyte associated genes and collagen (Mackay et al. 1998). The use of nanoscaffolds, which mimick the ECM, in combination with human BM-MSCs in the presence of growth factors was observed to achieve successful chondrocyte differentiation (Wise et al. 2008).

In addition to BM-MSCs, MSCs derived from other sources such as adipose derived MSCs (Diekman et al. 2009), synovial fluid MSCs (Lee et al. 2012) and umbilical cord derived Wharton's jelly MSCs (Fong et al. 2012), have also been successfully differentiated into chondrocytes. Intra-articular injection of BM-MSCs in sheep resulted in the regeneration of cartilage with normal histoarchitecture, especially when supplemented with growth factors such as TGF-β3 and insulin-like growth factor-1 (Al Faqeh et al. 2012). However, the use of adult derived MSCs can be limiting in that isolation of total MSCs from human tissue varies in number as a result of variability amongst individuals from which MSCs are derived and subculture of MSCs for expansion purposes induces morphological and phenotypic changes and may impact upon MSC senescence. MSCs derived from ESCs provide an additional stem cell source with great proliferation capabilities unlike adult MSCs and hence offer enormous potential for biomedical research. Chondrogenic differentiation of MSCs derived from ESCs with a combination of TGF-β1 and BMP-7 led to increased mRNA expression of collagen type 2, aggrecan and SOX9 compared to differentiation of MSCs with TGF-β1 alone. The enhanced chondrogenesis with a combination of TGF-β1 and BMP-7 was due to increased expression of TGFβR2 and the production of endogenous TGF-β (Lee and Li 2017).

3.4 Stem Cell Initiative for Cartilage Differentiation at King Abdulaziz University

Our stem cell unit in the department of orthopaedics at King Abdulaziz University hospital Jeddah Saudi Arabia, is a nascent facility and given our interests in this emerging field we have derived MSCs from bone marrow (BM) and synovial fluid (SF) samples obtained from both healthy and OA patients. Although, both BM-MSCs and SF-MSCs fulfilled the minimal criteria stipulated by ISCT (Dominici et al. 2006) their expansion *in vitro* was limited and demonstrated cellular senescence after subpassages (Fig. 2a). This could be attributed to the age of the patients (50–75 years) from which cells were derived. As microenvironmental cues are reported to play an essential role in effective chondrocyte differentiation (Watt and Huck 2013), we evaluated an explant culture model (Fig. 2b) where the osteochondral plugs obtained from OA patients were subjected to microdrill defect and the cells (BM-MSCs, cartilage pellet or a combination of both) obtained from the same patient were used for chondrocyte differentiation (Abbas 2017). Combination of both BM-MSCs and cartilage pellet demonstrated good differentiation into chondrocytes with increased cartilage matrix proteins secretions indicating that micro-environmental cues effectively contribute to the differentiation process (Abbas 2017). With insights obtained from both *in vitro* studies we then evaluated the combined effects of BM-MSCs and cartilage pellet

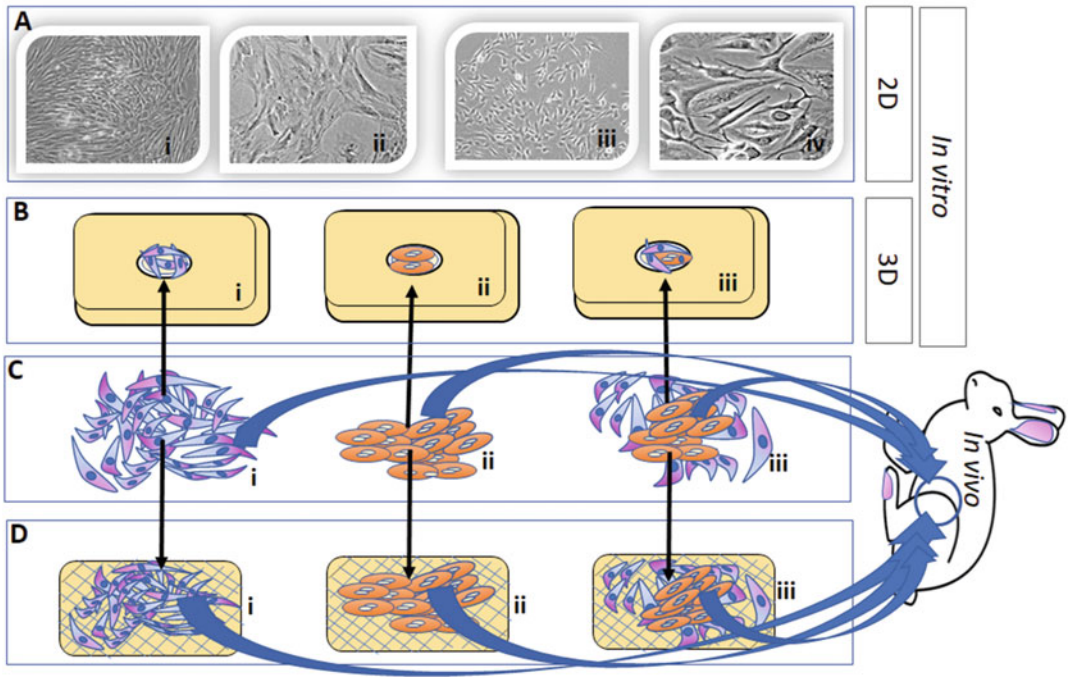

Fig. 2 Diagrammatic representation of the *in vitro* and *in vivo* models to evaluate cartilage regeneration. **Ai and Aii**: Phase contrast images of the bone marrow mesenchymal stem cells(BM-MSCs) at early (P0) and late (P6) passages. **Aiii and Aiv**: Phase contrast images of the synovial fluid mesenchymal stem cells (SF-MSCs) at early (P0) and late (P5) passages. The cells in late passages of both BM-MSCs and SF-MSCs showed broad flattened morphology that were not actively dividing in culture which was indicative of cellular senescence. **B:** Diagram representing the osteochondral plugs obtained from OA patients undergoing total knee arthroplasty (TKA). A central drill defect of 2 mm was made and filled with **(Bi)** – pelleted BM-MSCs; **(Bii)** – homogenized cartilage pellet (CP); or **(Biii)** – a combination of BM-MSCs and cartilage pellet to evaluate cartilage repair *in vitro*. The *In vivo* cartilage repair was evaluated following full thickness surgical defect of the articular cartilage over the Tibial surface in NZW rabbits. The defects were filled with either **(Ci)** – BM-MSCs; **(Di)** – BM-MSCs and Hyalofast™; **(Cii)** – CP; **(Dii)** – CP and Hyalofast™; **(Ciii)** – combination of BM-MSCs and CP; or **(Diii)** – Combination of BM-MSCs, CP and Hyalofast™

(CP) *in vivo* in rabbits (Fig. 2c), which also demonstrated improved cartilage regeneration. In addition, we also evaluated the efficiency of Hyalofast™, a biodegradable scaffold, together with BM-MSCs and (CP) in repair/regeneration of full-thickness cartilage defect *in vivo* in rabbits (Fig. 2d). Efficient and accelerated regeneration/repair of the defective cartilage was observed with combinations of Hyalofast™, BM-MSCs and CP, rather when used separately [unpublished results].

In general, the current cell-based repair strategies largely remain unsuccessful, especially when cartilage repair is undertaken in an autologous setting given the limitations in cell expansion and the excessive cost involved with the use of human grade culture media and supplements. Therefore, other alternative avenues of research are also being explored for cartilage repair/regeneration.

4 Cartilage Tissue Engineering

4.1 Bioprinting of Articular Cartilage

Despite significant advances using various approaches including tissue engineering and regenerative medicine, repair of articular cartilage and the osteochondral interface specifically

remains a major challenge (Apelgren et al. 2017; Daly et al. 2016; Zhang et al. 2017). Most of the inefficiency is derived from artificial matrices currently available often leading to inadequate healing and tissue regeneration. Various classes of biomaterials, such as hydrogels, can be tuned to provide mechanical stability and bioactivity (Duarte Campos et al. 2012; Memic et al. 2015; Schon et al. 2017; Yang et al. 2017). Furthermore, current three-dimensional (3D) approaches with cell-laden biomaterials are a significant step forward from conventional two-dimensional (2D) methods. However, for clinical translation these tissue engineered constructs often require large numbers of cells with complex structural hierarchies (Mouser et al. 2017; Richardson et al. 2016; Yang et al. 2017). In this regard, technologies focusing on 3D bioprinting approaches have the potential to offer several advantages that could address the limitations of traditional biomanufacturing methods. Ultimately, these novel constructs could prove to be better mimics of the native environment and provide improved clinical outcomes.

To develop clinically relevant tissue mimics, several 3D bioprinting strategies exist (Apelgren et al. 2017; Mouser et al. 2017; Schon et al. 2017; Zhang et al. 2017). By combining several biomaterial sources and cell types we can develop better articular cartilage tissue mimics. Similarly, these bioprinting methods could be used to recapitulate and study the diseased state, having characteristics that better mimic *in vivo* conditions (Arslan-Yildiz et al. 2016; Kang et al. 2016; Mehrali et al. 2016; Memic et al. 2017; Murphy and Atala 2014; Ozbolat et al. 2016; Zhang et al. 2017). As such they could provide answers related to the underlying biology that plays a role in articular cartilage repair and regeneration. In addition to providing modifiable biophysical cues, mechanical stiffness and bioactivity, 3D bioprinting allows for assembly of complex native-like architectures (Duarte Campos et al. 2012; Mouser et al. 2017; Yang et al. 2017). This is one of the major advantages of 3D bioprinting technologies whereby well-defined geometries with gradient composition of biomaterials and cells can be achieved during construct manufacturing with complex structural features (Jang et al. 2016). Ultimately, 3D bioprinting could be combined with patient derived cells, custom bioinks and biosensing technologies that could be the stepping stone for the development of truly personalized medicine applications (Bertassoni et al. 2014; Hasan et al. 2015; Park et al. 2016; Vaidya 2015).

A recent report showed that MSCs could be bioprinted within silk fibroin-gelatin hydrogel constructs (Das et al. 2015). The 3D bioprinted constructs were crosslinked in situ by tyrosinase or sonication maintaining good viability of encapsulated MSCs over 30 days during *in vitro* culture. Next, these MSCs could be guided into either chondrocyte and/or osteoblast lineage by using differentiation medium. Similarly, other studies have shown that bioprinting cell-laden alginate-based biomaterials, fabrication of cartilage-like tissue with a complex geometry could be possible (Markstedt et al. 2015). In this study, the bioprinted chondrocytes had long-time viability. Other strategies focus on bioprinting parameters that allow fabrication of gradient compositions and structures via active and efficient mixing of complex fluids. Such techniques have improved bioprinting efficiency and thus hold much potential towards the development of tissue-engineered zonal osteochondral/cartilage complex structures (Ober et al. 2015).

In future, once the convergence of several bioprinting technologies takes place (Daly et al. 2016; Duchi et al. 2017; Memic et al. 2017; Schon et al. 2017), we expect progress in the development of constructs with patient specific cells and custom bioinks that have improved structural resolution and mechanical integrity during bioprinting. Taken together advances in the field of articular cartilage repair and regeneration may be quickly translated into the clinic with significantly improved biological, physiological and personalized treatments and outcomes.

4.2 Cell Free Biomaterial Approaches for Articular Cartilage Repair

Although cell-based techniques using both MSCs and autologous chondrocytes have shown a lot of potential for clinical translation and application, challenges remain in developing novel clinical products. Often cell-based approaches are expensive and require a substantial amount of time for extraction, proliferation and differentiation of primary cells and/or MSCs (Foyt et al. 2018; Makris et al. 2015; Murphy et al. 2017). If these products are ultimately considered as biologicals, instead of as devices, by regulatory agencies it could further delay their approval and significantly increase the cost associated with their clinical translation. Considering these challenges, it appears that biomaterials built on cell-free platforms might provide substantial advantages for cartilage repair and regeneration (Calabrese et al. 2017; Makris et al. 2015; Mathis et al. 2017; Murphy et al. 2017).

Cell-free based biomaterial methods usually rely on controlled release of growth factors, serum or small bioactive molecules such as drugs that are able to modulate the local microenvironment in order to stimulate cartilage healing (Armiento et al. 2018; Duarte Campos et al. 2012; Makris et al. 2015). For example, biomaterials can rely on selective stimulation and recruitment of MSCs in order to exert therapeutic effects. If these biomaterials are further coupled with controlled release of tailored growth factors they could be able to activate chondrocytes in the local healthy microenvironment promoting tissue remodeling, repair and ultimately closure of the cartilage defect (Armiento et al. 2018).

One cell-free strategy is autologous matrix-induced chondrogenesis (AMIC) that aims to provide both early mechanical stability and long-term regeneration of cartilage. The procedures after the initial cleaning are based on inducing microfractures that release MSCs, blood, and serum, at the defect site that can then be sealed with a biomaterial (Patrascu et al. 2010). For example, a biomaterial implant that combines platelet-rich plasma or autologous serum with hyaluronic acid is placed at the defect site after initial microfractures are generated (Patrascu et al. 2010). The role of this biomaterial combination is twofold; first, the platelet-rich plasma and/or autologous serum can aid in the recruitment of bone marrow MSCs found in the underlying subchondral bone, (Patel et al. 2013) and secondly hyaluronic acid might promote their differentiation into chondrocyte-like phenotype at the defect site (Patrascu et al. 2013). In a recent clinical trial based on this treatment procedure involving 52 patients 1-year post-surgery an improvement in patient-reported Knee injury and Osteoarthritis Outcome Scores (KOOS) was observed. At the defect site hyaline-like repair tissue appeared to be present when analyzed using histological methods (Siclari et al. 2012). However, to demonstrate long term effectiveness of this approach more controlled clinical trials are required with extended time periods to prove the durability of this new tissue. Another similar clinical trial looked at the placement of clot-like structures made from the combination of chitosan and autologous whole blood that was drawn immediately before placement into the defect site (Buschmann et al. 2007). One year follow up reported that the chitosan-based approach had increased lesion fillings and improved tissue repair compared to microfractures alone (Stanish et al. 2013). However, in terms of a functional outcome there was little difference in the two sets of patients. This is especially confounding when considering that microfractures begin to degenerate in as little as 2 years post-surgery requiring extended follow up.

Significant room remains for improvement of AMIC and other cell-free biomaterials-based approaches for repair and regeneration of articular cartilage. More in-depth studies are required in assessing how different concentrations, release parameters and combinations of chemoattractants and chemokines including bone morphogenetic proteins and growth factors incorporated within biomaterial platform affect the action of MSCs in cartilage repair (Filardo et al. 2012; Richter 2009). Perhaps, personalized strategies can be developed for a certain set of

conditions and/or anatomical locations. Although some of these approaches like AMIC are FDA-approved additional carefully designed pre-clinical studies are needed to investigate their efficacy.

5 Conclusions

Very few connective tissues have an inherent regenerative ability and if they do, this repair and regeneration potential decreases with age. Tissue engineering and regenerative medicine is intended to facilitate the restoration of many tissues including those of the musculoskeletal system. Effective articular cartilage regeneration with the use of stem cells has still not been achieved, and more fibrotic/hyperplastic changes tend to occur with time. In addition, the problems associated with cell expansion, culture induced phenotypic changes and the high cost of using of human grade culture media and supplements appear to delay the progress of research in this area. Harvest of multinucleated cells from within the bone-marrow and direct injection to the cartilage defect area using the bone-marrow aspirate concentrate (BMAC) system is gaining prominence amongst the clinicians as it helps to circumvent the labour-intensive protocols with cell culture and the associated high cost. However, the interaction of the transplanted cells with the host tissue, their differentiation into articular cartilage, fulfilment of the bio-mechanical properties and long-term benefits remains to be understood. Continued research involving cellular therapies and/or biomaterials therefore is mandatory for identifying a successful technique to regenerate cartilage.

Acknowledgements The authors acknowledge the financial support provided by the "Sheikh Salem Bin Mahfouz Scientific Chair for Treatment of Osteoarthritis by Stem Cells" and the stem cell laboratory facility at CEGMR and King Abdulaziz University Hospital.

Conflicts of Interest The authors declare no conflict of interests.

Competing Interests and Disclosures The authors declare no competing interests.

Author's Contributions G. Kalamegam and A. Memic were involved in intellectual contribution and manuscript writing. MA and EB were involved in intellectual contribution and editing of the manuscript. A. Mobasheri contributed to the synthesis and editing of the manuscript.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 37–46
https://doi.org/10.1007/5584_2018_258

Published online: 8 August 2018

Exploring Stem Cells and Inflammation in Tendon Repair and Regeneration

A. Vinhas, M.T. Rodrigues, and M.E. Gomes

Abstract

Tendon injuries are frequent and are responsible for substantial morbidity both in sports and in the workplace. Despite the endogenous mechanisms of tendon repair and regeneration, tendon healing upon injury is slow and often insufficient to restore complete biomechanics functionality.

Inflammation has a pivotal role in tendon healing and failed healing responses contribute to the progression of tendinopathies. However, the molecular and cellular mechanisms involved are poorly understood requiring further insights.

During inflammation, bioactive molecules such as cytokines secreted locally at the injury site, influence resident stem cells that contribute as modulatory agents over the niche towards homeostasis, holding great promise as therapeutic agents for tendon pathological conditions associated to unresolved inflammation and failed healing.

This review overviews the role of cytokines and resident cells, focusing on the participation of tendon stem cell population in inflammation and tendon healing upon injury and their potential action in resolution of pathological conditions.

Keywords

Cytokines · Healing · Inflammation · Mechanical stimulation · Pathology · Repair · Tendon · Tenocytes

Abbreviations

CTGF	connective tissue growth factor
ECM	extracelular matrix
MMPs	matrix metalloproteinases
MSCs	mesenchymal stem/stromal cell
NO	nitric oxide
STAT3	activator of transcription 3
TDSCs	tendon-derived stem cells

1 Introduction

1.1 Tendon Niche

Tendons are dense connective tissues that connect muscles to bone and transmit the mechanical forces generated during contraction to the

A. Vinhas, M. T. Rodrigues, and M. E. Gomes (✉)
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal
e-mail: megomes@i3bs.uminho.pt

skeleton, therefore essential for locomotion (Liu et al. 2014; Subramanian and Schilling 2015). Tendons are hypocellular tissues mainly composed of tenocyte, and a stem and progenitor cell population (Bi et al. 2007). However, other cell types may be also present, for instance endothelial cells, mast cells, chondrocytes, synovial and vascular cells. Tenocytes are responsible for extra cellular matrix (ECM) maintenance, which is mainly composed of collagen, in particular fibrillar collagens, namely collagen type I and III, although other types of collagen are also present, such as collagen type III, V, VI, XII, XIV and XV (James et al. 2008; Millar et al. 2015; Rodrigues et al. 2013). Tendon ECM is also composed of proteins and proteoglycans, such as decorin, biglycan, aggrecan and elastin (Aparecida de Aro et al. 2012; Docheva et al. 2015; James et al. 2008; Tan et al. 2015; Subramanian and Schilling 2015).

Morphologically, tendons follow a hierarchical architecture of collagen molecules that gather to form collagen fibrils. These fibrils assemble into fibers to form collagen fiber bundles. Finally, the bundles organize into tendon fascicles. The presence and alignment of collagen fibers is oriented for providing resistance to tendon and increased tensile strength (Docheva et al. 2015; Killian et al. 2012; Lui 2015; Durgam and Stewart 2016), while reducing stress during muscle contraction (Ho et al. 2014).

In tendon milieu, tendon cells and the ECM coordinate actions in promoting damage repair and tissue regeneration. Since vascular supplies in tendon are confined to endotenon and epitenon, it is likely that stem cell recruiting through the vascular system may be restricted to the surrounding areas of these layers. Thus, the resident cell populations, stem cells and tenocytes, have a critical role in physiological homeostasis and regulation of the tendon matrix. When this delicate fine-tuned balance is disturbed the susceptibility to tendon damage increases (Cadby et al. 2014).

Growing evidence supports tendon stem cells, rather than tenocytes, as the main responsible for the healing response in acute injuries. Beyond the self-renewal capacity, proliferation and multilineage potential, stem cells are a secretory source of cytokines and growth factors with paracrine and autocrine activities. These soluble factors support the growth and differentiation of stem and progenitor cells and have a angiogenic, chemotatic, anti-apoptotic, anti-scarring or immunomodulatory activity (Meirelles et al. 2009) in local environments. The secretion of a broad range of bioactive molecules with paracrine effects resulting from the dynamic communication between stem cells and niche environment is believed to be the main mechanism by which mesenchymal stem cells achieve their therapeutic effect (Meirelles et al. 2009).

Other intrinsic agents such as age, genetics, nutrition, body habitus and metabolic diseases are also involved in homeostasis as well as extrinsic factors namely pharmacological influences and mechanical stresses, including loading, disuse, compression and exogenous damage (Abate et al. 2009; Riggin et al. 2015). When tendon injuries occur, there is a local failure in physiological conditions, whose attempt to be solved is mediated by tendon healing and regeneration processes.

2 Tendon Repair Mechanisms

The healing process is a prolonged and complex response of the host to injury and is crucial for the mechanisms of tissue regeneration. It is also a window of opportunity envisioning new therapies for improving impaired healing (Stalman et al. 2015) and understanding the molecular entities and mechanisms behind regeneration. Because tendons possess a limited intrinsic regeneration capacity with low cellularity, low vascularization and poor innervation, tendon healing results in healed tissues with impaired mechanical capabilities. The potential of tendon healing also depends on the anatomical location and local environment (Thomopoulos et al. 2015).

Most tendons heal spontaneously upon injury but the load-bearing functions are frequently dominated by fibrotic scaring, which can result

in adhesion formation and consequent failure to achieve proper biomechanics (Sharma and Maffulli 2005). Thus, a major challenge in tendon healing is to control the scar tissue formed (over scar remodeling) that deeply compromises the normal function (Rodrigues et al. 2013). Overuse or repetitive stretching during physical activities, which are the major cause of tendon lesions leading to microdisruption of tendon fibers, are known to trigger the release of pro-inflammatory mediators (Rileya et al. 2002; Yang et al. 2005). Increase levels of inflammatory cytokines have been associated to tendon degeneration and disease (Millar et al. 2009).

The lack of understanding on the cell mediated mechanisms disturbing the endogenous repair/regeneration process results in limited knowledge for effective treatment. The inflammation process has a pivotal role in the healing upon injury and failed healing responses contribute to the progression of tendinopathies, which represent a significant medical problem worldwide. The development of tendinopathy compromises tendon structure and function and is characterized by pain, swelling and dysfunction (Magnusson et al. 2010; Zhang et al. 2016), affecting athletes and general population.

The mechanisms supporting tendon healing are still a subject of debate. Two types of tendon healing were proposed: intrinsic and extrinsic. In intrinsic healing, tenocytes from the epitenon and endotenon migrate and proliferate into the site of injury, reorganizing the ECM and giving support to the internal vascular networking (Muller et al. 2015). Conversely, extrinsic healing is achieved by the invasion of cells from the surrounding sheath and synovium. Extrinsic healing has been associated to facilitate scar formation and, consequently inferior biomechanics. Other studies suggest that both intrinsic and extrinsic pathways are fundamental to the early stages of tendon healing (Harrison et al. 2003).

The tendon healing process typically includes three main phases: inflammation, proliferation and remodeling (Docheva et al. 2015) influenced by a temporal and spatially controlled array of mediators and the microenvironment events (Thomopoulos et al. 2015) (Fig. 1).

The first phase is often rapid and of short duration and characterized by the infiltration of inflammatory cells like monocytes, macrophages, neutrophils and platelets. These cells release chemotactic factors that favor the migration and activation of tendon cells from nearby regions of the injury and from tendon sheaths. In the next phase, tendon cells proliferate and produce a collagen rich ECM, re-establishing the alignment of tenocytes between collagen fibrils. Finally, during the remodeling phase, ECM becomes more organized with axial arrangement of collagen fibers. In rat flexor tendons, MMP-9 and MMP-13 mediate tissue degradation, while MMP-2, MMP-3 and MMP-14 were associated to the remodeling phase (Buono et al. 2013). The remodeling of the ECM is a crucial process of tendon healing for gaining biomechanical competence.

During healing, the inflammatory mediators such as Il-6 and TNF-α are secreted by tendon cells (Bauge et al. 2015; Wynn and Vannella 2016) assisting the crosstalk between cells and the ECM synthesis and arrangement contributing for the reparative versus degenerative process that drives tendon remodeling (Dakin et al. 2014; Dean et al. 2017).

Inflammation is the physiological response to injuries and is part of tendon healing process. If the injury is not resolved, the response becomes chronic and pathologic. The magnitude and duration of the inflammatory response is adjusted by regulatory mechanisms at the injury site (Andia et al. 2010; Prisk and Huard 2003).

Persistent inflammation disrupts the balance between MMPs and TIMPs contributing to scared tendon healing and chronic matrix degradation (D'Addona et al. 2017; Tarafder et al. 2017). Scared tissue results in poor rearrangement of collagen fibrils and separation of collagen bundles. The rupture on collagen fibers may be resulted in calcifications (Zabrzyński et al. 2016). Thus, modulation of the inflammatory response is necessary for recovery of tendon function (Shen et al. 2016).

Conservative treatments for tendon healing fight inflammation with anti-inflammatory drugs for tissue recovery in an attempt to diminish an

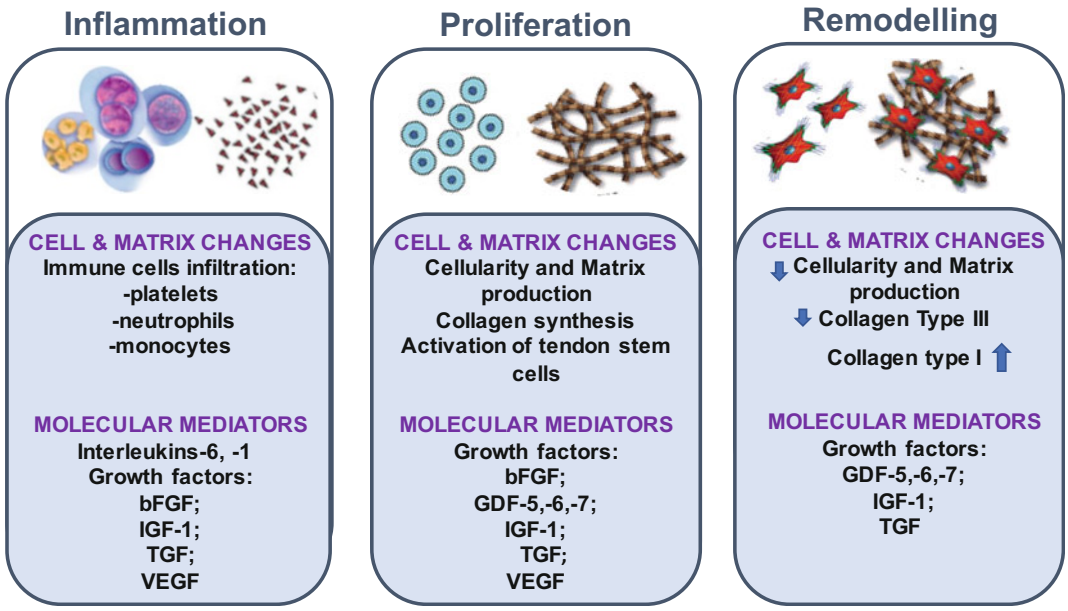

Fig. 1 Representation of the main phases of tendon repair. Inflammation, proliferation and remodeling phases and molecular, cellular and matrix changes during these phases

abnormal or prolonged inflammation often associated to pathophysiology conditions. However, interrupting inflammation overruns important beneficial effects that are required for proper healing to occur.

The role of inflammation in tendinopathy is a subject of debate. Although several studies point a relation between inflammation and tendinopathy (D'Addona et al. 2017; Dakin et al. 2014; Dean et al. 2017; Rees et al. 2014), the onset and development of tendinopathy are poorly understood. Growing evidence suggests that inflammation may not be the cause of several tendinopathies but the failure to resolve inflammation will likely contribute to a complex environment of inflammatory mechanisms (stromal, immune-sensing and infiltrating compartments such as immune cells) (Millar et al. 2017) affecting tendon homeostasis and exacerbating symptoms and tissue degeneration.

Tendinopathies are associated to changes in cellularity and in the remodeling activity of tendon ECM resulting in significant structural and biomechanical alterations of the host niche (Lui and Chan 2011; Tempfer and Traweger 2015). Histological examination of tendinopathy tissues showed collagenolytic injuries and an active healing process, focal hypervascularity and metaplasia. Moreover, the collagen fibers show unequal and irregular crimping, loss the transverse bands, separations and rupturing of the fibers with an increase of type III collagen. The type III collagen is deficient in the number of cross-links between and within the tropo-collagen units (Abate et al. 2009; D'Addona et al. 2017; Zabrzyński et al. 2016).

Inflammatory mediators including, IL-1, IL-6 and COX-2 were reported to be increased in Achilles tendinopathy (Legerlotz et al. 2012). In a degenerative tendon model, the expression of IL-6, IL-11, IL-15 and TNF-α was up-regulated and accompanied by increased expression of MMP-13 and IL-1β (Dakin et al. 2014; Legerlotz et al. 2012). MMP-13 levels were also increased in human cuff tendon injuries. MMP-13 together with MMP-1 and MMP-8 participate in the cleavage of type I collagen present in tendons. The excessive collagen degradation during turnover results in chronic injuries (Buono et al. 2013).

2.1 Role of Cytokines in Tendon Healing

Cytokines are small proteins with the ability to influence and regulate biological activities (Dakin et al. 2014; Evans 1999) of cells that contribute to the healing response (Barr and Barbe 2004; Lin et al. 2006; Muller et al. 2015). Cytokines constitute the major mediators of inflammatory response with a relevant role in cell signaling and communication, holding potent immunomodulatory properties. An endogenous expression of inflammatory cytokines, namely TNF-α, IL-1β, IL-6, IL-4 and IL-10 has been demonstrated in human injured and healthy tenocytes (Ackermann et al. 2013; Mobasheri and Shakibaei 2013). Additionally, some of these cytokines may also be involved in the (self)regulation of tenocyte processes as IL-6 stimulation was reported to increase the proliferation capacity of tenocytes and inhibition expression of tendon cell markers (Thomopoulos et al. 2015). Cytokine expression is also affected by external stimuli as mechanical stimuli/exercise, which impacts the cytokine profile (including IL-1β and TNFα) during the tendon healing process (Morita et al. 2017).

The biochemical profile within a tissue niche during healing and other physiological events is of ultimate importance as it can be indicative of homeostatic, inflammatory or pathological conditions. Thus, the biochemical relevance of soluble factors as cytokines in tendon niches anticipates their application as potential diagnosis and therapeutic tools for repair and regeneration strategies (Table 1).

3 Tendon Derived-Stem Cells (TDSCs) to Modulate Inflammation and Improve Tendon Healing

The interplay of mesenchymal stem/stromal cell (MSCs) with the tendon niche is essential for the modulation of the inflammatory response following injury (Lui and Chan 2011; Zhou et al. 2010) and is strongly dependent on a balance of soluble factors, cell-cell communication and cell-matrix interactions. During inflammation, MSCs interact with resident cells to promote cell migration and proliferation, which could allow a faster re-colonization of the defect, and matrix synthesis (Proffen et al. 2013).

The crosstalk between inflammation cues and stem cells is important to elucidate the mechanisms of how stem cells respond to tissue damage avoiding scar formation and tuning cell-based mechanisms for regenerative approaches.

Previous studies with non-tendon mesenchymal stem/stromal cell suggested that a MSCs treatment could attenuate scar formation and compromised function by improving tissue strength after ligament and tendon injuries. This was due to a paracrine-mediated immunosuppressive effect, through which MSCs modulate macrophage phenotypes (Proffen et al. 2013; Thomopoulos et al. 2015). It is expected that local stem cell populations within different tissues may also exert a similar effect. Thus, tendon stem/progenitor cells hold potential to contribute to the resolution of inflammation and pathophysiology of tendinopathies modulating biological responses at the injury site (Fig. 2).

A local tendon stem cell population could be beneficial over other stem cell sources due to their inherent pro-tenogenic abilities, which are likely more prone to produce tendon components under the influence of tendon environments (Dakin et al. 2014; Snedeker and Foolen 2017).

In 2007, a population of tendon stem/progenitor cells was firstly identified in tendons from mouse and humans, by Bi et al. (Bi et al. 2007). Tendon-derived stem cells (TDSCs) present universal stem cell characteristics such as the ability to self-renewal, clonogenicity and multi-lineage differentiation capacity (Bi et al. 2007). TDSCs were reported to *in vitro* differentiate into tenocytes, chondrocytes, osteocytes and adipocytes and to originate tendon, cartilage, bone and tendon-to-bone tissues in several animal models such as, nude mouse and rat, rabbit patellar and Achilles tendon (Lui 2013; Zhang and Wang 2010).

Table 1 Cytokines in homeostasis, inflammation and pathogenesis of tendon tissues

Cytokine	Function in homeostasis	Function in inflammation	Pathogenesis	References
IL-1β	Regulation of temperature, sleep and feeding	Stimulation of MMPs production	Acute and chronic inflammatory disorders	Ren and Torres (2009)
		Synthesis of pro-inflammatory cytokines (e.g. TNF-α, IL-6 and IL-8)		
	Modulation of cellular metabolism			
TNFα		Favors ECM degradation (MMPs)	Participates in degeneration of tendon	Dohnert et al. (2015)
		Induction inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-10)		
		Suppression of collagen type I		
		Increases the elasticity (reduction of ECM stiffness)		
IL-6	Maintain metabolic homeostasis	Increases collagen synthesis	Induces acute-phase responses	Ackermann et al. (2013)
		Amplifies the inflammatory response		
		Induction of VEGF and IL-10		
IL-4	Associated to ECM homeostasis in may disease models	Modulates and suppresses pro-inflammatory cytokines		Courneya et al. (2010), John et al. (2010), Schulze-Tanzil et al. (2011), Wojdasiewicz et al. (2014)
		Decreases the synthesis of inflammatory cytokines (TNF-α, IL-1β, IL-6) and inhibits the secretion of MMPs		
		Reduces tendon strength		
IL-10		Inhibition of the synthesis of inflammatory cytokines (TNF-α and IL-2) and MMPs		Ackermann et al. (2013)
		Synthesis of collagen type II and aggrecan		
		Induces proliferation and survival of tenocytes		
IL-17A		Promote tissue destruction and degeneration	Early inflammatory response in human tendinopathy	Millar et al. (2015)
		Induces the production of cytokines (IL-1, IL-6, TNF-α), MMPs and NO synthase in tenocytes	Mediates inflammation and tissue remodeling in human tenocytes	

NO nitric oxide, *ECM* extracellular matrix, *MMPs* matrix metalloproteinases

TDSCs have also shown evidence as cell source for tendon repair (Lui et al. 2016; Mienaltowski et al. 2014; Tarafder et al. 2017) (Table 2). TDSCs cultured in fibrin glue constructs were shown to promote earlier and improved tissue repair assessed by increased collagen production and fiber alignment in a patellar tendon window defect model (Ni et al. 2013). The TDSCs seeded in knitted silk-collagen sponge scaffolds also demonstrated ability to promoting regeneration of rotator cuff in rabbit model by inducing tenogenic differentiation and secretion of anti-inflammatory cytokines that prevented immunological rejection. (Shen et al. 2012).

The resident stem cell populations present in different regions of the tendon can be subject to

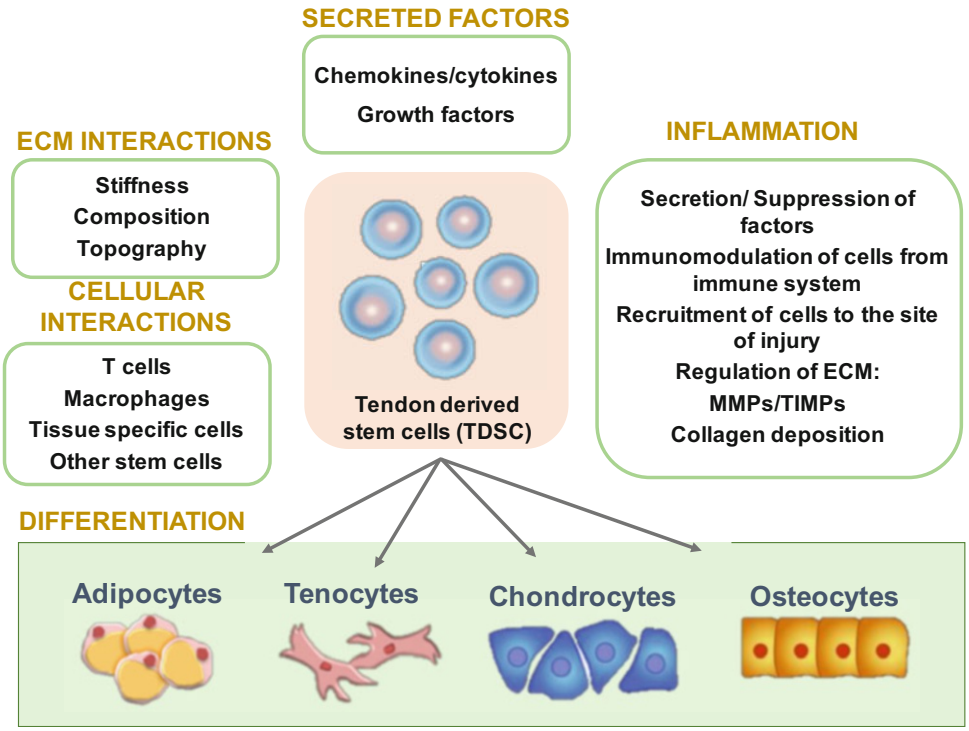

Fig. 2 Schematic representation of the TDSCs in tendon niches as modulators of tissue repair and regeneration

different biochemical stimuli and contribute in distinctive ways for the reparative response to injury, and thus play different yet interactive roles in inflammation and healing.

A study by Mienaltowski *et al.* compared the properties of proper-(TPs) and peritenon-(TPes) derived progenitor cells from embryonic Achilles tendon in an *in vitro* regenerative tendon construct model. The anatomical origin of TSCs (TPs or TPes) contributed differently for tendon-like tissue formation and the secretome of TPes bolster the expression of tenogenic differentiation markers and matrix assembly genes in TPs and tenocytes. These findings highlight an additional potential role of TPes in tendon repair besides the synthesis of provisional matrix (Mienaltowski et al. 2014).

TDSCs also participate in the regulation of inflammation during healing of acute tendon injuries (Tarafder et al. 2017). Connective tissue growth factor (CTGF) enriched CD146+ TDSCs were shown to reduce pro-inflammatory M1 cells in the early healing phase and express anti-inflammatory IL-10 and TIMP-3 vis JNK/signal transducer and activator of transcription 3 (STAT3) signalling (Tarafder et al. 2017).

The immunomodulatory action and trophic signaling on cytokine modulation are TDSCs parameters to be taken in consideration as they are proposed to impact cellular immunity and immune associated processes, controlling cell responses and holding great promise for a variety of pathologies where inflammation and failed healing could be problematic. Therefore, TDSCs therapy is promising for regenerative medicine approaches aiming to repair tendon injuries to tissues pre-injury functional stage.

4 Conclusions and Future Directions

Despite the insights from recent years on the cellular and molecular cues involved in tendon healing, the knowledge on biological mechanisms to recapitulate tendon regeneration remains at the infancy.

Table 2 The role of tendon derived stem cell populations in tendon healing and repair

TDSCs in tendon healing and repair		Model	References
TPes	Reinforces tendon differentiation genes synthesis of matrix	*In vitro*	Mienaltowski et al. (2014)
CTGF enriched CD146^{+} TDSCs	Induced anti-inflammatory factors: IL-10 and TIMP-3 expression Reduced pro-inflammatory M1 in the early healing phase	Full-transected patellar tendons (rat)	Tarafder et al. (2017)
TDSCs in fibrin glue	Increased collagen production and fiber alignment Earlier and improved tissue repair	Patellar window defect (mouse)	Ni et al. (2013)
Allogenic TSC in silk–collagen scaffold	Reduced number of lymphocytes	Rotator cuff (rabbit)	Shen et al. (2012)

TPes peritenon-(TPes) derived progenitor cells from embryonic tendon, *CTGF* connective tissue growth factor, *TDSCs* tendon-derived stem cells

Findings on tendon (stem cell) biology will likely contribute for better understanding of tendon homeostasis and proper healing. Inflammation as a necessary step for healing to occur should not be blocked but modulated and controlled. New studies are required to insight on the role of the mediators involved in unresolved and chronic inflammation to unveil new homeostatic or pathological markers and assist diagnosis tools for the treatment of tendon conditions. Ultimately, the knowledge gathered would enable the control of tendon healing response to injury toward a complete restoration of functional biomechanical cues.

Acknowledgements The authors acknowledge the financial support from Fundação para a Ciência e Tecnologia (FCT) for the doctoral grant PD/BD/128089/2016, the project NORTE-01-0145-FEDER-000021 supported by Norte Portugal Regional Operational Programme (NORTE 2020) and HORIZON 2020 under the TEAMING GRANT agreement No 739572 – The Discoveries CTR.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 47–57
https://doi.org/10.1007/5584_2018_219

Published online: 18 May 2018

Therapeutic Potential of Mesenchymal Stem Cell-Derived Exosomes in the Treatment of Eye Diseases

C. Randall Harrell, Bojana Simovic Markovic, Crissy Fellabaum, Aleksandar Arsenijevic, Valentin Djonov, Nebojsa Arsenijevic, and Vladislav Volarevic

Abstract

Mesenchymal stem cells (MSCs) were, due to their immunomodulatory and pro-angiogenic characteristics, extensively explored as new therapeutic agents in cell-based therapy of uveitis, glaucoma, retinal and ocular surface diseases.

Since it was recently revealed that exosomes play an important role in biological functions of MSCs, herewith we summarized current knowledge about the morphology, structure, phenotype and functional characteristics of MSC-derived exosomes emphasizing their therapeutic potential in the treatment of eye diseases.

MSC-derived exosomes were as efficient as transplanted MSCs in limiting the extent of eye injury and inflammation. Immediately after intravitreal injection, MSC-derived exosomes, due to nano-dimension, diffused rapidly throughout the retina and significantly attenuated retinal damage and inflammation. MSC-derived exosomes successfully delivered trophic and immunomodulatory factors to the inner retina and efficiently promoted survival and neuritogenesis of injured retinal ganglion cells. MSC-derived exosomes efficiently suppressed migration of inflammatory cells, attenuated detrimental Th1 and Th17 cell-driven immune response and ameliorated experimental autoimmune uveitis. MSC-derived exosomes were able to fuse with the lysosomes within corneal cells, enabling delivering of MSC-derived active β-glucuronidase and consequent catabolism of accumulated glycosaminoglycans,

C. R. Harrell and C. Fellabaum
Regenerative Processing Plant-RPP, LLC, Palm Harbor, FL, USA
e-mail: dr.harrell@regenerativeplant.org; crissy@regenerativeplant.org

B. Simovic Markovic, A. Arsenijevic, N. Arsenijevic, and V. Volarevic (✉)
Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, University of Kragujevac, Kragujevac, Serbia
e-mail: bojana.simovic@gmail.com; aleksandar@medf.kg.ac.rs; arne@medf.kg.ac.rs; drvolarevic@yahoo.com

V. Djonov
Institute of Anatomy, University of Bern, Bern, Switzerland
e-mail: valentin.djonov@ana.unibe.ch

indicating their therapeutic potential in the treatment of Mucopolysaccharidosis VII (Sly Syndrome). Importantly, beneficent effects were noticed only in animals that received MSC-derived exosomes and were not seen after therapy with fibroblasts-derived exosomes confirming specific therapeutic potential of MSCs and their products in the treatment of eye diseases.

In conclusion, MSC-derived exosomes represent potentially new therapeutic agents in the therapy of degenerative and inflammatory ocular diseases.

Keywords
Mesenchymal stem cells · Exosomes · Eye · Injury · Inflammation

1 Introduction

Due to their capacity to produce trophic and immunosuppressive factors, mesenchymal stem cells [MSCs] were extensively explored as new therapeutic agents in cell-based therapy of uveitis, glaucoma, retinal and ocular surface diseases (Joe and Gregory-Evans 2010). Although obtained results were promising, safety issues regarding MSCs-based transplantation are still a matter of debate, especially in the long-term follow up. The primary concern is unwanted differentiation of the transplanted MSCs induced by cellular and cytokine milieu of local microenvironment in which MSCs were engrafted (Volarevic et al. 2018). It was recently reported that three women suffering from macular degeneration developed complications including vision loss, detached retinas, and bleeding resulting in total blindness in stem cell-treated eyes as a consequence of unwanted differentiation of transplanted stem cells (Kuriyan et al. 2017).

As far as we know to date, beneficial effects of MSCs in cell-based therapy of degenerative and autoimmune disease are largely due to the activity of MSC-derived trophic and immunosuppressive soluble factors (Volarevic et al. 2017). Plenty of evidence indicate that MSCs conditioned medium (MSC-CM) attenuate progression of immune mediated diseases and promote regeneration of ischemic tissues in almost completely the same way as transplanted MSCs indicating that paracrine mechanisms are mainly responsible for MSC-based therapeutic effects and that therapeutic use of MSC-derived products may overcome safety concerns regarding unwanted differentiation of transplanted MSCs (Volarevic et al. 2017, 2018).

Since it was recently revealed that exosomes play an important role in biological functions of MSCs and MSC-CM (Yu et al. 2014; Rani et al. 2015; Lai et al. 2015), herewith we summarized current knowledge about the morphology, structure, phenotype and functional characteristics of MSC-derived exosomes emphasizing their therapeutic potential in the treatment of eye diseases. An extensive literature review was carried out in April 2018 across several databases (MEDLINE, EMBASE, Google Scholar), from 1990 to present. Keywords used in the selection were: "mesenchymal stem cells", "exosomes", "eye", "degenerative diseases", "inflammatory diseases", "regeneration", "immunosuppression". Studies that emphasized molecular and cellular mechanisms responsible for beneficent effects of MSC-derived exosomes in the therapy of eye diseases were analyzed in this review.

2 MSC Derived Exosomes: Morphology and Structure

MSC-derived exosomes are nano-sized extracellular vesicles that originate via the inward budding of the late endosome membranes called multivesicular bodies (MVBs). Upon the fusion of MVBs with the plasma membrane, MSC-derived exosomes are released into the extracellular milieu and can be either taken up by target cells residing in the microenvironment of engrafted MSCs or may be carried to distant sites via biological fluids where, in endocrine manner, modulate function of immune cells, endothelial cells (ECs), pericytes and other tissue-resident cells (Hyenne et al. 2015)

MSC derived exosomes have a narrow diameter range of 40–100 nm and a density of 1.13–1.19 gml^{-1} in sucrose solution. Their membranes are enriched in cholesterol

sphingomyelin ceramide and lipid raft proteins (de Gassart et al. 2003) which enable protection of exosome's cargo from degradative enzymes or chemicals and facilitates uptake of exosome's content into target cells through endocytosis or membrane fusion regardless of biological barriers (Lai et al. 2011). MSC-derived exosomes express evolutionary conserved set of proteins including tetraspanins (CD81 CD63 CD9-involved in MSC proliferation and signaling) heat-shock proteins (HSP60 HSP70 HSP90-involved in MSC respond to stress) ALG-2-interacting protein X (Alix-apoptosis regulating protein) tumor susceptibility gene 101 (TSG101-having role in cell growth and proliferation) and adhesion molecules (CD29 CD44,CD73- enabling migration of exosomes to the inflamed and injured tissues) (Fig. 1). The incorporation of all these proteins in exosomes is thought to be controlled by lipid-dependent mechanisms primarily by the activity of the endosomal sorting complex required for transport (ESCRT) (Colombo et al. 2014).

Exosome content may vary according to the physiological and pathological conditions of the tissue microenvironment in which engrafted MSC is exposed. In this regard, the exosomal cargo can reveal the state of the donor MSC and can also influence in a paracrine and/or endocrine manner the fate of the recipient cell (Schey et al. 2015; Villarroya-Beltri et al. 2014). The reliability of intercellular communication between MSC and target cell is maintained and translated by specific components within the MSC-derived exosomes. These components are generally made of

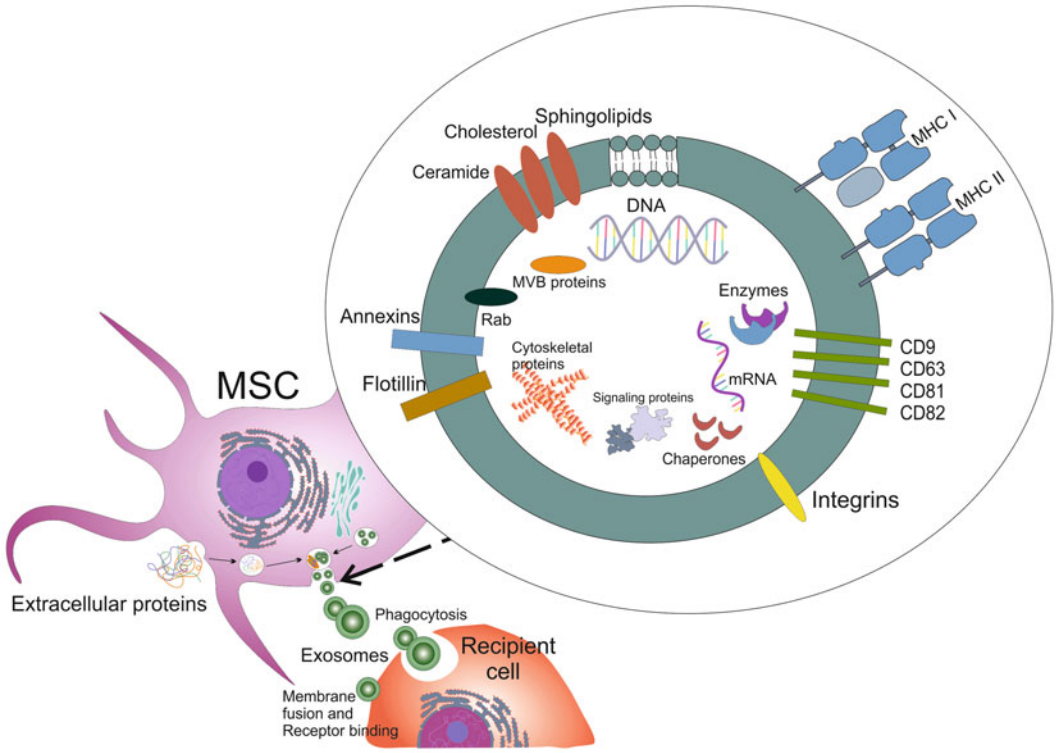

Fig. 1 MSC-derived exosomes: morphology and structure. MSC-derived exosomes are nano-sized extracellular vesicles which are released from MSCs into the extracellular milieu and taken up by target cells. Their membranes are enriched in cholesterol, sphingomyelin, ceramide and lipid raft proteins which enable protection of exosome's cargo from degradative enzymes or chemicals and facilitates uptake of exosome's content into target cells through endocytosis or membrane fusion, regardless of biological barriers. MSC-derived exosomes express evolutionary conserved set of proteins, including tetraspanins (CD81, CD63, CD9), heat-shock proteins (HSP60, HSP70, HSP90), and adhesion molecules (CD29, CD44,CD73) and carry nucleic acids, proteins (cytokines, chemokines) and lipids having important role in immunomodulation and tissue regeneration

proteins, lipids, DNA fragments, mRNA and small RNA species (Villarroya-Beltri et al. 2014; Vlassov et al. 2012). The cargo is not randomly distributed into exosomes: strictly regulated mechanisms determine the "information" that will be distributed from MSC to the recipient cell by exosome (Villarroya-Beltri et al. 2014).

MSC derived exosomes carry nucleic acids proteins (cytokines chemokines) and lipids. In cargo of MSC-derived exosomes 4850 unique gene products and 4150 miRNAs have been detected and identified by mass spectrometry antibody array and microarray analysis (Lai et al. 2012; Chen et al. 2010). Furthermore, proteasome subunits were observed in MSC-derived exosomes (Carayon et al. 2011). It has been revealed that the 20S proteasome is responsible for degradation of intracellular oxidative damaged proteins which may partly contribute to the cardioprotective activity of MSC-derived exosomes (Lai et al. 2012). Through the activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathway MSC-derived exosomes increased levels of adenosine triphosphate (ATP) reduced oxidative stress attenuated myocardial ischemic injury and promoted myocardial viability and cardiac function (Lai et al. 2010; Li et al. 2013), indicating their potential therapeutic use in the treatment of myocardial ischemia (Arslan et al. 2013)

The presence of nucleic acids inside the exosomes had the crucial role in altering the fate of recipient cells. Within the nucleic acids spectrum, miRNA sequences become the most intensively investigated (Zaharie et al. 2015; Berindan-Neagoe and Calin 2014). Several miRNAs, detected in MSC-derived exosomes, including miR-191, miR-222, miR-21, miR-222, and miR-6087 were responsible for increased differentiation of ECs enabling modulation of angiogenesis (Merino-González et al. 2016). Similarly, through the activity of miR-494, MSC-derived exosomes accelerate muscle regeneration and promote myogenesis and angiogenesis (Nakamura et al. 2015).

MSC based modulation of vascular endothelial growth factor (VEGF)-driven angiogenesis is mediated by miR contained within MSC-derived exosomes (Merino-González et al. 2016; Nakamura et al. 2015). Lee and coworkers (Lee et al. 2013) revealed that MSC-derived exosomes enriched with miR-16 suppress tumor progression and angiogenesis via down-regulation of the VEGF expression in tumor cells. Opposite results were reported by Zhu and colleagues (Zhu et al. 2012) who showed that *in vivo* application of MSC-derived exosomes activated extracellular signal-regulated kinase1/2 (ERK1/2) pathway in tumor cells that resulted with the enhanced expression of VEGF, increased neo-angiogenesis and accelerated tumor growth.

Intravenous transplantation of MSC-derived exosomes improves neurogenesis, neurite remodeling and angiogenesis after ischemic brain injury (Xin et al. 2013). Therapy based on the delivery of MSC-derived exosomes promoted axonal growth and significantly increased the number of neuroblasts and ECs in ischemic and injured regions of central nervous system (CNS) (Xin et al. 2013). MSCs communicate with brain parenchymal cells and regulate neurites outgrowth by transferring miR-133b in neurons and astrocytes via exosomes (Xin et al. 2012) which could be a promising therapeutic strategy in the treatment of spinal cord injury.

3 Modulation of Immune Response and Inflammation in the Eye by MSC-Derived Exosomes

MSCs have capacity to synthesize and secrete a broad spectrum of exosomes, significantly more than other exosome producing cells of mesodermal origin (Yeo et al. 2013). MSC-derived exosomes are involved in important physiological and pathological processes such as disposal of unwanted proteins, genetic exchange, modulation of immune response and inflammation (Théry et al. 2009; Zöller 2009).

Immediately after engraftment, MSCs through the release of exosomes interact with multiple cell types to elicit appropriate cellular responses: affect the support of stromal cells enabling maintenance of dynamic and homeostatic tissue microenvironment (Lai et al. 2015) and modulate

immune response by delivering immunosuppressive factors to the effector immune cells (Lai et al. 2010; Li et al. 2013; van Koppen et al. 2012; Zhang et al. 2014; Kordelas et al. 2014).

It was recently revealed that exosomes derived from amniotic fluid derived MSCs (AF-MSCs) contain immunosuppressive factors TGF-β and HGF. TGF-β suppresses activation of Jak-Stat signaling pathway in T cells, causing the G1 cell cycle arrest (Volarevic et al. 2017; Bright et al. 1997) while HGF acts synergistically with TGF-β enabling suppression of T cell proliferation and attenuation of T cell-mediated inflammation (Volarevic et al. 2017; Di Nicola et al. 2002). In line with these findings, when phytohemagglutinin-stimulated peripheral blood mononuclear cells [PB-MNCs] were cultured in the presence of TGF-β and HGF-containing AF-MSCs-derived exosomes, proliferation of PB-MNCs was notably reduced and their apoptosis was significantly enhanced (Balbi et al. 2017). Similarly, maturation and proliferation of B cells was reduced and their capacity for production of antibodies was suppressed, indicating strong immunosuppressive potential of AF-MSCs-derived exosomes (Balbi et al. 2017). Interestingly, among PB-MNCs, AF-MSC-derived exosomes did not attenuate proliferation of immunosuppressive CD4 + CD25 + FoxP3+ T regulatory cells, confirming significance of AF-MSC-derived exosomes as potentially new therapeutic agents in the therapy of inflammatory diseases.

In line with these findings, we recently developed immunomodulatory ophthalmic solution ("Derived Multiple Allogeneic Proteins Paracrine Signaling "D-MAPPS") the activity of which is based on the activity of AF-MSC-derived exosomes, cytokines and growth factors capable to attenuate inflammation in the eye: IL-1 receptor antagonist [IL-1Ra], indoleamine 2,3-dioxygenase (IDO) and growth related oncogene (GRO). Based on our preliminary results, this product had beneficent effects in treatment of corneal injuries and dry eye syndrome (DED).

Corneal injuries are usually complicated with the influx of immune cells and consequently developed inflammation (Dana et al. 2000). During the early stage of corneal damage, injured epithelial cells secrete the inflammatory cytokine IL-1, which is stored in epithelial cells and released when the cell membrane is damaged by external insults (Yamada et al. 2003). IL-1Ra has an important anti-inflammatory role in corneal protection and regeneration. When IL-1Ra binds to the IL-1 receptor (IL-1R), interaction between inflammatory IL-1 and IL-1R is prevented. Accordingly, various pro-inflammatory events, initiated by IL-1:IL-1R interaction, including the synthesis and release of chemokines, and enhanced influx of neutrophils, macrophages, and lymphocytes in injured corneas are inhibited (Balbi et al. 2017). In line with these observations, our preliminary findings suggest that IL-1Ra containing AF-MSC-derived ophthalmic solution significantly attenuated inflammation in patients suffering from corneal injury.

Similarly, as for progression of corneal injury, inflammation has crucial role in the pathogenesis of DED, multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability (Gayton 2009). It is well known that Th17 cell-driven inflammation plays important role in the pathogenesis of DED (Théry et al. 2009). Inflammatory dendritic cells (DCs), in IL-1, IL-6, and IL-23 dependent manner induce differentiation of naïve T cells into Th17 cells which reduce tear production and promote progression of DED in IL-17 dependent manner (Gayton 2009; De Paiva et al. 2009). AF-MSCs, through the production of immunomodulatory GRO, attenuate maturation and antigen-presenting function of inflammatory *DCs and suppress Th17 immune response. At the same time,* AF-MSC-derived GRO promote generation of regulatory DCs capable to produce high levels of anti-inflammatory IL-10 (Merino-González et al. 2016; Nakamura et al. 2015; Yi and Song 2012) creating immunosuppressive microenvironment. Similarly, MSC-derived IDO acts as a critical molecular switch that simultaneously blocks re-programming of Tregs into inflammatory, IL-17 producing effector Th17 cells having important role in Treg-based immunosuppression of Th17 driven inflammation (Volarevic et al.

2017). In line with these observations, our preliminary results showed that AF-MSC-derived ophthalmic solution, which contains a high concentration of immunosuppressive GRO and IDO, significantly attenuated dryness, grittiness, scratchiness, soreness, irritation, burning, watering, and eye fatigue in patients suffering from DED, indicating therapeutic potential of AF-MSC-derived secretomes in the treatment of DED.

4 MSC Derived Exosomes in the Therapy of Retinal Injury

Damage of retinal cells caused by injury, infection or ischemia triggers degeneration in neighboring neural cells, resulting with the spread of morphological and functional retinal damage and irreversible visual impairment (Yoles and Schwartz 1998). Till now, there is no effective neuroprotection therapy currently available for retinal injury and, accordingly, transplantation of stem cells and their products have been extensively tested as new therapeutic approach for retinal regeneration. By using animal model of laser-induced retinal injury, Yu and coworkers recently demonstrated therapeutic potential of MSC-derived exosomes in attenuation of retinal damage and inflammation (Fig. 2) (Yu et al. 2016). One hour after their intravitreal injection, MSC-derived exosomes diffused rapidly throughout the neural retina, retinal pigment epithelium and gradually spread to the outer layers. Importantly, MSC-derived exosomes were as efficient as transplanted MSCs in limiting the extent of retinal damage. MSC-exosome-treated and MSC-treated eyes showed equivalent attenuation of laser-induced retinal injury with milder disorganization of the tissue, more residual photoreceptor cells, smaller retinal disordered areas, and reduced loss of nuclei in the outer nuclear layers compared with the eyes that were treated with vehicle only. Furthermore, application of MSC-derived exosomes significantly reduced infiltration of immune cells, particularly CD68+ macrophages and attenuate consequent apoptosis of retinal cells when compared to vehicle-treated group. MSC-derived exosomes managed to significantly alleviate expression of inflammatory mediators in the injured retinas involved in migration of monocytes in the eye: cytokine (TNF-α), chemokine (monocyte chemoattractant protein-1, MCP-1) and adhesion molecule (intercellular Adhesion Molecule 1, ICAM-1). Application of MCP-1 abolished effects of MSC-derived exosomes suggesting that they reduce retinal injury and inflammation mainly by targeting MCP-1-dependent migration of monocytes (Yu et al. 2016). In accordance to the attenuated retinal injury and inflammation, the significant improvement of dark- and light-adapted electroretinogram response in laser-injured mice treated with MSC-derived exosomes was observed, indicating functional recovery of retinal cells (Yu et al. 2016).

In line with result obtained by Yu and colleagues (Yu et al. 2016) are findings recently reported by Mead and Tomarev (Mead and Tomarev 2017) who demonstrated therapeutic potential of bone marrow MSCs [BM-MSCs]-derived exosomes in the regeneration of injured retinal ganglion cells [RGCs]. RGCs are the sole projection neurons and their axons make up the optic nerve, making them susceptible to traumatic (optic nerve crush; ONC) and degenerative (glaucoma) diseases. Since RGC are CNS neurons, they are neither replaceable nor capable of axon regeneration and their loss or dysfunction results with irreversible blindness. BM-MSC-derived exosomes efficiently promoted survival and neuritogenesis of RGCs *in vitro* and *in vivo*, in ONC experimental model. In compared to untreated animals where, 3 weeks after ONC, more than 80% of RGCs are lost, cell death of RGCs was reduced to 30% in rats treated with BM-MSC-derived exosomes. Moreover, in BM-MSC exosome-treated retinas, over 50% of RGC function was maintained, suggesting that exosomes managed not only to protect RGC from death but also to preserve their function. Importantly, this was significantly higher

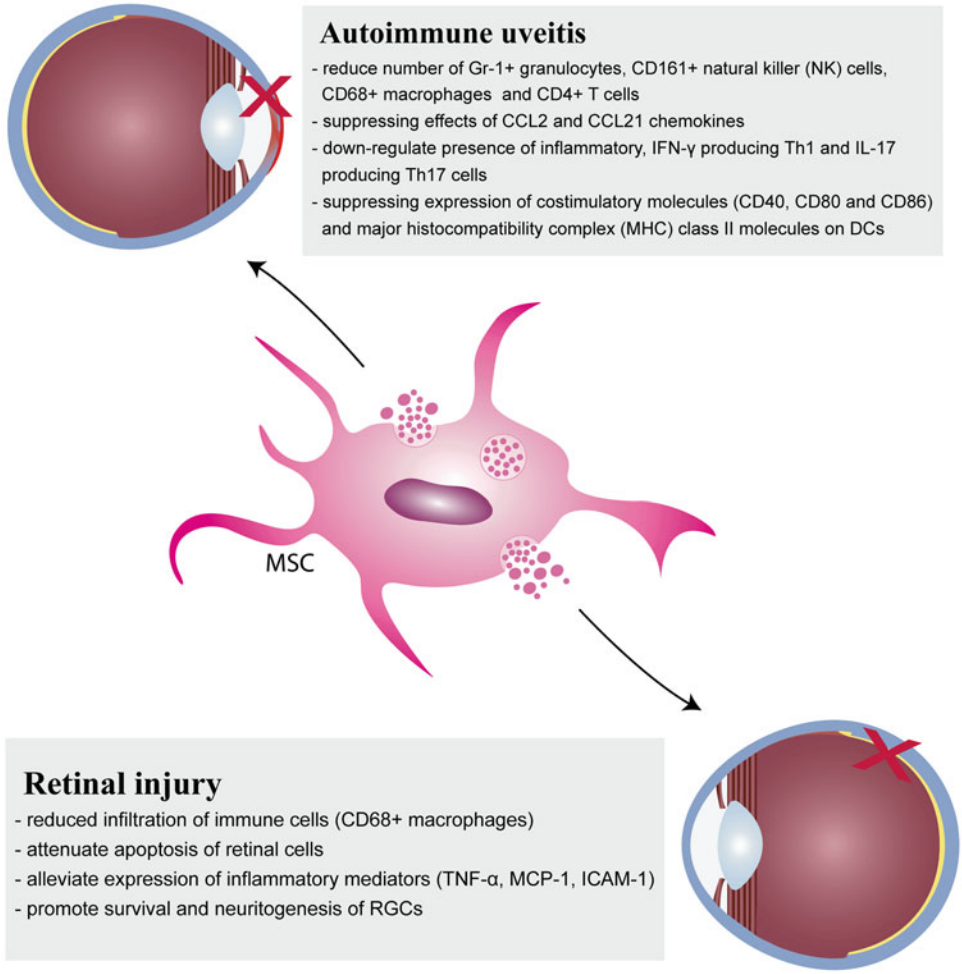

Fig. 2 Mechanisms responsible for therapeutic effects of MSC-derived exosomes in the therapy of autoimmune uveitis and retinal injury. MSC-derived exosomes deliver immunomodulatory enabling suppression of detrimental immune response in autoimmune uveitis. By providing trophic support, MSC-derived exosome attenuate apoptosis of retinal cells and promote survival of retinal ganglion cells (RGCs) enabling regeneration of injured retinal tissue

neuroprotection of RGCs than those observed after transplantation of BM-MSCs. BM-MSCs lack the capacity to integrate into the retina and they remain in the vitreous after application. On contrary, within 1 hour after intavitreal injection, MSC-derived exosomes diffused rapidly and successfully delivered their cargo to the inner retina, including the RGCs where they elicited their therapeutic effects through miRNA dependent mechanisms (particularly through miR-17-92 and miR21 that regulate phosphatase and tensin homolog (PTEN) expression which is an important suppressor of RGC axonal growth and survival and through miR-146a that targets expression of epidermal growth factor receptor (EGFR) involved in inhibition of axon regeneration. Importantly, beneficial effects of MSC-derived exosomes in the treatment of retinal injury and in protection of RGCs were noticed only in animals that received MSC-derived exosomes and were not seen after therapy with fibroblasts-derived exosomes confirming specific

therapeutic potential of MSCs and their products in retinal regeneration (Yu et al. 2016; Mead and Tomarev 2017).

5 Therapeutic Potential of MSC-Derived Exosomes in the Treatment of Autoimmune Uveitis

Autoimmune uveitis represents one of leading causes of visual disability. Since long-term use of immunosuppressive drugs and corticosteroids is limited due to the serious side effects and possible development of glaucoma and cataract, new therapeutic approaches for attenuation of autoimmune reaction in the eye are urgently needed. Most recently, Bai and colleagues demonstrated that MSC-derived exosomes efficiently attenuated experimental autoimmune uveitis (EAU), well established murine model of autoimmune uveitis (Bai et al. 2017), indicating their potential therapeutic use in the treatment of this disease (Fig. 2). Both clinical and histological analysis revealed that periocular injection of MSC-derived exosomes significantly ameliorated EAU, protect retinal structure and rescue retinal function in experimental rats. This was followed with notably reduced number of Gr-1+ granulocytes, CD161+ natural killer (NK) cells, CD68+ macrophages and CD4+ T cells in the inflamed retinas. Application of MSC-derived exosomes inhibited influx of leukocytes in the eye by suppressing effects of CCL2 and CCL21 chemokines which are involved in chemotaxis of inflammatory cells in the injured eyes. Interestingly, MSC-exosomes did not affect proliferation of activated T cells but managed to remarkably down-regulate presence of inflammatory, IFN-γ producing Th1 and IL-17 producing Th17 cells in the retinas, without affecting total number of immunosuppressive CD4 + CD25 + FoxP3+ T regulatory cells. Similar to these results are findings obtained by Shigemoto-Kuroda and colleagues (Shigemoto-Kuroda et al. 2017) who demonstrated that intravenous injection of MSC-derived exosomes, given immediately after immunization, prevented development of EAU in the same way as intravenously transplanted MSCs. Little structural damage of retinas with few inflammatory infiltrates were noticed in the eyes of EAU mice that received MSCs or MSC-derived exosomes while EAU mice that received vehicle showed severe disruption of the retinal photoreceptor layer accompanied with massive infiltration of inflammatory cells. Total number of retinal-infiltrating CD3+ T lymphocytes was significantly reduced both in MSCs and exosome-treated EAU mice when compare to vehicle-treated animals with EAU. In similar manner as it was observed by Bai and colleagues (Bai et al. 2017), MSC-derived exosome efficiently attenuated Th1 and Th17 immune response in the eye, without affecting total cell number, phenotype and function of immunosuppressive T regulatory cells (Shigemoto-Kuroda et al. 2017). The transcript levels of Th1 and Th17 related inflammatory cytokines (IFN-γ, IL-17A, IL-2, IL-1β, IL-6, and IL-12) and total number of eye-infiltrated Th1 and Th17 cells were significantly lower in the eyes of MSCs- and exosome-treated mice when compared with the vehicle-treated controls, while there was no significant difference in total number of T regulatory cells and immunosuppressive IL-10 (Shigemoto-Kuroda et al. 2017). Mixed lymphocyte reaction and flow cytometry analysis of DCs revealed that MSC-derived exosomes attenuated Th1 and Th17 immune response directly, by attenuating proliferation, effector function and cytokine production of CD4+ T lymphocytes and indirectly, by suppressing expression of costimulatory molecules (CD40, CD80 and CD86) and major histocompatibility complex (MHC) class II molecules on DCs, inhibiting their capacity for antigen presentation (Shigemoto-Kuroda et al. 2017). Results obtained by Bai and colleagues (Bai et al. 2017) and Shigemoto-Kuroda and coworkers strongly suggest that MSC-derived exosomes efficiently suppress migration of inflammatory cells in inflamed retinas, attenuate detrimental Th1 and Th17 cell-driven immune response and, accordingly, should be further explored as novel therapeutic agents for the treatment of human autoimmune uveitis, for which local non-corticosteroid therapy is urgently needed.

6 Effects of MSC-Derived Exosomes in the Treatment of Sly Syndrome

Mucopolysaccharidosis (MPS) is a group of seven related disorders caused by a mutation in one of the lysosomal exoglycosidases required for the sequential degradation of glycosaminoglycans (GAGs), resulting with lysosomal storage in several organs, including eyes. MPS VII, also known as Sly syndrome, manifested by corneal clouding, hepatomegaly, skeletal dysplasia, short stature, and delayed development is caused by a mutation of β-glucuronidase which leads to the accumulation of heparin sulfate, dermatan sulfate and chondroitin-4- and -6-sulfate. Current treatment for MPS VII is transplantation of bone marrow-derived stem cells or enzyme substitution therapy, but neither of these two therapeutic approaches are effective for ameliorating the corneal clouding due to corneal avascularity. Therefore, corneal clouding is ultimately treated by corneal transplantation (keratoplasty) which requires general anesthetic which is usually not possible in MPS VII patients suffering from respiratory dysfunction and/or severe cardiomyopathy. Accordingly, new therapeutic approaches are urgently needed for these patients. Most recently, Coulson-Thomas and colleagues proposed MSC-derived exosomes as potentially new agents for the treatment of MPS VII patients (Coulson-Thomas et al. 2013). Results obtained in this study suggest that β-glucuronidase-containing exosomes released from umbilical cord derived MSCs (UC-MSCs) are able to successfully enter into host corneal keratocytes and ECs of MPS VII mice (Coulson-Thomas et al. 2013). Furthermore, UC-MSC-derived exosomes managed to fuse with the lysosomes within recipient cells, enabling delivering of MSC-derived active β-glucuronidase and consequent catabolism of accumulated GAGs in MPS VII mice. These findings strongly support the hypothesis that UC-MSCs-derived exosomes have great potential for being successful in treating MPS VII and other human corneal congenital metabolic diseases.

7 Conclusion

Although transplantation of MSCs has enormous perspective in regenerative medicine, accumulating evidence indicates that treatment using MSC-derived exosomes have multiple advantages over MSC-based therapy. The risks of allogeneic immunological rejection, unwanted differentiation, and obstruction of small vessels by intravenously injected MSCs might be avoided by therapeutic application of MSC-derived exosomes that have similar effects and migration potential as MSCs. Additionally, exosomes, due to their nano-dimension, can easily pass through biological barriers and enter all target organs (Yu et al. 2016).

Nevertheless, there are still some challenges that need to be addressed in order to develop MSC-derived exosomes as an effective therapeutic agent in the treatment of eye diseases. Further studies are needed to optimize the injection frequency and dose to maintain the long-lasting effects of MSC-derived exosomes. Moreover, having in mind that exosomes are highly heterogeneous depending on the tissue origin of MSCs from which they were derived, pre-selection of the most effective tissue source of MSCs-derived exosomes is of crucial importance for their further successful use in ophthalmology. Finally, precise exosome-containing factors responsible for therapeutic effects of MSC-derived exosomes should be defined for each eye disease. In this way, defined therapeutic factor could be overexpressed in MSCs-derived exosomes prior to application in patients maximizing their therapeutic potential and efficacy.

Acknowledgment Study was supported by Swiss National Science Foundation project (SCOPES IZ73Z0_152454/1), Novartis foundation for medical-biological research (Grant No.16C197), Serbian Ministry of Science (ON175069, ON175103), and Faculty of Medical Sciences University of Kragujevac (JP02/09).

Conflict of Interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 59–72
DOI 10.1007/5584_2018_177

Published online: 8 March 2018

Transplantation and Alternatives to Treat Autoimmune Diseases

Pravin Shende, Bernice Rodrigues, and R.S. Gaud

Abstract

Transplantation is considered as one of the methods for the treatment of autoimmune diseases. There are different sorts of transplantation which improved the situation for the cure of different kinds of autoimmune diseases. Cord blood transplantation is favored over other transplant techniques. The propelled treatments incorporate interferon administrative elements and mesenchymal stromal cells for the management of immune system issue particularly in the treatment of rheumatoid joint inflammation. According to the studies conducted, it was proven that cord blood/UC mesenchymal cells along with DMARDs, without consistent organization expanded the level of administrative regulatory T-cells of the peripheral blood which might be a protected and huge technique for the treatment of patients experiencing rheumatoid joint inflammation. This review article focusses on different organ transplantation and alternative methods to treat autoimmune condition like rheumatoid arthritis. Using 3D printing and artificial intelligence are some of the recent trends that may be used for the management of autoimmune diseases.

P. Shende (✉), B. Rodrigues, and R. S. Gaud
Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM'S NMIMS, Mumbai, India
e-mail: shendepravin94@gmail.com

Keywords

Stem cells · Mesenchymal stromal cells · Rheumatoid arthritis · Transplantation

Abbreviations

DMARDs	Disease-Modifying Antirheumatic Drugs
UC	Umbilical Cord
MSCs	Mesenchymal Stromal Cells
RA	Rheumatoid Arthritis
SCs	Stem Cells
HSCs	Hematopoietic Stem Cells
GVHD	Graft Versus Host Disease
RBCs	Red Blood Cells
IRFs	Interferon Regulatory Factors
HLA	Human Leukocyte Antigen
FLSs	Fibroblast-like synoviocytes
TH1	T helper 1
IL-18BP	Inter-leukin18 Binding Protein
TNFα-	Tumor Necrosis Factor alpha
NF_KB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
cJUN	c-Jun N-terminal kinase
ACPAs	Anticitrullinated protein antibodies
EULAR	European Group Against Ailment
DAS	Disease Activity Scores
UC-MSC	Umbilical Cord-derived MSC
NOTA	National Organ Transplant Act

OPTN	Organ Procurement and Transplantation Network
PSDA	Patient Self-Determination Act
SGB V	Sozialgesetzbuch Fünftes Buch
TPG	German Transplantation Law
ZTCC	Zonal Transplant Coordination Center
ANNs	Artificial Neural Network
ESCs	Embryonic Stem Cells
siRNA	small interfering Ribonucleic Acid
NOC	No Objection Certificate
NGO	Non-Governmental Organization
3D	3 Dimensional

1 Introduction

Transplantation is an act of surgical transfer of healthy organ, tissue or cell from one place to another or from one organism to another. Transplantation is obligatory for patients who have an organ failure or the organs have been damaged due to some accident, injury or disease or no alternative conventional treatment available. In conventional treatment, the disease is controlled to refrain the immune system with immunosuppressant like DMARDs, cytotoxic drugs, azathioprine. These medicines reduce few signs of the illness but cause side effects like weakness, melancholy, prone to infection and may even cause cancer. In autoimmune diseases the response to immunosuppression is observed 60–70% primarily because of either disease progression or non-response to the drug used. The autoimmune diseases, in some cases, may show clinical remission to relapse (Chandrashekara 2012; Kooij et al. 2007). Presently, use of site-targeted drugs explores to reduce the toxicity of immunosuppressant drugs and also offers more robust immunosuppression effect (Feldmann and Steinman 2005). Such targeted immunosuppressive treatments do not escalate the remission rate considerably inclusive of rheumatoid arthritis (Böhm et al. 2006). Due to associated side effects of conventional drugs and treatment options, transplantation is an alternative for the treatment and survival for human being.

The body parts like kidney, heart, lungs, liver, pancreas, intestines, stomach and testis can be transplanted and are shown in Fig. 1. The tissues, cells and fluids that can be transplanted like hand, cornea, face skin and face transplant, an islet of Langerhans, bone marrow or SC, blood cells and blood parts transfusion, blood vessels, heart valve and bone.

1.1 History

During the 500 BCE- 600 CE, the rebirth of the Lord Ganesha by cephalic transplant as depicted in Hindu scriptures (Nanda et al. 2016) is the first example of xenotransplantation on the earth as shown in Fig. 2. In 1743, William Hunter (1718–1783) stated that "ulcerated cartilage is a troublesome disease which when destroyed, it is not recovered from Hippocrates down to the present age" (Hunter et al. 1743). Since onward the efforts were made by orthopedics to develop reliable methods to restore the cartilage (Hunter 1744). Henri Judet first reported the implantation of osteochondral grafts in animals (Judet 1908) but the clinical use of allograft joint transplants was first introduced by Lexer in 1908 (Nikalaou and Giannoudis 2017; Langer and Gross 1974). A patient in 1838 underwent the first corneal xenotransplantation (from a pig), whereas transplantation of the first corneal allograft (human-to-human) was carried out in 1905 (Cooper 2012). Hara and Cooper reviewed the field of corneal xenotransplantation (Hara and Cooper 2010, 2011). By 1925, Lexer had documented 34 hemi or whole knee allogenic implants in humans and reported a 50% success rate (Lexer 1925). By the 1960s, Keith Reemtsma said that kidney transplant other than that of human might function in human recipients and thus be a successful treatment for renal failure. Earlier transplantation of kidney from a deceased person to a patient with a kidney failure was less as the numbers of deaths were less. So he performed a research wherein he

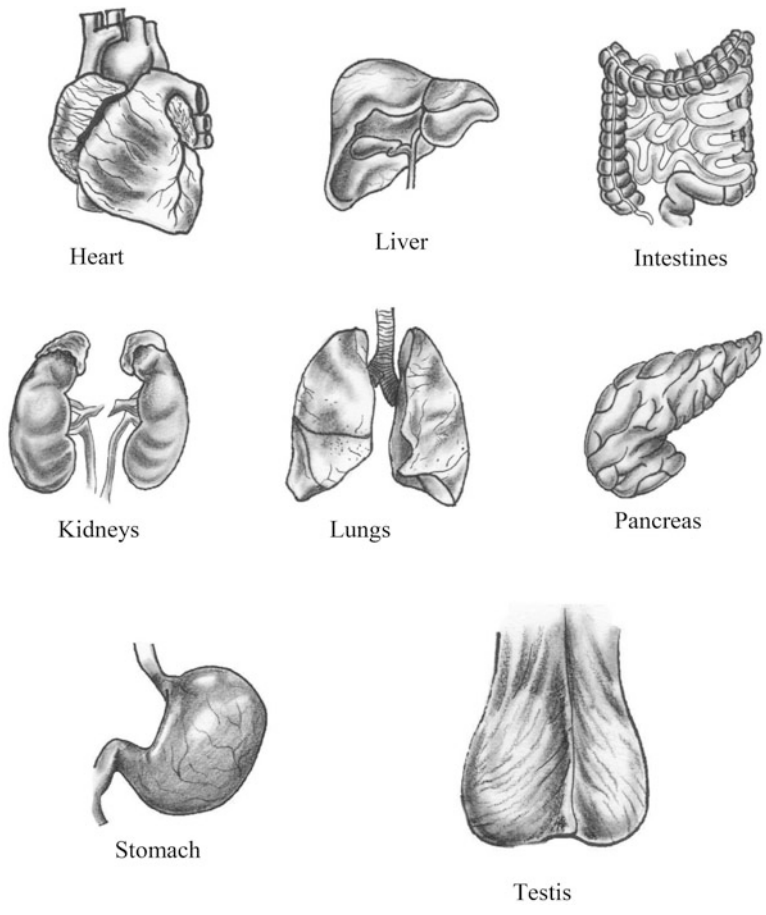

Fig. 1 Major organs used for transplantation

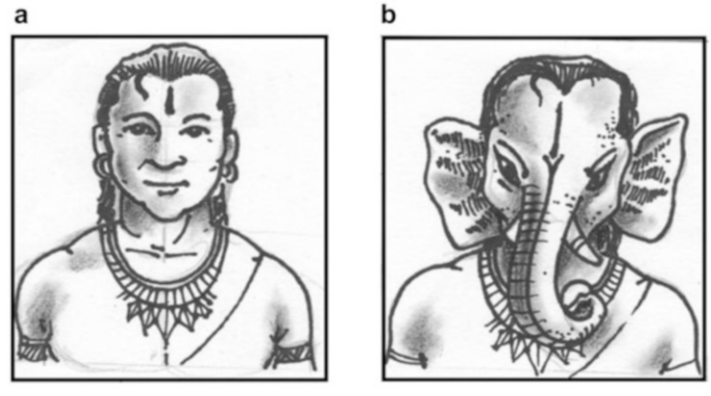

Fig. 2 Picture of Lord Ganesha (**a**) before and (**b**) after transplantation

said he could give life to a patient by providing him with a kidney from a non-human primate. In this case, kidney of a chimpanzee was used to transplant it into the patient (Reemtsma et al. 1964) but most of the patients died within few weeks. Fortunately, one patient survived for few months and then expired. After autopsy, it was found out that no signs of acute or chronic rejection appeared in the chimpanzee kidneys. The patient had suffered from electrolyte imbalance because the transplant was from a non-human primate. In 1967, Barnard and his colleagues established the procedure of cardiac allotransplantation (Barnard 1967), two cardiac xenotransplants were also carried out by them (Barnard et al. 1977). In 1983, Leonard Bailey performed a surgical procedure which involved the transplantation of a baboon heart known as Baby Face into an infant girl. It was technically successful but the patient died in 20 days later as the graft underwent acute rejection (Starzl et al. 1966). It was concluded that the rejection was caused due to the blood type 'O' which is primarily not found in baboon. Tom Starzl, performed liver transplants from a nonhuman primate to a human which was unsuccessful in the 1960s (Starzl 1969; Starzl et al. 1974; Giles et al. 1970). He performed two liver transplants in the 1990s but failed again as the patient lasted for just a few days (Starzl et al. 1993). In 1993, a Swedish group first attempted to transplant a pig islet in patients with diabetes was carried out. The group was headed by Carl Groth (Groth et al. 1994; Alexander BR P OFM 2008). The timeline of successful transplants started from 1905 and is illustrated in Fig. 3.

1.2 Advantages

Societal Duty Everyone has a societal duty to contribute for the betterment of the community. The donation of organs to the patients in need and help them to endure is a noble cause which can serve the society.

Supports the Family of the Deceased For a healthy and a productive life of a person who is in need of an organ transplant.

Imparts a New Hope for Normal Life Organ transplant gives a new hope to the recipients.

Recovers Life In the case of kidney dysfunction, the patients depend on dialysis due to kidney failure for their entire life but when a kidney transplant is done they get a new life.

1.3 Disadvantages

Complications During and After Surgery The complications include severe hemorrhage, blood clot formation or development of infection at the site where the surgical procedure was performed. In some cases the doctor can control such complication but in exceptional cases such complications may be fatal to the donor.

The Overall Health in the Long Term Individual may face some adverse reactions depending on the organ of live donor. For example, hypertension or kidney failure may be caused if a healthy person donates one kidney.

Financial Problems High cost in surgery in transplantation.

Trade of Human Vital Organs The prohibited and immoral trade of organs in illicit business is the most negative impact that has affected the society.

Transplantation is classified on basis of organs or tissues to be transplanted and the anatomical site and is depicted in Fig. 4.

Organ transplantation is a major task due to lack of organ donors and large number of recipients. In order to overcome such problems, various alternatives are used for treating autoimmune diseases without organ transplantation include bone marrow replacement therapy, SC

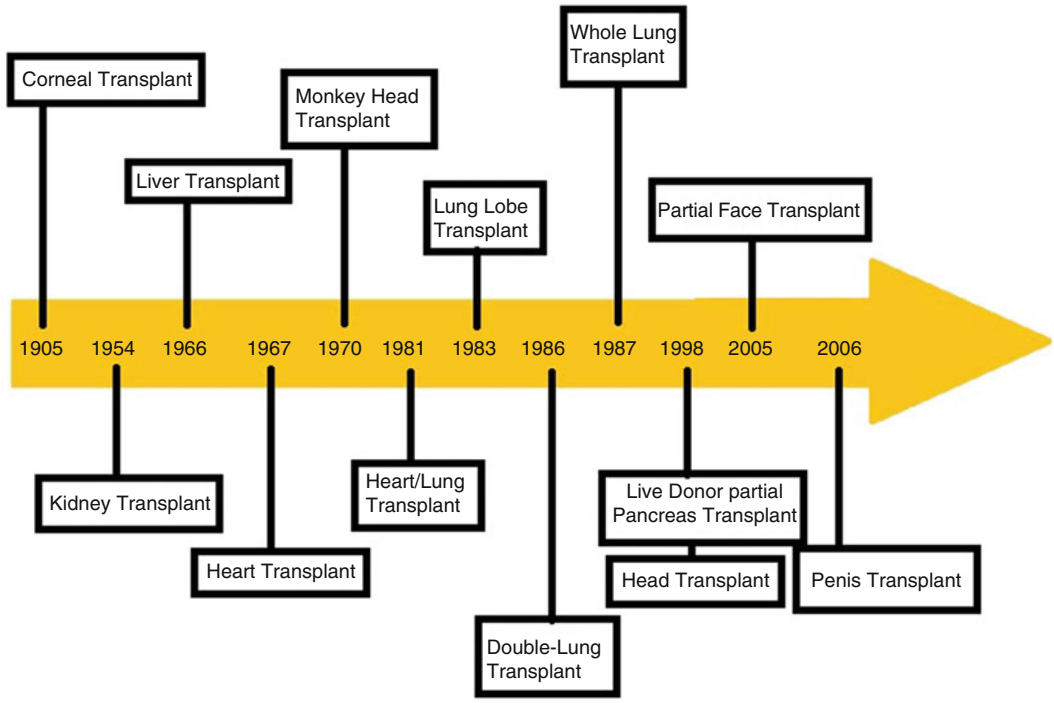

Fig. 3 The time-line of successful transplants

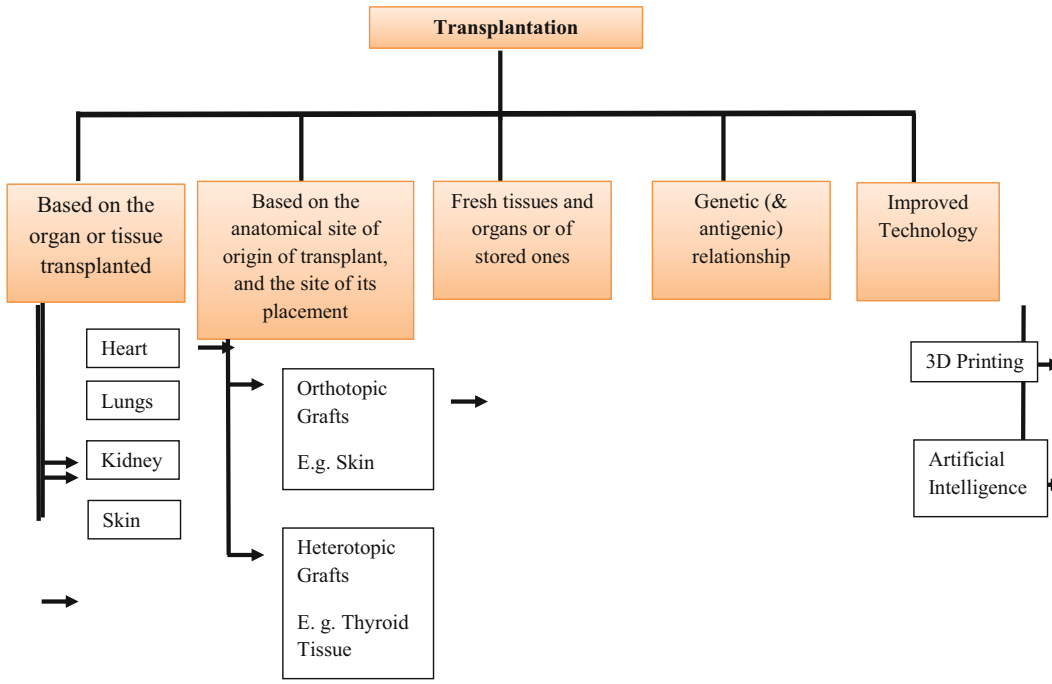

Fig. 4 Classification of transplantation

transplantation, cord blood cells some medical devices, and medicines like immunosuppressant.

2 Bone Marrow Transplantation

A fatty tissue and spongy structure that is present inside bones which contains immature cells known as SCs is called the bone marrow. The bone marrow produces red platelets which are utilized to transport oxygen, white platelets which act against disease and platelets which are in charge of the arrangement of clumps. The structure of bone marrow incorporates juvenile blood-framing undifferentiated cells known as hematopoietic foundational microorganisms, or HSCs which can possibly duplicate through cell division and either remain immature microorganisms or separate and develop into a wide range of blood cells. A bone marrow is a methodology to supplant a man's flawed or harmed foundational microorganisms with solid cells. The beneficiary gets solid foundational microorganisms either from a sound donor or they can originate from a similar individual's body. Ailments like leukemia, serious blood sicknesses, for example, thalassemia, aplastic iron deficiency and sickle cell weakness including various myeloma and certain resistant lack diseases. There are various types of bone marrow transplants which include autologous transplant that use its own SCs, allogenic transplants (cells from a donor and/or UC blood transplant where right after birth the SCs from a newborn UC are removed). The SC procedure is usually performed after completion of chemotherapy and radiation therapy. The delivery of SCs into the bloodstream is carried out using central venous catheter. The SCs flow through the blood to the bone marrow and no surgery is required. The way a donor cell is collected can be classified in two ways as shown in the Table 1.

Bone marrow procedure is performed in the cases of certain cancers like leukemia, diseases that affect the production of bone marrow cells like aplastic anemia, severe immune system illnesses like sickle cell anemia and if chemotherapy has destroyed the bone marrow. Chest pain, hypotension, taste disturbances, shortness of breath etc. may be some risks that are involved in bone marrow transplantation. The complications of bone marrow therapy may depend on the treatment of the disease, whether chemotherapy or radiation is conducted before the bone marrow transplant and the dosages of such treatments, the match with the donor and the type of bone marrow transplant received etc. Delayed growth in children who receive a bone marrow transplant, GVHD, infections, which can be very serious, stomach problems, early menopause are few of the complications that may occur due to bone marrow transplant.

The various terminologies of bone marrow transplant are shown in the Fig. 5.

2.1 UC Transplantation/Cord Blood Cells

The UC is being cut after a baby is born. The placenta contains some amount of blood vessels and also the part of the UC that is in contact of the baby contains these blood vessels. After birth, this extra blood is not needed by the baby. This blood is called placental blood or UC blood "cord blood". This is the motivation for line blood can be utilized for transplantation as a contrasting option to bone marrow. This system has been utilized as another option to bone marrow transplant. Cord Blood transplants have mostly been used for patients suffering from blood and immune system diseases (Ballen 2005; Lubin and Shearer 2007; Meyer et al. 2005).

2.1.1 The Advantage to Patients

Availability The prescreening and testing of selected cord blood and was frozen for the future use.

Table 1 Differentiation in collecting donor cell

Bone marrow harvest	Leukapheresis
Minor surgery where the donor is given general anesthesia and is mostly a pain-free technique.	The blood is withdrawn from the donor through the IV line.
The region at the back and of both the hips is considered as a site for bone marrow removal and the amount of bone marrow removal depends on the weight of the recipient.	The SCs are given to the recipient prior to which they are separated from the part of white blood cells by a machine. The RBCs are then returned back to the donor.

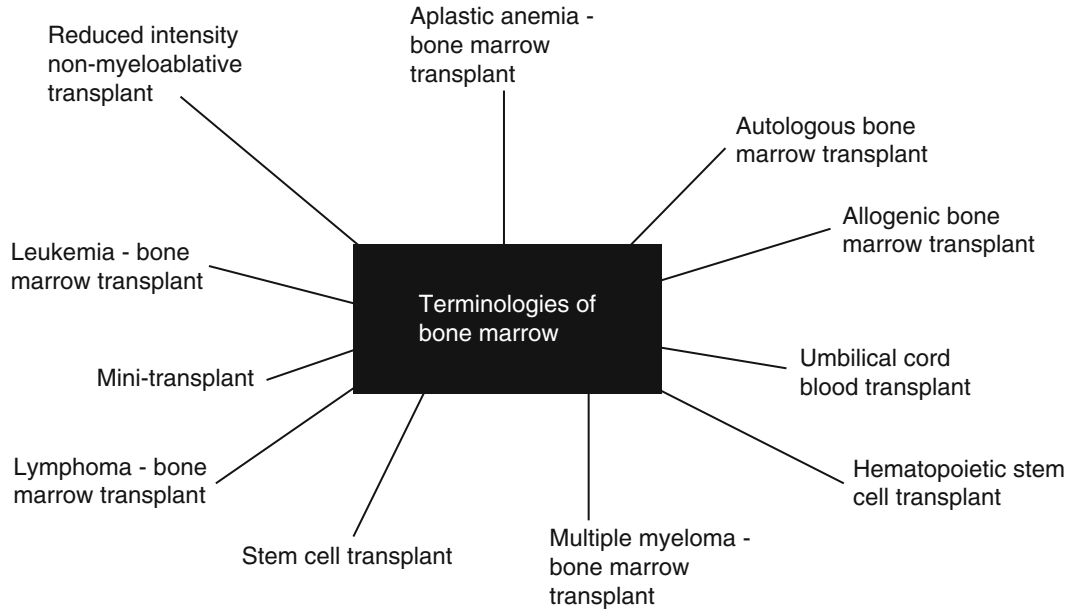

Fig. 5 Terminologies used for bone marrow transplant

GVHD Lesser people are suffering from this disease.

Infectious Disease Transmission Transplantation with the cord blood SC limits the diseases caused by blood-blood transfusion.

HLA Matching Transplants are subjected to the level of HLA coordinating among the transplant beneficiary and the contributor string blood. HLA coordinating assumes an essential part of effective engraftment, the seriousness of GVHD and general survival. A patient's result after transplantation can be by enhancing a nearby match amongst the individual and the cord blood unit.

2.1.2 Disadvantage

Storage The effectiveness of storage of cord blood can be frozen or stored is not known. The successful transplantation of cord blood samples that have been preserved for 10 years have been reported.

Engraftment Individual's weight, age and illness status decides the quantity of cells required for transplant in a patient. Due to the presence, fewer undeveloped cells in the cord blood unit the engraftment of cord blood SC is better from bone marrow or peripheral blood. Until the point that

engraftment happens, patients are in danger of creating hazardous diseases. Cord blood transplant may make beneficiaries be defenseless against diseases for a normal of 1–2 months which is more than marrow and fringe blood undeveloped cell beneficiaries.

Clinical Data Transplantation is carried out to a patient via donor cord blood SCs, even if the genetic diseases present in the individual that are not evident at the time of birth, hence routine checkup must be conducted.

3 Advanced Therapy

3.1 IRFs

These were first identified as regulators of virus-induced type I interferon (IFN A and B) gene expression (Ning 2014; Matta et al. 2017). Several findings stated that IRFs play a major role in regulating both innate and adaptive immune responses, and are also involved in the activation and differentiation of distinct immune cell populations (Tamura et al. 2008; Battistini 2009). Currently, 10 IRFs have been discovered in vertebrates of which some are inactive in humans and in mice. (Nehyba et al. 2009).

3.1.1 RA

RA is a systemic autoimmune diseases that is caused by expansion of synovial tissue, chronic inflammation and progressive damage of a joint. Several immune cell subsets such as macrophages, monocytes, neutrophils, dendritic cells, natural killer cells, T-cells, and B-cells perform an important part in RA pathogenesis. The major pro-inflammatory cytokines that show a prominent function in RA development are TNFα, IL1b and IL6. The major constituents of synovial hyperplasia are FLSs and FLS-dependent effector molecules are known as important mediators of RA. FLS show critical part to occupy cartilage and bone tissue (Ganesan and Rasool 2017). IL18, a cytokine for RA pathogenesis, (Gracie et al. 1999) promotes T-cells to produce TH1 type cytokines and helps in the differentiation of TH1 cells. The biologic activity of IL18 is controlled by IL-18BP, which has an IRF1 binding site in the IL-18BP promoter (Hurgin et al. 2002). During joint replacement or synovectomy, the fibroblasts were isolated from synovium of RA patients and an *in vitro* knockdown system was utilized to analyze the effect of TNFα-induced IRF1 nuclear translocation and regulate IL-18BP expression when NF_KB or JNK2 signaling pathway was blocked, IRF1 nuclear translocation was reduced. Further, it was shown that IRF1 forms a complex with NF_KB and cJUN in the nucleus (Marotte et al. 2011). ACPAs found to persuade IRF4 and IRF5 protein expression. IRF5 siRNA weakened ACPA movement fundamentally while ACPAs prompted IRF5 action and prompted M1 macrophage polarization. (Zhu et al. 2015).

3.2 MSC Treatment for Autoimmune Disease

The first effective therapeutic use of MSCs was stated in year 2000 for bone marrow graft enhancement in humans (Koc et al. 2000). There are various factors like diverse and multiple are responsible for the practical biological effects of MSC. The first *in vivo* model showed a positive effect of MSC on a murine skin graft model similar positive reports have been seen in many autoimmune diseases like RA, multiple sclerosis (Bartholomew et al. 2002; Tyndall and Uccelli 2009). Besides pharmacological advantages, it has been used in cosmetic surgery in the treatment of burns.

3.2.1 RA

Four patients suffering from RA accepting allogeneic or bone marrow-determined MSC IVI was basically negative, however no adverse condition was observed. Two of the three had a EULAR direct reaction at a half-year yet encountered backslide at 7 and 23 months, separately. Two patients had no EULAR reaction to MSC

transplant. No patient accomplished the DAS-28-characterized abatement in the follow-up period (Liang et al. 2012; Djouad et al. 2005). Not with standing, a moment bigger, non-randomized relative trial in 172 RA patients with dynamic RA who had deficient reactions to conventional prescription was distributed in which 136 patients received 40 x106 allogeneic UC-MSC and 36 patients received just the cell-dissolvable without the cells. The two treatment choices were: DMARDs in addition to medium without UC-MSC, or DMARDs in addition to UC-MSC gather through intravenous infusion. No genuine unfriendly impacts were seen amid or after imbuement. The helpful impacts kept up for 3–-6 months without consistent organization, associating with the expanded level of administrative WBCs in fringe blood. Conversely, there were no such advantages saw in the control gathering of DMARDS in addition to medium without UC-MSC. No patients demonstrated intense genuine reactions either amid or after UC-MSC imbuement, and 4% indicated mellow unfriendly impacts amid the mixture, for example, chill and additionally fever (< 38.5 8C), which vanished inside 2 h with no treatment. No major strange discoveries in hematologic or serum science profiles were found in the investigation. It was reasoned that treatment with DMARDs in addition to UC-MSC may give sheltered, noteworthy, and industrious clinical advantages for patients with dynamic RA (Wang et al. 2013; Ra et al. 2011).

3.3 Dendritic Cells-Derived Exosomes

Resemblance to biology of the cell from which they are derived, dendritic cells have gained importance in the treatment of autoimmune diseases and tumor (Sousa et al. 2017). They overcome barriers like synovial membrane and the blood-brain. These molecules can interfere with different pathways; they are likely to possess more target and long-lasting therapeutic effect due to hyper branched structure (Aryani and Denecke 2016).

4 Worldwide Regulatory Aspects

4.1 USA

In 1968, the Uniform Anatomical Gift Act was enacted to solve the problems of different rules of transplantation in states and provided an outline of even laws in the United States related to organ and tissue transplantation. The Uniform "Organ Contributor Card" was authorized in 1972 which was an authoritative report in every one of the 50 states under the Uniform Anatomical Blessing Act. This engaged any individual matured 18 years or more to lawfully make a promise to give his organs upon death. The OPTN in 1984 by the NOTA expressed that the purchasing and offering of organs are precluded. Also, the installment of "the costs of movement, lodging, and lost wages brought about by the (living) contributor" is allowed in segment 301(Arthur 2008). The various acts along with their roles are shown in Table 2.

4.2 South Africa

On 2nd May 2005, the National Wellbeing Act 61 of 2003 (South Africa, 2003) became effective. In 1983, the Human Tissue Act 65 states that any person of 16 years or more should make a will or a record with his/her marks and 2 witnesses demonstrating the desire for organ gift. (Slabbert and Mnyongani 2011).

4.3 Europe

The Mandate 2010/45/EU on the norms of value and security of human organs required for transplantation was received on seventh July 2010. The point was to absorb the gauges of value and security and grow more proficient transplantation frameworks. The European Parliament presented the Activity Anticipate Organ Gift and Transplantation (2009–2015) notwithstanding the order. In Article 19 et. Seq. the Gathering of Europe made extraordinary standards on organ expulsion.

Table 2 Various Acts and their roles

Year	Act	Role
1987	Uniform anatomical gift act (amended)	Expressed to encourage a helpful and uniform lawful condition of organ gift all through the nation
1991	PSDA	Engages and advances the utilization of propelling mandates, for example, living wills and sturdy forces of the lawyer for human services.
1999	Organ donor leave act	Leave of 7 days for bone marrow gift and 30 days for strong organ gift is accommodated an organ benefactor who is the government workers.
1999	Organ procurement and transplantation network final rule	The reason for the last govern is to help accomplish the most evenhanded and therapeutically compelling use of human organs that are given in trust for transplantation
2000	Children's health act	The law tends to the uncommon need of youngsters who are beneath the age of 18 years.
2004	Organ donation and recovery improvement act	Gives subsidizing to the states, in the accompanying cases: Bolster organ gift programs so that there are more organ benefactors.
		Give money related help to living contributors which includes travel allowances and other benefits
		Accidental non-restorative costs.

4.4 Germany

On 5th November 1997 the Act was passed by the German Bundestag which came into force on 1st December 1997; on 4th September 2007 and on 1st August 2012, an amended version of the act was published wherein the later stated that all health insurance members who are 16 years or older will be asked frequently if they are willing to donate their organs.

4.4.1 Law for Post-Mortal Organ Donation under the TPG

The law stated that if the individual give prior permission for organ donation after his/ her death only then the organs will be transplanted.

4.4.2 Law for Living Donation under the TPG

Once a living donation is carried out, the recipient has to be ensured that he/ to no expected hazard past that of the operation. Organs that make them recharge properties can be given to obscure people, the gift of non-regenerative organs (e.g. kidney, parts of the liver) is allowable to transplant to relatives or with whom the donor has a very close personal relationship. The code of SGB V state expresses that a living donor has an expansive case against the health care coverage of the organ recipient, for example, therapeutic treatment, recovery, travel costs, ailment pay. On the other hand, the organ recipient can guarantee for repayment of wages in the event that he can't work.

Guidelines and opinions of the German Medical Association.

The German Medical Association (Bundesärztekammer) establishes Guidelines for specific areas of transplantation medicine. The "Permanent Committee for Organ Transplants of the German Medical Association" (Ständige Kommission Organ transplantation der Bundesärztekammer) elaborates these guidelines and keep it updated at regular intervals.

4.5 Regulatory Aspects in India

In 1994, the transplantation of Human Organs Act was passed. The aim was to regulate the removal, storage and transplantation of human organs for therapeutic purposes and also to prevent the commercial dealings of human organs. The 3 states Maharashtra, Himachal Pradesh and Goa initiated the act (who therefore adopted it by default) and which was subsequently adopted by all states except Jammu and Kashmir and Andhra Pradesh. In 2009 the states Goa, Himachal Pradesh and West Bengal proposed an amendment to address inadequacies in the efficacy,

Table 3 Amendments of 2009 and 2013

Transplantation of human organs (Amendment) Bill, 2009	Transplantation of human organs (Amendment) Bill, 2013
This amendment bill offers regulation of the transplantation of human tissue along with organ transplant	A composite set of guidelines for dealing with deceitful practices and for countering illegal organ transplant
It was necessary that every organ donation case should go to the authorization committee first.	Along with an authorization committee, there will be a 'verification committee' to check the details provided by the donor and recipient.

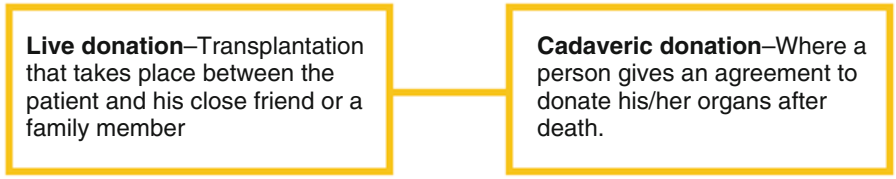

Fig. 6 Types of donation

relevance and impact of the Act. In 2011, the amendment to the Act was passed by the parliament, and in 2014 the rules were notified.

The various amendments done in the act are shown in Table 3.

4.6 Types of Donations

The types of donation are live donation and cadaveric donation. It is shown in Fig. 6.

4.6.1 Steps Involved in Organ Donation

The steps for organ transplant is shown in the Table 4.

4.6.2 Requirements for Recipient

- Get an NOC by registering yourself with the ZTCC for cadaveric donations.
- Your physician can assist or guide you through the process of registering.
- Cross-matching is a must for every organ transplant

5 Future Perspective

5.1 Artificial Intelligence

Studies demonstrated that computerized reasoning can be utilized as an apparatus to distinguish donor beneficiary by utilizing ANNs for benefactor coordinating in liver transplantation (Briceño et al. 2014).

5.2 3D Printing

This innovation has been utilized to make blood manufactured vessels. Chen and his group created advanced 3D microstructures that copy the perplexing plans and elements of organic tissues. The veins that were embedded were not yet equipped for different capacities like transporting supplements and waste. In any case, researchers say that it can be enhanced sooner rather than later. (Zhu et al. 2017).

A biotechnology organization, in particular, Sichuan Revotek situated in Chengdu, China, has effectively embedded a printed segment of the vein into a monkey. Organovo, a firm in San

Table 4 Steps for organ donation

Donating to your own family member	In case of cadaver donation
Individual needs an NOC from the government if he/she wants to donate an organ	An organ donation card will be arranged for an individual after registration with any NGO, if you want to donate an organ.
Individual has to undergo a complete health check- up along with a blood cross matching test before the operation.	Your family must be informed about your willingness so that after the death of the individual doctor may go for organ harvesting.
	The body has to be covered properly after transplantation and returned to the family members in an aesthetic manner
	After being declared brain dead or after death the organs of an individual are tested for their usability.

Diego, declared that it had transplanted printed human-liver tissue into mice and that this tissue had survived and worked. Johnson and Johnson, L'Oréal, Proctor and Gamble, and BASF are chipping away at printing human skin. They propose to utilize it to test their items for unfavorable responses.

5.3 Nuclear Transplantation, ESCs and the Potential for Cell Therapy

Nuclear transplantation is also known as nuclear cloning or nuclear transfer. It alludes to a procedure where a cloned fetus is created by the presentation of a core from a grown-up benefactor cell into an enucleated oocyte. The incipient organism show in the female uterus has the ability to develop into a newborn child which would be a clone of the grown-up contributor cells. This developing life produces embryonic foundational microorganisms that can possibly turn out to be any sort of cells shown in the grown-up body. As embryonic undifferentiated organisms are inferred by atomic exchange they are hereditarily indistinguishable to the contributor and are along these lines valuable for some remedial applications, this procedure is called "nuclear transplantation therapy" or "therapeutic cloning." Restorative cloning may be utilized to enhance the treatment of blood issue, neurodegenerative sicknesses or diabetes. (Hochedlinge and Jaenisch 2003).

6 Conclusions

Autoimmune disease is a condition wherein body's own cells attack individual's own cells and disrupt the immune system. Various techniques of transplantation like bone marrow transplantation and cord blood cells have been employed for the treatment of autoimmune diseases. Cord blood method along with the use of DMARDs is most effective technique used in the management of rheumatoid arthritis. Newer approaches like IRFs and mesenchymal stromal cells have been used in the treatment of autoimmune diseases. The combination of MSCs with DMARDs is considered to be therapeutically effective as compared to the individual technique.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 73–85
https://doi.org/10.1007/5584_2018_212

Published online: 17 May 2018

Therapeutic Applications of Mesenchymal Stem Cells for Systemic Lupus Erythematosus

Jianyong Xu

Abstract

Mesenchymal stem cells (MSCs) have been intensively studied and applied in regenerative medicine and tissue engineering. Recently, their immune modulation functions make them as attractive potential approaches for autoimmune disease treatment. Systemic lupus erythematosus (SLE) is one type of chronic autoimmune diseases with multi-organ damaged by the immune system. Although current available treatments are effective for some patients, others are refractory for these therapies. The immunomodulatory and regenerative characteristics of MSCs make them as one promising candidate for treating SLE. Thus, we would discuss their immune modulation effects, pre-clinical and clinical applications, and the potentials for immune tolerance re-establishment in SLE here.

Keywords

Autoimmune diseases · Mesenchymal stem cells · MSCs · SLE · Systemic lupus erythematosus

Abbreviations

BAFF	B cell activating factor
BM	bone marrow
Breg	regulatory B cell
CCL2	C-C motif chemokine ligand 2
HSCs	hematopoietic stem cells
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-gamma
IL	interleukin
iNOS	inducible nitric oxide synthase
MSCs	mesenchymal stem cells
OAZ	olfactory 1/early B cell factor-associated zinc-finger protein
PD	programmed death
PGE2	prostaglandin E2
SLE	systemic lupus erythematosus
Tfh	follicular helper cell
TGF-β	transforming growth factor beta
Th	T helper cells
TNF-α	tumor necrosis factor alpha
Treg	regulatory T cells
UC	umbilical cord

1 Introduction

Mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, are spindle-shaped

J. Xu (✉)
Department of Immunology, Shenzhen University School of Medicine, Shenzhen University, Shenzhen, People's Republic of China
e-mail: xujianyong@szu.edu.cn

cells with multi-potent (chondrocyte, osteoblast, and adipocyte) and self-renewal abilities (Dominici et al. 2006; Pittenger et al. 1999). They could be derived from various adult tissues (Uccelli et al. 2008; Wang et al. 2014c), attach to tissue culture dishes and express certain cell surface markers (positive for CD73, CD90 and CD105; negative for CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR) (Dominici et al. 2006). MSCs have been proposed as effective and safe cell source for stem cell therapy. They have low immunogenicity and could be safely harvested with no major ethical concerns (Wang et al. 2014c).

In addition to their applications in regenerative medicine and tissue engineering (Uccelli et al. 2008; Wang et al. 2014c), their immune modulation functions make them as attractive potential approaches for autoimmune disease treatment (Bernardo and Fibbe 2013; Keating 2012). Autoimmune diseases are characterized by local tissue destruction and chronic inflammation, which are induced by aberrant immune responses to self-constituents. This process normally includes both innate and adaptive immune responses, T and B cell auto-activation, and eventually immune tolerance collapse (Banchereau et al. 2017; van Kempen et al. 2015). So far, MSCs have been successfully applied in treating many autoimmune diseases, including systemic lupus erythematosus (SLE) (Liao et al. 2015).

Although many questions are still unsolved, the immune modulation effects of MSCs make them as the promising target for immune tolerance re-establishment in SLE. Thus, we would discuss their immune modulation effects, pre-clinical and clinical applications, and the potentials for immune tolerance re-establishment in SLE here.

2 Immune Modulations by MSCs

It has been demonstrated that the MSCs therapy is safe, without obvious side effects and malignancy in stem cell therapies for many disease (Tyndall 2014; Wang et al. 2014c). Theoretically, the transplanted MSCs should migrate into the inflamed tissues. However, the cell number is very low (Karp and Leng Teo 2009). Thus the paracrine effects have been proposed. And the immune tolerance re-construction is another promising theory for the therapeutic effects of MSCs (Ko et al. 2016). It is suggested that the MSCs induce monocytes/macrophages tolerance to autoimmunity in the eye (Ko et al. 2016). MSCs have been investigated to promote HSCs (hematopoietic stem cells) engraftment and prevent graft rejection in graft-versus-host disease (Gao et al. 2016; Le Blanc et al. 2008; Le Blanc et al. 2004; Polchert et al. 2008). MSCs also promote immune tolerance in organ transplantation (Contreras-Kallens et al. 2017). Furthermore, the MSCs have been applied in treating SLE (Sun et al. 2010).

The immune modulation activities of MSCs are through both cell-cell direct contact and also secretome, which is composed of extracellular vesicles and soluble factors (Heldring et al. 2015; Phinney et al. 2015). The soluble factors include IDO (indoleamine 2,3-dioxygenase), PGE2 (prostaglandin E2), soluble HLA-G5, TNF-stimulated gene-6, heme oxygenase-1, nitric oxide, IL-10 (Interleukin-10) and TGF-β1 (transforming growth factor beta 1) (Bernardo and Fibbe 2013; Keating 2012; Uccelli et al. 2008).

MSCs are low immunogenic or have immune privilege resulting from low expression of major histo-compatibility complex class I, II and the lack of co-stimulatory factors (Bernardo and Fibbe 2013, Keating 2012, Uccelli et al. 2008). Thus allogenic MSCs transplantation should be feasible with minimal immune rejections. However, it has been found that the allogenic MSCs would be rejected in the mice model (Ankrum et al. 2014). And the immune environments affect the therapeutic effects of MSCs significantly (Carrion et al. 2010). Thus understanding how the MSCs interact with the immune system is critical before clinical applications.

3 MSCs Therapy for Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is one type of chronic autoimmune diseases with multi-organ damaged by immune system (Bernatsky et al. 2006; Lee et al. 2016; Murphy et al. 2013; Tsokos 2011; Tyndall 2009). Several factors, such as genetic and environmental factors, could induce self-tolerance loss and immune system over-activation, resulting in tissue and organ injuries (Lisnevskaia et al. 2014; Tsokos et al. 2016). Current available treatments for SLE have been demonstrated to reduce morbidity and mortality effectively, such as non-steroidal anti-inflammatory drugs, anti-malarial agents, glucocorticoids, immunosuppressive agents and target depleting B cells (Xiong and Lahita 2014). Although they are effective for some patients, others are refractory for these therapies. The immuno-modulatory and regenerative characteristics of MSCs make them as one promising candidate for treating SLE.

3.1 MSCs Defects in SLE

The bone marrow MSCs derived from SLE patients have impaired proliferation capability and immune modulation functions (Che et al. 2014; Feng et al. 2014; Gao et al. 2017; Nie et al. 2010; Sun et al. 2007). They are defective in cell migration and more prone to cell death (Gu et al. 2014a; Gu et al. 2014b; Li et al. 2012; Shi et al. 2014). Furthermore, they fail to suppress the T and B cell activities (Feng et al. 2014; Wang et al. 2014a). Thus the MSCs isolated from SLE patients have no therapeutic benefits (Carrion et al. 2010). Furthermore, it has been demonstrated that in the MRL/lpr mice model of SLE, the impairment of bone marrow MSCs and their niche deficiency contribute to the pathogenesis of SLE-like diseases (Sun et al. 2009).

MSCs from SLE patients have cytokine secretion abnormality (Sun et al. 2007) and are more prone to senescence and apoptosis (Nie et al. 2010) through up-regulating Wnt/β-catenin and p53/p21 pathways (Gu et al. 2014b) or down-regulating Bcl-2 (Li et al. 2012) and IDO (Wang et al. 2014a). The MSCs derived from the bone marrow of lupus-like mice and SLE patients have decreased abilities to suppress the B cell proliferation, differentiation, and activity, resulting from CCL2 (C-C motif chemokine ligand 2) reduction. And the CCL2 over-expression could restore their immune suppression abilities in the SLE MSCs (Che et al. 2014). Furthermore, the gene OAZ (olfactory 1/early B cell factor-associated zinc-finger protein) is highly enriched in MSCs and up-regulated in the SLE patients. Knocking down OAZ could also restore the impaired immune suppression function of SLE MSCs via up-regulating CCL2 expression (Feng et al. 2014). Later studies have demonstrated that the SLE MSCs have increased reactive oxygen species production, DNA damage and repair, p53 and p16 expression, pro-inflammatory cytokine production through the mitochondrial antiviral signaling protein-Interferon-β feedback loop (Gao et al. 2017). Thus the autologous MSCs derived from SLE patients have impaired therapeutic applications and are not the suitable cell source for cell therapy. And the allogenic MSCs from healthy donors are the preferred cell source in treating SLE patients.

3.2 Pre-clinical Studies of MSCs Therapy for SLE

In the mice model of SLE, MSCs transplantation improves renal functions, reduces renal and lung pathology, decreases serum levels of autoantibody and proteinuria, and prolongs the survival of lupus prone mice through inhibiting monocyte, B cell, follicular helper cell (Tfh), and inducing Treg (regulatory T cells) and IL-10 (Table 1) (Chang et al. 2011; Choi et al. 2012; 2015; Deng et al. 2015; Gu et al. 2010; He et al. 2016; Jang et al. 2016; Ji et al. 2012; Ma et al. 2013; Park et al. 2015; Schena et al. 2010; Sun et al. 2009; Thiel et al. 2015; Zhang et al. 2014; 2017; Zhou et al. 2008).

Table 1 Therapeutic applications of MSCs for SLE in pre-clinical studies

Disease Model	Cell Origin	Delivery Method	Follow-up Period	Outcomes	References
MRL/lpr mice	hBM-MSCs	1×10^6 cells; tail vein; once	16 weeks	Auto-antibody reduction; proteinuria reduction; renal pathology reduction	Zhou et al. (2008)
MRL/lpr mice	mBM-MSCs	0.1×10^6 cells/10 g; tail vein; twice	11 weeks	Osteoblastic niche reconstruction; multiple organ function improvement	Sun et al. (2009)
MRL/lpr mice	hUC-MSCs	1×10^6 cells; tail vein; three times	11 weeks	Auto-antibody reduction; proteinuria reduction; renal pathology reduction	Gu et al. (2010)
(NZBxNZW) F1 mice	mBM-MSCs	$1.25x10^6$ cells; tail vein; three times;	5 weeks	Reduction in glomerular immune complex deposition and lymphocytic infiltration	Schena et al. (2010)
(NZBxNZW) F1 mice	hUC-MSCs	1×10^6 cells; tail vein; once	8 weeks	Auto-antibody reduction; proteinuria reduction; renal pathology reduction; prolonged the life span	Chang et al. (2011)
(NZBxNZW) F1 mice	hAD-MSCs	0.5×10^6 cells; tail vein; every 2 weeks	54 weeks	No adverse effects; higher survival rate; auto-antibody reduction; proteinuria reduction; renal pathology reduction	Choi et al. (2012)
MRL/lpr mice	mBM-MSCs	0.2×10^6 cells/10 g; tail vein; twice	4 weeks	Disease activity reduction; T cell proliferation inhibition	Ji et al. (2012)
MRL/lpr mice	mBM-MSCs	1×10^6 cells; tail vein; once	8 weeks	Decreased BAFF expression; auto-antibody reduction; proteinuria reduction	Ma et al. (2013)
MRL/lpr mice	hUC-MSCs	1×10^6 cells; tail vein; three times	11 weeks	Interstitial pneumonitis, lung peribronchiolar lesion, and lung perivascular lesion reduction	Zhang et al. (2014)
MRL/lpr mice	hAD-MSCs; CTLA4Ig-overexpressing hAD-MSCs	1×10^6 cells; tail vein; every 2 weeks	18 weeks	Auto-antibody reduction; proteinuria reduction; kidney inflammation reduction	Choi et al. (2015)
MRL/lpr mice	hUC-MSCs	1×10^6 cells; tail vein; once	4 weeks	Improved the proportion of $CD206^+$ macrophages and their phagocytic activity	Deng et al. (2015)
Roquin (san/san) mice	hAD-MSCs	1×10^6 cells; tail vein; once weekly	5 weeks	Auto-antibody reduction; proteinuria reduction; decreased $ICOS^+CD44^+$ Tfh cells, Th1 cells and Th17 cells; increased Treg cells; Bregs expansion induction	Park et al. (2015)
(NZBxNZW) F1 mice	hESC-MSCs	0.5×10^6 cells; tail vein; 3 times weekly	12 weeks	Auto-antibody reduction; proteinuria reduction; preserved renal architecture; kidney inflammation reduction	Thiel et al. (2015)
MRL/lpr mice	mAD-MSCs	1×10^6 cells; tail vein; every 2 weeks	10 weeks	Auto-antibody reduction; proteinuria reduction; kidney inflammation reduction	He et al. (2016)
(NZBxNZW) F1 mice	hBM-MSCs	1×10^6 cells; retro-orbital; every 2 weeks, 3 times	11 weeks	Attenuated glomerulonephritis; auto-antibody reduction; proteinuria reduction; improved survival; Tfh cell reduction	Jang et al. (2016)

(continued)

Table 1 (continued)

Disease Model	Cell Origin	Delivery Method	Follow-up Period	Outcomes	References
MRL/lpr mice	hUC-MSCs	1×10^6 cells; tail vein; once	4 weeks	Tfh cell reduction; auto-antibody reduction	Zhang et al. (2017)

hBM-MSCs human bone marrow derived mesenchymal stem cells, *hUC-MSCs* human umbilical cord derived mesenchymal stem cells, *hAD-MSCs* human adipose tissue derived mesenchymal stem cells, *hESC-MSCs* human embryonic stem cell derived mesenchymal stem cells, *mAD-MSCs* mouse adipose tissue derived mesenchymal stem cells, *mBM-MSC* mouse bone marrow derived mesenchymal stem cells

One single dosage of MSCs transplantation shows significant therapeutic effects, which could be further promoted by multiple treatment (Choi et al. 2012; Gu et al. 2010). And the long-term serial administrations do not show any adverse effects (Choi et al. 2012). Furthermore, it would be more effective at the early stage of SLE progress than the late stage (Choi et al. 2012). The transplanted cells could be detected in the kidney even after 3 months post cell infusion (Gu et al. 2010).

MSCs prevent disease associated inflammation, protein cast deposition, and infiltration of $CD3^+$ lymphocytes and complement C3 in the kidneys (Thiel et al. 2015; Zhou et al. 2008). This therapy also leads to significant reductions in serum levels of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), two inflammatory cytokines associated with SLE (Chang et al. 2011; He et al. 2016; Thiel et al. 2015). They also decrease T-helper (Th) 1 cytokines (Interferon-γ, IL-2), Th17 cytokine (IL-17) and increase Th2 cytokines (IL-4, IL-10) (Chang et al. 2011; Choi et al. 2012; He et al. 2016).

MSCs can significantly decrease marginal zones, T1, T2, activated B cells, and plasma cells (Ma et al. 2013; Park et al. 2015). Moreover, serum levels of B cell activating factor (BAFF) decrease significantly after MSCs transplantations (Ma et al. 2013). MSCs inhibit antigen-dependent proliferation and differentiation to plasma cells of follicular and marginal zone B cells. This inhibitory effect is dependent on cell-to-cell contact, interferon-gamma (IFN-γ) and the programmed death 1 (PD-1)/PD ligand pathways (Schena et al. 2010). The infiltration of long-lived plasma cells into the inflamed kidney is also reduced in the hBM-MSCs (human bone marrow derived mesenchymal stem cells) treated mice (Jang et al. 2016).

The auto-antibodies are predominantly produced with the help of Tfh cells and then form immune complexes that trigger widespread inflammatory damages, including nephritis (Jang et al. 2016). MSCs decrease the percentage of Tfh cells, which is increased in lupus and positively correlated to plasma cell proportions and serum total IgG as well as anti-dsDNA antibody levels (Park et al. 2015; Zhang et al. 2017). The proliferation and differentiation of Tfh cells are markedly suppressed by MSCs through iNOS (inducible nitric oxide synthase) production and a cell-contact-dependent manner (Jang et al. 2016; Park et al. 2015; Zhang et al. 2017). In addition, MSCs decrease the Th1 cells and Th17 cells while increase the Foxp3-expressing Treg cells and IL-10 expressing Breg (regulatory B cell) cells (Choi et al. 2012; He et al. 2016; Park et al. 2015; Zhou et al. 2008). MSCs also inhibit the G1/S transition of the abnormal lupus T lymphocytes through up-regulating p21 and p27 and down-regulating cyclin-dependent kinase 2 (Ji et al. 2012)

MSCs transplantation increases the proportion of $CD206^+$ and $CD68^+$ macrophages and their phagocytic activities (Deng et al. 2015; He et al. 2016). What is even more interesting is that the MSCs transplantation is capable of reconstructing the bone marrow osteoblastic niche and more effectively reverses the multi-organ dysfunction when compared with medical immuno-suppression with cyclophosphamide (Sun et al. 2009).

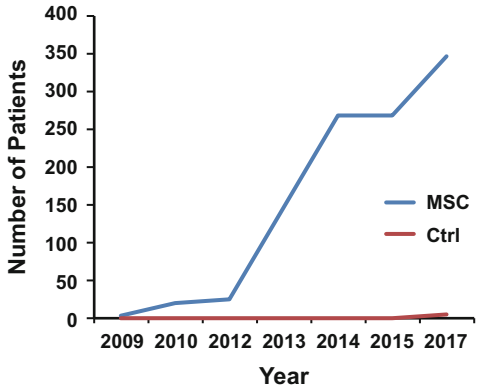

Fig. 1 Number of SLE patients subjected to MSCs therapy in clinical studies. *SLE* systemic lupus erythematosus, *MSCs* mesenchymal stem cells, *Ctrl* control

3.3 Clinical Studies of MSCs Therapy for SLE

In clinical studies, MSCs transplantation is safe and effective in treating SLE patients, with improved renal functions and decreased autoantibody productions (Fig. 1, Table 2; Deng et al. 2017; Gu et al. 2014a; Li et al. 2013; Liang et al. 2010; Phillips et al. 2017; Shi et al. 2012; Sun et al. 2009; 2010; Wang et al. 2013, 2014b, 2015, 2017a, b, c).

Allogenic bone marrow or umbilical cord (UC)-derived MSCs transplantation has shown the disease activity amelioration and renal function improvement in the SLE patients refractory to conventional treatment (Gu et al. 2014a; Sun et al. 2009; Wang et al. 2013). MSCs also reduce many SLE markers significantly. And the improvement is followed by peripheral Treg up-regulation and the balance re-establishment between Th1- and Th2-related cytokines (Sun et al. 2010). The MSCs transplantation has cured the SLE patients with diffuse alveolar hemorrhage, who was refractory to the methylprednisolone and immunoglobulin treatment (Liang et al. 2010; Shi et al. 2012), and SLE patients with refractory cytopenia (Li et al. 2013). Another study also shows that the umbilical cord derived MSCs have the therapeutic effect in severe and refractory SLE patients (Wang et al. 2014b). And this therapeutic effect is partially through up-regulating Treg and down-regulating Th17 cells by MSCs (Li et al. 2013; Sun et al. 2010; Wang et al. 2015, 2017a). The up-regulation of Treg is mediated by increasing the serum levels of TGF-β while the down-regulation of Th17 cells is by PGE2 up-regulation (Wang et al. 2017a). Moreover, the higher levels IFN-γ could predict a good response to MSCs therapy in active lupus patients (Wang et al. 2017c).

3.4 Mechanisms of MSCs Therapy for SLE

Thus both pre-clinical and clinical studies have shown that MSCs are safe and effective for treating SLE. However, most of the underlying mechanisms still remain unclear. Now it is clear that MSCs could suppress the immune response, reduce the pro-inflammatory factors and up-regulate anti-inflammatory factors (Chang et al. 2011; Choi et al. 2012; He et al. 2016; Thiel et al. 2015). Furthermore, they could suppress the B cell (Jang et al. 2016; Ma et al. 2013; Park et al. 2015; Schena et al. 2010), Th1 and Th17 cells (Choi et al. 2012; He et al. 2016; Park et al. 2015; Zhou et al. 2008). And they increase Treg and Breg cells (Choi et al. 2012, He et al. 2016, Park et al. 2015, Zhou et al. 2008; Fig. 2).

MSCs suppress the B cell activities through inhibiting Tfh cells via iNOS production and cell contact (Jang et al. 2016; Park et al. 2015; Zhang et al. 2017). Furthermore, the inhibition of B cell proliferation and antibody production by MSCs is mediated by both $CD4^+$ and $CD8^+$ T cells. Moreover, the cell-cell contact between the MSCs and T cells, but not between the MSCs and B cells, is necessary to inhibit the B cell proliferation. (Rosado et al. 2015).

It has been demonstrated that the bone marrow derived MSCs could induced the T cell apoptosis via the FAS ligand-dependent FAS pathway, resulting in disease phenotype ameliorates. MSCs could secrete monocyte chemotactic protein 1, recruit T cells and mediate T cell apoptosis. The apoptotic T cells then induce macrophages to produce high levels of TGF-β, which up-regulates of $CD4^+CD25^+Foxp3^+$ regulatory T cells and, ultimately, immune tolerance (Akiyama et al. 2012).

Table 2 Therapeutic applications of MSCs for SLE in clinical studies

	No. of Patients					
Cell origin	**MSCs treatment**	**Ctrl**	**Delivery method**	**Follow-up period**	**Outcomes**	**References**
hBM-MSCs	4	0	Intravenously	12–18 months	Disease activity reduction; renal function improvement; serologic markers improvement	Sun et al. (2009)
hUC-MSCs	1	0	Intravenously	5 weeks	Improvements in the clinical condition, oxygenation level, radiographic and hematological status	Liang et al. (2010)
hUC-MSCs	16	0	Intravenously	3–28 months	Significant improvements in the SLEDAI score, levels of serum ANA, anti-dsDNA antibody, serum albumin, complement C3, and renal function	Sun et al. (2010)
hUC-MSCs	4	0	Intravenously	9–24 months	Dramatic improvements of their clinical manifestations; amelioration of oxygen saturation as well as hematological and serologic changes	Shi et al. (2012)
hBM-MSCs hUC-MSCs	35	0	Intravenously	6–45 months	Decline of disease activity; increased Treg and decreased Th17; reverse hematological aberration in SLE patients with refractory cytopenia	Li et al. (2013)
hBM-MSCs hUC-MSCs	87	0	Intravenously	4 years	Significant changes in the SLEDAI score, levels of serum auto-antibodies, albumin, and complements	Wang et al. (2013)
hBM-MSCs hUC-MSCs	81	0	Intravenously	12 months	Obvious amelioration of renal function; lomerular filtration rate improved; no adverse event	Gu et al. (2014a)
hUC-MSCs	40	0	Intravenously	12 months	No adverse events; proteinuria declined; serum antinuclear antibody and anti-double-stranded DNA antibody decreased	Wang et al. (2014b)
hHSCs plus hBM-MSC	1	0	Intravenously	36 months	Clinical symptoms caused by SLE were remitted; $CD4^+CD25^+FoxP3^+$Treg cells increased	Wang et al. (2015)
hUC-MSCs	12	6	Intravenously	6 months	No obvious positive treatment effect.	Deng et al. (2017)
hUC-MSCs	1	0	Intravenously	12 months	Auto-antibodies declined	Phillips et al. (2017)
hUC-MSCs	30	0	Intravenously	12 months	Treg increased; Th17 decreased; increase in serum TGF-β; decrease in serum TNF-α	Wang et al. (2017a)
hUC-MSCs	9	0	Intravenously	6 years	No adverse events; long-term safety proved	Wang et al. (2017b)
hUC-MSCs	26	0	Intravenously	12 months	IFN-γ predicts clinical response to MSCs in SLE	Wang et al. (2017c)

hBM-MSCs human bone marrow derived mesenchymal stem cells, *UC-MSCs* human umbilical cord derived mesenchymal stem cells, *hHSCs* human hematopoietic stem cells, *Ctrl* control, *SLE* systemic lupus erythematosus, *IFN-γ* interferon-gamma, *TNF-α* tumor necrosis factor alpha, *TGF-β* transforming growth factor beta, *Treg* regulatory T cells, *Th17* T helper cells, type 17

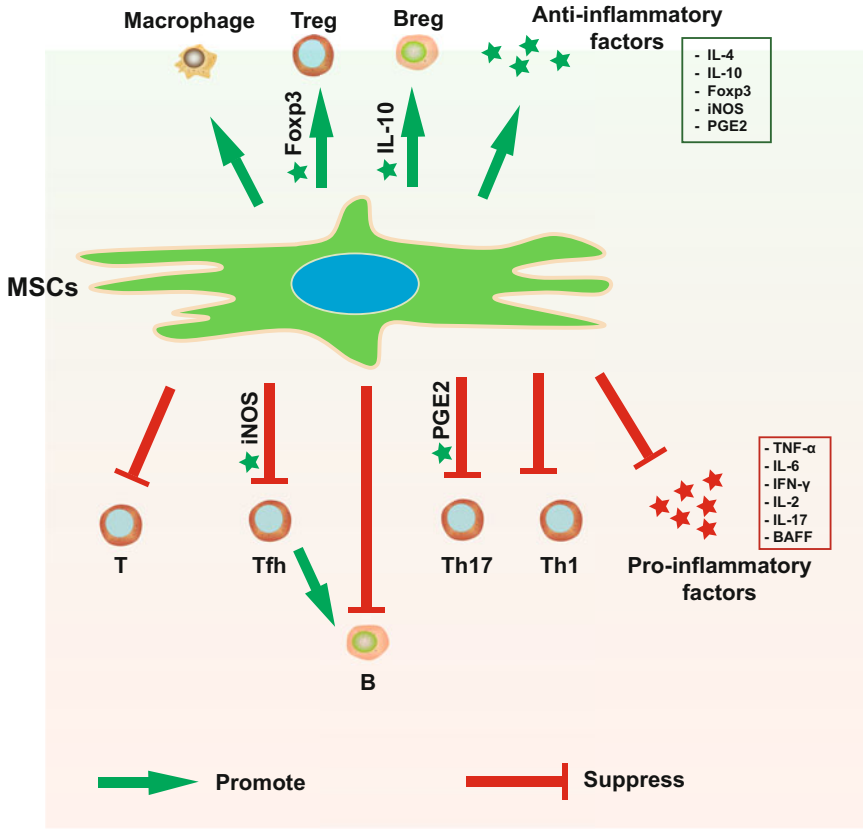

Fig. 2 Mechanisms of MSCs therapy for SLE. MSCs could up-regulate the expression and secretion of anti-inflammatory factors, and promote the immune suppressive functions of macrophage, Treg and Breg cells. MSCs induce Treg production through up-regulating Foxp3 expression and Breg via IL-10 expression. On the other hand, MSCs also could suppress the functions of T, B, Tfh, Th1, and Th17 cells with decreased the expression of pro-inflammatory factors. MSCs suppress the Tfh through iNOS production and Th17 via PGE2. In addition to the direct inhibition, MSCs also suppress the B cell activities through Tfh inhibition. *MSCs* mesenchymal stem cells, *Treg* regulatory T cells, *Breg* regulatory B cells, *T* T cells, *B* B cells, *Tfh* follicular helper T cells, *Th1* helper T cell, type 1, *Th17* helper T cell, type 17

4 Targeting MSCs for Re-Establishing the Immune Tolerance

SLE is characterized by local tissue destruction and chronic inflammation, which are caused by aberrant immune responses to self-constituents. This process normally includes both innate and adaptive immune responses, T and B cell auto-activation, and eventually immune tolerance collapse (Banchereau et al. 2017; van Kempen et al. 2015) (Fig. 3). The chronic inflammation would induce genomic instability and produce mutated proteins, which might generate cross-reactivity against the native proteins according to the "antigen mimicry" theory (Joseph et al. 2014; van Kempen et al. 2015). Thus inflammation suppression is important for autoimmune disease treatment as this process might continually produce new auto-antigens.

Conventional therapies for SLE, such as hydroxychloroquine, corticosteroids, cyclophosphamide, belimumab, mycophenolate mofetil and others, have significantly improved the survival of SLE patients (Hahn 2013). However, they often cause serious side effects including infection, secondary malignancy, bone marrow suppression, and disease relapses after drug

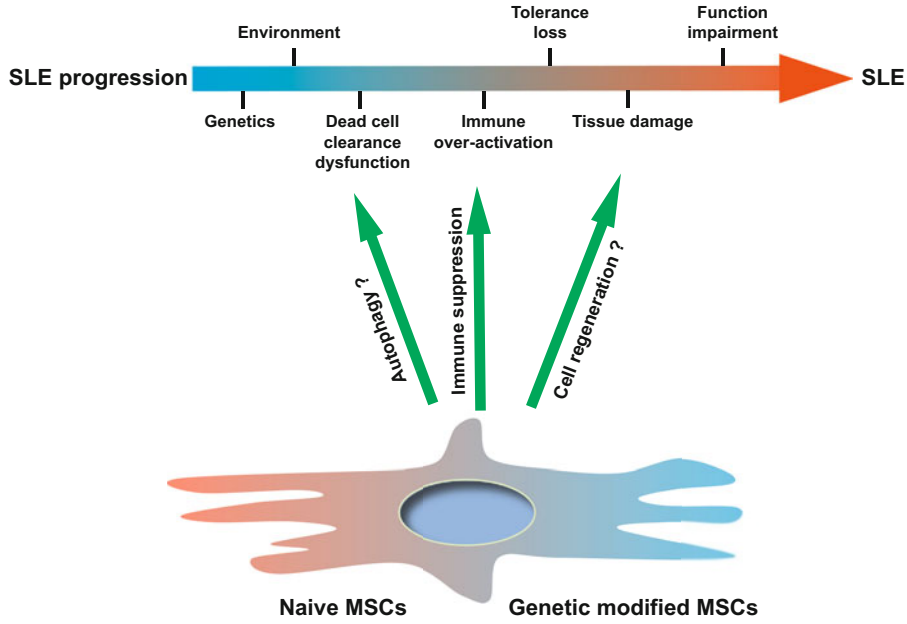

Fig. 3 Targeting MSCs for re-establishing the immune tolerance. During the SLE progression, dysfunctions in the dead cell debris clearance, over-activation of the immune system, and the tissue damaged by self-attack are critical. Naïve or genetic modified MSCs could suppress the immune response to alleviate the SLE symptoms. Potentially, MSCs might also promote the self-antigen clearance through autophagy and cell regeneration via stem cell differentiation. *SLE* systemic lupus erythematosus, *MSCs* mesenchymal stem cells

withdrawal (Bernatsky et al. 2006; Lee et al. 2016). Immune cell targeted therapy, such as Treg transplantation and B cell depletion have been evaluated in animal models or SLE patients. Regulatory T cell ($CD4^+CD25^+$ Treg) based immunotherapy shows effectiveness in the treatment of SLE animal models (Liao et al. 2015). And B cell depletion therapy has also been demonstrated as effective and safe treatment for SLE patients (Kamal and Khamashta 2014). Although they are effective for some patients, others are refractory for these therapies. Thus, novel alternative therapies are needed (Murphy et al. 2013). Both tissue regeneration with healthy stem cells and immune tolerance re-construction are important for treating SLE. In addition to their immune suppression abilities, the autophagy and cell regeneration might also contribute to the SLE therapy. Thus MSCs might provide a promising candidate for immune tolerance re-construction in SLE treatment (Fig. 3).

MSCs stimulated with strong inflammation signals or bacteria have enhanced immune suppressive activities (Chan et al. 2016). In contrast, low inflammatory signal, such as in SLE mice model or patients, reduces significantly the immune suppressive effects of MSCs (Che et al. 2014; Rasmusson et al. 2007). Thus, genetic modified or chemical primed MSCs would be more optimal for treating SLE (Fig. 3).

5 Limitations and Future Perspectives

MSCs are multi-potent stem cells isolated from many tissues (Bianco et al. 2008). However, there is no specific cell surface markers for MSCs so far and diverse markers have been applied to isolate MSCs (Lv et al. 2014; Mo et al. 2016). MSCs are composed of phenotypically and functionally diverse cells (Mo et al. 2016). Genome-wide

methylation, transcription, and *in vivo* analysis have shown that the MSCs isolated from different origins have different characteristics, although they share similar MSC cell surface markers and differentiation abilities *in vitro* (Acar et al. 2015; Reinisch et al. 2015). Thus more efforts must be made to screen MSCs specific markers. Purifying the MSCs subpopulation with specific cell surface markers may enhance their differentiation or immune modulation abilities. MSCs lineage and subtype analysis would also help to define the pure populations and the mechanisms of MSCs for their therapeutic applications.

Another concern is that the MSCs might differentiate into myofibroblast and cause organ fibrosis (El Agha et al. 2017; Trial et al. 2016). Thus carefully characterization and function assessment should be critical analyzed before clinical applications.

Acknowledgements This work was supported by Natural Science Foundation of SZU (2017083), Natural Science Foundation of Shenzhen (JCYJ20170302152735071) and Medical Foundation of Guangdong (A2018308).

Conflict of Interest The authors declare no commercial or financial conflict of interest.

Ethical Approval The authors declare that this article does not contain any studies with human participants or animals.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 87–96
https://doi.org/10.1007/5584_2018_250

Published online: 27 July 2018

Potential Use of Stem Cells in Mood Disorders

Gabriela D. Colpo, Laura Stertz, Breno S. Diniz, and Antonio L. Teixeira

Abstract

Mood disorders are heterogeneous conditions characterized by complex genetics, unclear pathophysiology, and variable symptomatology. Currently, there is no biomarker for the diagnosis or prognosis of mood disorders, and the treatments are of limited efficacy in a significant proportion of patients. Furthermore, the disease models are not able to recapitulate their complexity. In this scenario, stem cells may have different applications in mood disorders. Circulating stem cells may be regarded as potential biomarkers. Mesenchymal stem cells are a promising therapeutic strategy for mood disorders as they promote neurogenesis and increase the expression of neurotrophic factors that enhance the survival and differentiation of neurons. In addition, induced pluripotent stem cells, cells reprogrammed from somatic cells of healthy subjects or patients, offer a great opportunity to recapitulate both normal and pathological development of human brain tissues, thereby opening a new avenue for disease modeling and drug development in a more disease-relevant system.

Keywords

Bipolar disorder · iPSCs · Major depressive disorder · Mood disorder · MSCs · Stem cells

1 Introduction

Mood disorders, specifically, major depressive disorder (MDD) and bipolar disorder (BD), encompass a constellation of symptoms involving emotional, cognitive, and behavioral domains. Mood disorders are highly prevalent worldwide, being recognized by the World Health Organization (WHO) as a major source of morbidity and mortality (Kessler et al. 2006; WHO 2012). These conditions are frequently chronic and debilitating (Whiteford et al. 2013; Marotta et al. 2015). Patients with mood disorder are also at higher risk for developing a wide range of medical conditions, including cardiovascular and cerebrovascular diseases, and metabolic syndrome (Cizza 2011; Goldstein et al. 2015).

The pathophysiology of MDD and BD is still unclear. Family studies have provided consistent evidence that genetic factors are implicated in

G. D. Colpo (✉), B. S. Diniz, and A. L. Teixeira (✉)
Neuropsychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA
e-mail: Gabriela.D.Colpo@uth.tmc.edu; Antonio.L.Teixeira@uth.tmc.edu

L. Stertz
Translational Psychiatry Program, Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, USA

their pathogenesis (York et al. 2005; Kendler et al. 2011). Environmental factors influencing pre- and post-natal development have also been associated with mood disorders (Pedersen et al. 2017). In addition, patients with mood disorders present changes in several brain structures (Strakowski et al. 2002; Lyoo et al. 2006; Wise et al. 2016), peripheral levels of inflammatory, oxidative stress and neurotrophic biomarkers (Pfaffenseller et al. 2013; Sayana et al. 2017; de Melo et al. 2017; Castren and Kojima 2017).

Although a reasonable number of therapeutic options exist for MDD and BD, their treatment is still challenging. Treatment response is variable and difficult to predict from the beginning. Treatment resistance is not uncommon, especially for those patients with depressive episodes. There is a critical need to identify more effective therapeutic strategies for mood disorders by targeting receptors and/or signaling pathways beyond the monoamine systems (Papakostas and Ionescu 2015; Colpo et al. 2017). Taking into account the heterogeneous and complex neurobiology and clinical presentation of mood disorders, it is important to develop treatments that modulate specific pathophysiological processes. For example, immune-based strategies should be used for inflammation-related depressive and bipolar illnesses, while circuit modulation for those with identified brain circuitry dysfunctions (Colpo et al. 2017; Riva-Posse et al. 2017).

Despite the great effort and resources invested in research for drug discovery and novel therapeutics, the drug attrition rate remains very high in psychiatry, and only a small percentage of new drugs enter the market (Waring et al. 2015). Accordingly, there is a growing clinical interest in the use of stem cells as therapy, especially for patients resistant to conventional treatments. The objective of the current review is to summarize the potential use of stem cells in mood disorders, highlighting their role as biomarkers, as a new therapy for mood disorders, and as a model for these psychiatric disorders (Fig. 1).

2 Circulating Stem Cells in Mood Disorder Patients

Under physiological conditions, low number of stem cell populations, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), as well as very small embryonic-like stem cells (VSELs) can be detected in the peripheral blood (Borkowska et al. 2014; Borkowska et al. 2016). These number can increase during stress or in response to tissue damage (e.g., heart infarct, stroke, intestinal inflammation, or skin burns) (Ratajczak et al. 2011). The release pattern of these cells into the blood differs according to the condition, therefore, they may be regarded as potential biomarkers.

Previous studies investigated the profile of circulating EPCs in different diseases. There are reduced numbers and/or impaired function of EPCs in cardiovascular diseases, diabetes mellitus and hypertension (Loomans et al. 2005). Some studies have also investigated EPCs in patients with depression, mostly in the context of cardiovascular diseases. They found that patients with cardiovascular diseases and depression present significantly lower levels of circulating EPCs compared with patients with the same cardiovascular problem but without depression (Di Stefano et al. 2014; Felice et al. 2015; Chen et al. 2013).

In 2009, Dome et al. was the first group to describe a decreased number of EPCs in patients with major depression (without medical comorbidity) when compared with healthy subjects (Dome et al. 2009). In 2011, Yang et al. confirmed these results, showing that depression was associated with lower levels of circulating EPCs (Yang et al. 2011). In a subsequent study, Dome et al. did not find any difference in the number of EPCs between baseline and after effective antidepressant treatment in patients with major depression (Dome et al. 2012). As EPCs are involved in vascular integrity (Khakoo and Finkel 2005), these results might suggest that

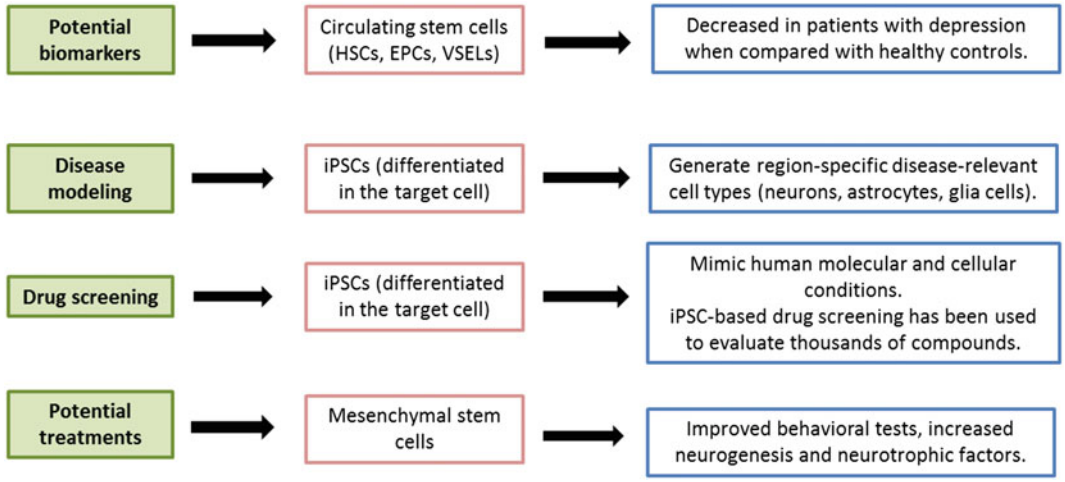

Fig. 1 Potential use of stem cells in mood disorders

mood disorders could contribute to the development of endothelial dysfunction and atherosclerosis. Interestingly, epidemiological studies show that patients with mood disorder are at increased risk of incident atherosclerosis and cardiovascular diseases (Baune et al. 2006).

In BD, there is only one study that investigated the effect of lithium treatment on circulating stem cells in the peripheral blood. In BD subjects not taking lithium, the number of VSELs, MSCs and EPCs was significantly higher than in control subjects. In lithium-treated patients, these values were similar to controls and the number of VSELs correlated negatively with the duration of lithium treatment and serum levels of lithium (Ferensztajn-Rochowiak et al. 2017). These results suggest that treatment with lithium can reduce the number of circulating VSELs, and VSELs may be regarded as a potential biological marker of the illness and its clinical progression.

3 iPSCs as a Tool for Disease Modeling

The identification of better therapeutics for mood disorders has been challenging, and very few effective strategies have been developed in the last decade. Identifying the pathological mechanisms underlying human diseases plays a key role in discovering novel therapeutic strategies. The incomplete understanding of the human brain and the complex genetic basis of mood disorders are some of the obstacles that hinder the progression of research in this area. Another major obstacle is the lack of a valid preclinical model that recapitulates all the aspects of the human disease, especially the different mood polarities of BD. Despite that, animal models have contributed to the understanding of the roles of specific genes, molecular and cellular signaling pathways in mood disorders (Slattery and Cryan 2014). Post-mortem brain tissue is an alternative for studying mood disorders, but it only provides an end-stage picture of long-standing changes in the brain structure at both cellular and molecular levels.

In the last years, the research in pathophysiological basis of diseases has been revamped by the emergence of cellular reprogramming technologies which can turn differentiated somatic cells into induced pluripotent stem cells (iPSCs) by inducing the expression of key transcription factors that define the embryonic stem cell state (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Kunisato et al. 2011). iPSCs have been generated from patients' fibroblasts, keratinocytes, hair follicles, peripheral blood and likely most other cell types (Soliman et al. 2017).

One of the greatest advantages of the iPSC technology for modeling human diseases is that iPSCs contain the entire genetic background of the donor, making the technology particularly suitable for addressing diseases with defined genetic causes. In this sense, these cells are ideally suited to identify alterations in cell behavior, distinguish the affected cell type(s), examine gene expression and identify and test novel signaling pathways. However, epigenetic changes are lost during the reprogramming process. For conditions like mood disorders, in which environmental factors play a major role in epigenetic modifications, this may be of great concern (Soliman et al. 2017). Another challenge in disease modeling with iPSC technology is to generate region-specific disease-relevant cell types. There is an enormous diversity of cell subtypes in the central nervous system, and different psychiatric disorders can target distinct subset of neurons. In order to model their pathogenesis with iPSC technology, it will be necessary to generate the specific neuronal cells targeted by the mood disorders. Due to the complex nature of mood symptoms, involving multiple affective, cognitive and behavioral domains, different subtypes of neuronal cells and glial cells, or even neural circuits, must be take into account for meaningful *in vitro* modeling (Hansen et al. 2011).

A group of researchers created iPSCs from the fibroblasts of patients with BD, and these iPSC model neurons were hyperexcitable, firing action potentials at a higher frequency than control neurons. Accordingly, these neurons showed altered expression of calcium signaling and mitochondrial genes (Mertens et al. 2015). Importantly, this hyperexcitability exhibited by hippocampal neurons was selectively reversed by lithium treatment only in neurons derived from patients who also responded to lithium treatment, but not by neurons derived from non-lithium responders.

Two independent groups proposed protocols for generating serotonergic neurons from iPSC cells. Xu et al. reported that human primary fibroblasts were directly converted to induced-serotonergic (i5HT) neurons by the expression of Ascl1, Foxa2, Lmx1b and FEV. They observed that i5HT neurons expressing markers for mature serotonergic neurons had Ca2 + −dependent serotonin release and selective serotonin uptake, and exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents (Xu et al. 2016). Vadodaria et al. showed that overexpressing the transcription factors NKX2.2, FEV, GATA2 and LMX1B in combination with ASCL1 (Achaete-scute homolog 1) and NGN2 (Neurogenin-2) directly and efficiently generated serotonergic neurons from human fibroblasts. Induced serotonergic neurons showed increased expression of specific serotonergic genes known to be expressed in raphe nuclei. These neurons displayed spontaneous action potentials, releasing serotonin *in vitro* and functionally responding to selective serotonin reuptake inhibitors (SSRIs) (Vadodaria et al. 2016). Serotonergic neurons are dysregulated in depression and are the target of commonly used antidepressants such as the SSRIs. These new methods may help the study of serotonergic neurons from patients, possibly contributing to a deeper understanding of the pathways involved in the phenotypic heterogeneity of mood disorders.

4 iPSCs for Drug Screening for Mood Disorders

Traditional high-throughput screenings for drug use either immortalized cell lines or rodent primary cells which have questionable validity either from artifacts of overexpression or by virtue of simply being nonhuman with poor track records for predicting results of potential therapeutics in clinical trials (Eglen and Reisine 2011; McGonigle and Ruggeri 2014).

The use of iPSCs has become a valuable tool for drug screening due to its specific advantages as a model more tailored to faithfully mimic

human molecular and cellular conditions. iPSCs are a valuable source of primary cells with relatively stable genomes that can be cultured, expanded and differentiated to model different tissue-systems. Also, its intrinsic ability to self-renew allows for scaling up stem cell colonies for high throughput assays and drug screening purposes. However, there are some limitations. The confirmation of the link between genotype and disease can be misleading due to the complexity of the individual's genetic composition and the potential influence of epigenetic changes on disease manifestation. These limitations can increase exponentially when studying complex disorders as mood disorders in which multiple genes and environmental factors seem to play a pathogenic role.

Overall, iPSC-based drug screening has been used to evaluate thousands of compounds for several diseases (Burkhardt et al. 2013; Corti et al. 2015), and some candidates have been identified (Bright et al. 2015; Naryshkin et al. 2014; Mullard 2015). So far no study using iPSCs for drug screening for mood disorder was published. This reflects at least in part the complexity and heterogeneity of these disorders.

Another application of disease-specific iPSCs is in drug repurposing, in which existing drugs already approved for specific diseases are tested to find new applications in other diseases. For example, the anti-epileptic drug ezogabine demonstrated efficacy in an iPSC model of amyotrophic lateral sclerosis and is now undergoing clinical evaluation for this latter indication (McNeish et al. 2015).

Although a great progress has been made with iPSCs technology and drug discovery platforms, there are still several challenges that need to be addressed. First, cell-type heterogeneity reduces the chances of identifying a positive hit, illustrating the need for better differentiation protocols. Second, the culture conditions need to be controlled and standardized. Third, phenotypic assays need to be robust and should be disease-relevant. Finally, animal models are still required for the validation of positive hits from iPSC-based screening platforms. Despite these challenges, this technology holds great promise as a new translational platform for drug testing using human neurons.

5 Stem Cells as an Alternative for Treatment Resistant Patients with Mood Disorders

Mesenchymal stem cells (MSCs) are possibly the best example of stem cells with potential therapeutic implication for mood disorders. MSCs are multipotent progenitor cells that have the capacity to differentiating into all lineages of mesodermal origin, e.g. cartilage, bone, and adipocytes (Pittenger et al. 1999). Studies have shown that MSCs are also able to differentiate into cells from sources other than the mesoderm, such as neurons and hepatocytes (Dezawa et al. 2004; Hermann et al. 2004).

Clinical interest in the use of MSCs has increased significantly in the last years as they present ideal characteristics for regenerative medicine such as plasticity and high self-renewal capacity. In addition, MSCs are known for their ability to promote the neurogenesis of primary neural progenitors and survival of neural cells by expressing soluble factors, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-1) (Crigler et al. 2006). As a result of their immunomodulatory properties, they can also prevent apoptosis and decrease inflammatory responses (Crigler et al. 2006; Yoo et al. 2008). Besides soluble factors, MSCs release membrane-derived extracellular microvesicles (ExMVs) that can deliver mRNA, miRNA, and functional proteins to target cells, thereby additionally promoting cell survival and proliferation (Ratajczak et al. 2016; Ratajczak and Ratajczak 2016, 2017). All these paracrine effects mediated by soluble factors and/or ExMVs seem to be the main factors responsible for the positive results observed in patients after systemic or local stem cell therapies (Ratajczak et al. 2016; Ratajczak and Ratajczak 2016).

Based on the evidence that mood disorders are associated with a low-grade inflammation and a reduced neurotrophic support (van den Ameele

et al. 2017; Zhang et al. 2016), MSCs may be a promising therapeutic strategy for those conditions. However, only a few studies have evaluated the effects of MSC in pre-clinical models of mood disorders.

Tfilin et al. showed that the intracerebroventricular administration of MSCs improved depressive-like behavior and increased hippocampal neurogenesis in a genetic animal model of depression (Flinders sensitive line, FSL rats). The increase in hippocampal neurogenesis suggests that this can be the antidepressant mechanism of MSCs transplantation in the rat brain (Tfilin et al. 2010). These results confirmed prior studies showing increased neurogenesis after implantation of human MSCs into the dentate gyrus of mice and after transplantation of neural progenitors into the hippocampus of prenatally heroin-exposed mice (Munoz et al. 2005; Ben-Shaanan et al. 2008). More recently, Shwartz et al. also treated FSL rats with intracerebroventricular MSCs. They showed that MSCs administration attenuated depressive-like behaviors as assessed by the forced swim test, novelty exploration test and sucrose self-administration paradigm (Shwartz et al. 2017). Conversely, another study showed that intra-hippocampal transplantation of MSC enhanced neurogenesis in wild-type rats. In order to examine the behavioral consequences of intrahippocampal MSC transplantation in the rats, they assessed locomotion, learning and memory, and anxiety-like and depression-like behavior, but they did not observe any behavioral change (Coquery et al. 2012).

To the best of our knowledge, no stem cell therapies have been performed in patients with mood disorders. Based on the promising results from studies with MSCs in patients with multiple sclerosis, amyotrophic lateral sclerosis (Llufriu et al. 2014; Connick et al. 2012; Karussis et al. 2010; Sykova et al. 2017) and Parkinson's disease (Venkataramana et al. 2010; Canesi et al. 2016), conditions marked by significant mood symptoms (Kummer and Teixeira 2009), the investigation of the therapeutic potential of MSC for mood disorders is definitely warranted.

There are significant challenges to treat these patients with stem cells. First, it is important to decide which the patients are eligible for receiving the MSCs (e.g. refractory to conventional treatments). Another challenge refers to the schedule of administration of autologous stem cells to maintain clinical improvement, i.e. whether single or repetitive doses are necessary for remission.

6 Conclusion

The understanding of the pathophysiology and the development of new therapies for mood disorders have been a great challenge in psychiatry. The heterogeneity and complexity of these disorders along with the lack of valid and reliable biomarkers have hampered the advance of the field.

Stem cells have already influenced several areas of medicine. Stem cells as therapy have been used to treat human diseases such as metabolic, cardiovascular and neurodegenerative diseases. Regarding mood disorders, stem cells have only been used in pre-clinical studies. Based in the results obtained in other areas, especially neurodegenerative diseases, stem cells seem to be promising, especially for treatment-resistant patients with mood disorders.

iPSCs can reveal new relationships between disease phenotypes and gene expression profiles, which have broadened and deepened the understanding of different diseases. However, investigating mood disorders that are seemingly caused by multiple genes and/or have multiple phenotypes remains a challenge for iPSC models. With further advancement in stem cell technology, iPSCs will probably contribute to the refinement of mood disorder pathophysiology.

Conflicts of Interest The authors declare that they have no conflict of interest.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 97–113
https://doi.org/10.1007/5584_2018_279

Published online: 26 September 2018

Cancer Stem Cells in Metastasis Therapy

Esra Aydemir Çoban and Fikrettin Şahin

Abstract

Tumors consists of subpopulation of cells in which each subtype has contributes to tumor progression. Specifically one subtype known as cancer stem cells are associated with the initiation, progression, resistance to conventional therapies and metastasis. Metastasis is leading cause of cancer related deaths. Overall it is important to consider cancer as a whole in which a mutated cell proliferating indefinitely and forming its hierarchy consisting of subgroups with different molecular signatures. To be able to target this disease we need to evaluate every step including initiation, progression, survival, angiogenesis and finally migration and repopulation. Cancer stem cells do play vital roles in each step however when metastasis can be stopped or eliminated we talk about saving a life or improving its quality. Considering how deeply these cancer stem like cells affect the tumor life and metastasis it is crucial to develop effective strategies against them. Metastatic cascade can also be directed by membrane derived vesicles specifically exosomes. Several studies show the role of exosomes in mediating cellular migration and pre-metastatic niche formation. During this chapter we wanted to explain in detail how the metastasis occur in tumor and how cancer stem cells contribute into the development of metastatic cascade and possibly suggest therapeutic approaches against cancer stem cells.

Keywords

Cancer stem cells · Metastasis · Cancer therapy

Abbreviations

CSCs	cancer stem cells
MMPs	matrix metalloproteinases
EMT	epithelial-to-mesenchymal transition
ALDH1A1	Aldehyde Dehydrogenase 1 Family Member A1
RTKs	receptor tyrosine kinases
MICs	metastasis-initiating cells
TICs	tumor-initiating cells
hWAPL	human wings apart-like
HPV	human papillomavirus
Pdcd4	programmed cell death protein 4
CXCL12	chemokine stromal cell-derived factor 1
VEGFR-1	vascular endothelial growth factor receptor 1
CDs	cluster of differentiation
CXCR4	chemokine receptor complex 4
CAFs	cancer associated fibroblasts
IGF-1	insuling growth factor 1

E. Aydemir Çoban and F. Şahin (✉)
Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey
e-mail: esra.aydemir@yeditepe.edu.tr; fsahin@yeditepe.edu.tr

IL-17A	interleukin 17A
DTC	disseminated tumor cell
MIF	migration inhibitory factor
RARRES3	Retinoic Acid Receptor Responder 3
NKX2-1	homeobox domain-containing transcription
MITF	microphthalmia-associated transcription factor
EVs	Extracellular vesicles
ABs	apoptotic bodies

1 Introduction

Tumor tissues are comprised of heterogeneous populations in which stem cells play important roles including the maintenance of the integrity of tissues. Since their discovery cancer stem cells are investigated deeply and thousands of studies have been done to target these stem like cells in cancer. Many therapeutical approaches that target those stem like cells hold promise in the fight of cancer. Heterogeneous tissue is composed of various subtypes of populations where cancer stem like cells exist in rare numbers but stay in quiescent state. Two distinct properties of these stem like cell population are the self-renewal capacity and differentiation into more mature and committed cells. Moreover stem like cells in tumor tend to cause more clonogenic and tumorigenic behavior overall. Interactions with stromal elements like fibroblasts and following various cascade of signaling events affect the stem cells physiology and may cause the initiation and continuation of tumors. Based on these knowledge cancer stem cell theory has been developed and studied for years. According to this, neoplasms are comprised of hierarchically organized cells and a group of cells called, cancer stem cells (CSCs) that may be the origin of initiation of the tumor, which can also recapitulate the entire tumor under favorable conditions.

CSCs are designated as the only subpopulation that are associated with tumor-initiation and they can also be responsible for the metastases. In a study done by Herman et al. it has been shown that CSC do play significant role in pancreatic tumor growth and they also present a side population of migrating stem like cells in the metastasis (Hermann et al. 2007). They finally concluded that CSCs may be the ones that initiate metastatic cascade.

Metastasis is a series of event where a circulating single or cluster of cancer cells detach from the primary tumor bulk by digesting extracellular matrix metalloproteins (MMPs) and through invasion reach the blood or lymphatic system and intravasation into the vasculature. In there, single or cluster of these cancer cells survive against harsh conditions of the blood and finally through extravasation leave the blood and settle into the final destination where a new colony will form. In this chapter we will focus entirely on the roles of CSCs in the metastatic cascade along with their association with the tumor microenvironment so called niche.

1.1 Stem Cells

Stem cells are special cells with potentials to self-renew and differentiate into other lineages in such conditions such as organ development and tissue repair. This mechanism is also being favored by cancer cells when needed.

In recent years a great effort has been made to be able to distinguish stem cells from non-stem cells. Especially in tumors it is very important to separate cancer stem cells in the population so that these cells can be targeted. Based on this many approaches such as differentiation and antibody directing therapies have been developed to target cancer stem cells. Presence or absence of certain markers were discovered and new ones are under investigation. But there is not a clear cut distinction between cancer stem cells and normal stem cells. However some markers are certainly more or less specific for different types of stem cells like the ones that separate embryonic stem cells from adult stem cells or pluripotent cells from progenitor ones. When it comes to cancer stem cells it is more difficult to distinguish them from non-cancerous stem cells due to the

presence of the same markers being expressed on both of the cell types. It is known that almost all the markers are expressed by normal stem cells as well as cancer stem cells therefore for scientists it is challenging to find new candidates as tumorigenic markers. Presently the ultimate choice for a therapeutic target depends on onco-fetal stem cell markers because they are not expressed on normal adult stem cells.

1.2 Cancer Stem Cells (Discovery and Its Origin)

The exact origin of CSCs is an unclear issue however hypotheses were recommend from time to time. Currently we know that there are three main hypotheses and the first one is the transformation of normal stem cells. The theory suggests that CSCs is transformed from normal stem cells, and through series of differentiation processes and accumulation of mutations they will contribute to cancer progression. These mutations are adequate enough to induce malignant transformation and bear tumor growth (Krivtsov et al. 2006; Scheel et al. 2011; Jordan 2009). While this theory encounters the clonal evolution theory, which indicates that all cancer cells have tumorigenic potential with a potential to recapitulate the entire tumor (Nowell 1988), development in the cancer stem cell field states that these two models share common mechanisms therefore it is suspense through which mechanisms one stem cell become cancer stem cell (Cabrera et al. 2015; Plaks et al. 2015). One can conclude that there is a dynamism in transformation of non-CSC to a CSC state, vice versa.

This transformation occurs very rarely and spontaneously and a variety of factors including inflammatory cell infiltration, chemokines and hypoxia can induce this event (Chen et al. 2016). Gaining stem cell characteristics also require signals from tumor niche (Pattabiraman and Weinberg 2014) as well as interactions amongst the cells within the tumor that might also regulate stemness of the tumor (Plaks et al. 2015). The former also is known as the second theory cancer stem cell in which of mature cancer cells dedifferentiate into cancer stem cells through a process called epithelial-to-mesenchymal transition (EMT) (Pattabiraman and Weinberg 2014). This transient state is critical not only for the survival of cancer cells, but also for metastatic progression. And the last hypothesis is the introduction of induced pluripotent cancer cells. Malignant transformation of adult stem cells into cancer stem cells were proposed by several researchers.

EMT is also another factor that affects the progression of cancer progression and eventual metastasis (Mani et al. 2008). During EMT cancer cells gain characteristics of normal stem cells including differentiation and self-renewal. Loss of polarity and cell-cell contact and alterations in the cytoskeletal structures cause cancer cells become motile and resistant to cellular death (Mani et al. 2008). These features allow CSCs to initiate new tumors that's why they are called tumor initiating cells (Reya et al. 2001). Up to date several surface markers were described as cancer stem cell markers including; CD44, CD24, CD34, CD133 and CD117, and Aldehyde Dehydrogenase 1 Family Member A1 (ALDH1A1) (Gottschling et al. 2012). Various signaling pathways and growth factor receptor tyrosine kinases (RTKs) induce EMT. One of the most important signaling pathway proteins is Transforming Growth Factor beta (TGF-β). It is the most and widely studied protein and it has been shown that TGF β phosphorylate and activate Smad2a and Smad3 which in turn form trimers with Smad4 and cause a translocation to the nucleus for the regulation of TGF-β target genes (Fuxe et al. 2010). Other proteins like Notch and Wnt also are collaborated with TGF-β to induce EMT (Shin et al. 2010; Eger et al. 2004; Timmerman et al. 2004). As major regulators of EMT SNAIL, SLUG, TWIST and ZEB transcription factors are well characterized and studied (De Craene and Berx 2013). When activated these factors can suppress epithelial markers like E-cadherin and upregulate mesenchymal markers such as N-cadherin and vimentin. Activation of other signaling molecules like

Ras/Raf/MAPK, PI3K/Akt can aid these processes (Valcourt et al. 2005; De Craene and Berx 2013).

It has been more than 50 years of research since the discovery of cancer stem cells based on the similarity between cancer and embryonic development processes. Throughout these years a number of tumors were found to be associated with cancer stem like cells as a driving force. Tumors that may contain the traces of cancer stem like cells include leukemia (Bonnet and Dick 1997) and solid tumors such as bladder cancer (Chan et al. 2009), breast cancer (Al-Hajj et al. 2003), malignant melanoma (Schatton et al. 2008), ovarian cancer (Zhang et al. 2008b), head and neck cancer (Prince et al. 2007), pancreatic cancer (Hermann et al. 2007), Central Nervous System (CNS) cancers (Singh et al. 2004), colon carcinoma (Dalerba et al. 2007), liver cancer (Zhang et al. 2008b), Ewing sarcoma (Suva et al. 2009), and chordoma (Aydemir et al. 2012). The primary studies based on hematological diseases show that a subset of cells found in the tumor heterogeneity drive the tumor development and relapse.

Unlike liquid tumors it is very challenging to detect cancer stem cells in solid tumors due to loss of specific markers present in cancer stem cells. Among specific markers CD133 is the one very speculative in which colorectal carcinoma studies indicate that CD133 negative colorectal cancer stem cells recapitulate the entire tumor. (Shmelkov et al. 2008; Ren et al. 2013).

Several analytical methods and approaches have been developed to detect and characterize CSCs. Most used techniques for detection and isolation CSCs are functional, molecular, and cytological and filtration approaches as well as functional methods and ultimately xenotransplantation studies hence animal modeling. Assays such as colony and sphere formation, side population analysis, aldehyde dehydrogenase activity and drug therapy resistance are being practiced regularly to distinguish cancer stem cells from non-CSCs. Innovative techniques like cell sorting based on magnetic capturing of fluorescent conjugated antibodies based on certain cell surface proteins pioneered a new era in the scientific world. With this technology it became possible to separate CSCs from non-CSCs among heterogeneous cell populations in a more reliable way. (Kentrou et al. 2011; Greve et al. 2006).

In addition to these techniques gene expression analysis by multiplex reverse transcription quantitation, immunocytochemistry, immunohistochemistry and immunofluorescence are the common molecular methods to characterize CSCs. (Lianidou and Markou 2011) As for functional assays the gold standard one is xenotransplantation into immune-deficient animals in which cancer stem cells being transplanted into immune-deficient animal and let them reform the original tumor. (Fulawka et al. 2014) All of these techniques and cons and pros based on their set up but the better and more reliable method is to combine them appropriately. Overall functionally CSCs are defined by their capability to begin tumors in immune-compromised/deficient mice upon serial injections which is the indicators of self-renewal, differentiation into multilineages to form the tumor entity. (Korkaya et al. 2008; Ginestier et al. 2007).

1.3 Cancer Stem Cells in Metastasis

In 1889 Paget proposed the hypothesis of Seed and Soil, which is coherent with the CSC model [45]. In his hypothesis, a CSC is the seed where it is nourished by the soil known as the metastatic site. This new environment is the niche where the growth of CSCs will be promoted. Epigenetic and genetic alterations will also take place to lead CSCs drive the tumor and these changes ultimately affect the phenotype of the primary tumor. So the new metastatic tumors are known to arisen from the CSCs. Based on a colorectal cancer model Brabletz et al. suggested that tumor entity possesses a heterogeneous bulk where part of cells play roles in proliferation and cell cycle arrest where others in epithelial to mesenchymal transition, cell adhesion and spread. He further proposed that all of these events are orchestrated to push for tumor progression by a subset of cells called “migrating cancer stem cells”(Brabletz et al. 2005).

Metastasis is a multistep process that begins with the invasion of cancer cells to nearby tissues locally. These metastasis-initiating cells (MICs) are able to seed clinically important metastatic colonies in other organs and tissues of the body. Like the tumor-initiating cells (TICs), MICs can take over some of the normal stem cell pathways, increase cellular plasticity and stem-ness. MICs also must hold additional competences which will allow them to survive the metastatic cascade and act as TICs in an organ microenvironment characteristically different from the primary tumor. These cells are exceptionally difficult to identify, capture, and characterize but they certainly create a relationship between the primary tumor and following metastasis. Even the source of MICs remains indefinable; they might occur at the primary tumors or appear later in the metastatic cascade or even acquire features when reach to final destination. MICs share common characteristics with cancer stem cells therefore tools to analyze and identify cancer stem cells are also used for MICs including *in vitro* tumor sphere assays, in vivo dilution tumor initiation studies, analyzing cancer stem cell (CSC) cluster of differentiation (CDs) markers (Celia-Terrassa and Kang 2016).

Malignant tumor cells first lose their cell-cell adhesion capacity and detaches from the primary tumor bulk. Through alterations between cell and their extracellular matrix interactions, cells find their way to invade the adjacent stroma, a process called invasion. Basement membrane and extracellular matrix are degraded by substances in addition with the expression as well as suppression of proteins associated in motility and migration (Cooper et al. 2003). In a breast cancer study done by Dustin et al. nuclear translocation was found to be a major rate limiting factor for CSC spreading. They further suggested that cytoskeletal elements like myosin IIB, which was upregulated in CSCs, might targeted against cancer stem cell dissemination from the primary tumor site (Thomas et al. 2016). One of the cancer stem cell characteristics, pluripotency was shown to decrease in cervical tumor spheres after knockdown with human wings apart-like (hWAPL) and human papillomavirus (HPV) indicating that suppression of hWAPL expression decreased HPV E6 levels and consequently inhibited tumor invasion in mice suggesting that hWAPL is a cervical CSC marker for proliferation and a promising target for therapeutics (Gong et al. 2017). CD133 and CD44 are discovered surface markers for the identification of colorectal cancer stem cells (O'Brien et al. 2007; Chu et al. 2009). It was shown that expression of both of these markers were found to be associated with liver metastasis in colon cancer patients (Jing et al. 2015). In a similar study done by Jiang et al. gastric cancer stem cells positive for CD26 and chemokine receptor 4 (CXCR4) were involved in invasion and metastatic ability (Jiang et al. 2017).

Next, cells must reach the nutrient and oxygen through a step called angiogenesis so that growing cancer cells will be nourished their toxic waste will be removed (Ellis and Fidler 1996). Cancer stem cell phenomenon clashed with the hypothesis of angiogenic behavior of tumor bulk as different parts of it show variety in levels of oxygen. This was shown by Folkman et al. that heterogeneous population of human liposarcoma cells reflect the angiogenic capacity variously when implanted into mice such that one subpopulation (so called cscs) give rise to highly angiogenic whereas others (non-cscs) develop poorly angionegnic tumors or even non angiogenic (Achilles et al. 2001). A different study has shown that the reason for tumor relapse and metastasis is linked to cancer stem cells (CSCs), under control of numerous mechanisms like elevated levels of angiogenesis (Folkins et al. 2009).

Intravasation is the step where cancer cells enter into circulatory system and survive in it. In a study investigated by Asangani et al. post-transcriptional regulators such as miR-21 plays in important role in invasion or intravasation by regulating and targeting programmed cell death protein 4 (Pdcd4) in colorectal cancer (Asangani et al. 2008). In the blood invaded cells resist despite the harsh condition such as high blood pressure rate and platelets by interacting with endothelial cells forming stronger bonds and by penetrating the base membrane and endothelium leaves the blood vasculature at a distant organ by extravasation (Chay et al. 2002), finally settle into

the new environment and build its colony (Chambers et al. 2002; Wirtz et al. 2011). Adaptation of the cells into new site is driven by CSCs (Reya et al. 2001; Tu et al. 2002).

Since CSCs have self-renewal and clonogenic capabilities they are more likely to develop metastatic behavior. In deed CSCs present a varying degree of motility and invasion Moreover, CSCs should have some degree of motility and invasion to spread a distant site (Brabletz et al. 2005). Based on the similarity in the migration of normal stem cells and cancer stem cells, it has been recently suggested they share a same mechanism, which is upregulation of the chemokine stromal cell-derived factor 1 (CXCL12) and its G-protein-coupled receptor CXCR4 (Kucia et al. 2005). Previous studies proposed that hepatocyte growth factor (HGF) and its receptor MET have a parallel function in driving the recruitment and migration of normal stem cells together with cancer stem cells. In the embryonic term, MET in response to HGF expression causes a migration of embryonic cells for a successful development as similarly observed in adults where bone marrow stem/progenitor cells (Andermarcher et al. 1996; Bladt et al. 1995; Takayama et al. 1996) express MET in response to HGF gradients to wounded tissues for repair. Upto date, it is not for certain that the overexpression of MET expression is associated with CSCs. However, according to the theory of stem cell plasticity caused by the malignant transformation of normal stem cells if cancer stem cells originate from the malignant transformation of normal stem cells, we can accept the fact that MET expression might enable CSCs to shift to the invasive program (Pardal et al. 2003; Reya et al. 2001). MET has been known as an oncogene and this brings with a dual role as in the initiation as well as clonal selection. It also has been proposed that independent of the oncogenic events wild-type MET can enhance motility, invasion and metastasis of CSCs (Lorenzato et al. 2002). When MET is overexpressed it causes cells to become sensitive to HGF and invasive signaling so that microenvironment can promote metastasis (Mueller and Fusenig 2004). When other oncogenes including Ras, RET, and ETS become activated and together with other mitogenic signals stimulating MET transcription (Boccaccio et al. 1994; Gambarotta et al. 1996; Ivan et al. 1997) occur, MET overexpression is considered as a consequence however it absolutely has a key role in cellular metastasis.

1.4 Tumor Niche and Cancer Stem Cells

The tumor microenvironment is embed in a non-cellular matrix and comprised of non-cancerous cells including fibroblasts, immune cells, endothelial cells. These components build the tumor stroma which alters as tumor progresses and grows and eventually become drug resistant (Egeblad et al. 2010; Junttila and de Sauvage 2013). Tumor niche nourishes the cancer stem cells by releasing a variety of factors that will protect them immune attach and keep their plasticity maintaining their properties (Lloyd et al. 2016). As for metastatic preference certain growth factors are begin released by tumor stroma for the direction of the primary tumor cells to the secondary tumor site as in the case of cancer associated fibroblasts (CAFs) in the primary breast cancer secreting CXCL12 and insuling growth factor 1 (IGF-1) which will stimulate bone metastasis (Zhang et al. 2013; Zhang et al. 2009). In a similar example, CAFs secrete hepatocyte growth factor that will stimulate CSCs to self-renew promoting the reprogramming of colorectal cancer progenitor cell into CSCs through the signaling of β-catenin pathway (Vermeulen et al. 2010). After chemotherapy treatment certain cytokines specifically interleukin 17A (IL-17A) is being released that contributes self-renewal trait of colorectal CSCs promoting invasion (Lotti et al. 2013). This is an indication of how chemotherapy re-shape the tumor niche and aid tumor progression therefore tumor microenvironment might be altered as chemically (Zeuner et al. 2014).

2 The Role of CSCs in Modulating the Tumor Microenvironment Through Secretion of EVs

It has been known for long that during apoptosis cells release vesicles to the extracellular environment. Comprehension of healthy cells secreting the similar vesicles is also considered currently by the researchers and they used the generic term for these vesicles as extracellular vesicles. Extracellular vesicles (EVs) contain at least three sub-classes namely exosomes, microvesicles (MVs), and apoptotic bodies (ABs). Exosomes are made by budding of endosomal membrane inwardly, while microvesicles (MVs) are formed by budding directly from the plasma membrane. Apoptotic buddies, on the other hand, are made during programmed cell death. Their size, structures and functions are being evaluated consistently. Origin of EVs whether derived from normal cells or cancer cells differ in molecular markers which will affect the function of it in the recipient cells. Differences in molecular signatures of these EVs may help in diagnosis as well as prognosis in a variety of cancers. Exosomes have a distinctive role as a cargo during cell to cell communication in which they carry almost any molecule. In cancer through exosomes cells contact one another which will aid in metastasis, drug resistance and even immunology (Milane et al. 2015). In a study done by Ono et al., increased expression miR-23b and decreased expression of MARCKS were found in bone marrow of a metastatic breast cancer patient suggesting that exosomal transfer of miRNAs from the bone marrow might be endorsing breast cancer cell latency in a metastatic environment (Ono et al. 2014).

Exosomes are carried from original cells to final destination through the circulatory system and localized there by binding to cell surface through their membrane proteins that will be recognized by the recipient cells. Taylor et al., showed that greater levels of exosomes were found in body fluids of cancer mouse models and cancer patients (Taylor and Gercel-Taylor 2008; Ghosh et al. 2010). Exosomes play active roles in cancer progression. Studies indicate that exosomes derived from mesenchymal stromal cells (MSC) or fibroblasts secrete various miRNAs and soluble factors which were delivered into tumor cells that enable cancer progression and cause drug resistance in several cancers including multiple myeloma, colorectal cancer, and gastric cancer cells (Roccaro et al. 2013; Hu et al. 2015b; Ji et al. 2015) advantaging tumor survival and growth. Cancer cell-derived exosomes can favorably fuse with the cells to form a pre-metastatic niche for metastasis (Hoshino et al. 2015). Also these cancer derived exosomes may turn normal epithelial cells into cancerous cells as shown in murines (Melo et al. 2014). Taken together, exosomal delivery to drive tumorigenesis is a very common and popular field of interest that capture researchers' attention for not too old. Exosomal delivery of therapeutics even became popular in cancer treatment (Seow and Wood 2009; Camussi and Quesenberry 2013).

Exosomes derived from cancer stem cells drive an activated angiogenesis, which will lead stimulation of normal endothelial cells to grow and form vessels resulting metastasis and tumor progression (Grange et al. 2011). Mesenchymal stem cells facilitate EMT and induce stem like properties which will allow cancer stem cells to increase survival in the circulatory system. The role of CSC derived exosomes in metastasis is that they cause tumor reseeding and pre-metastatic niche formation similar to MSC-derived exosomes. For instance in a study done by Wang et al., gastric cancer (GC) MSC-derived exosomes were detected to transport miR-221 to HGC-27 cells aiding proliferation and migration (Wang et al. 2014).

3 Organ Specific Metastasis

Tumors can prefer specific organs depending a number of factors. This selection of metastasis to certain organs is called organotropism (Tayyeb

and Parvin 2016). There are two main hypotheses that might enlighten organotropism one being an anatomic circulation system, which is tumor cells spread into lymphatic system and followed by a distant spread by the vascular system (Hess et al. 2006). The first hypothesis is logical but does not explain all that metastatic patterns of certain cancers in the body. According to the first hypothesis liver and brain take same amount of blood in volume but differ in metastatic patterns (Obenauf and Massague 2015; Budczies et al. 2015). This leads the scientific world to question whether other possible mechanism might have a role in selection organs to metastasize. The second hypothesis lays underneath the "seed-and-soil hypothesis" which, indicates that metastatic tumor cells can be fed and grow only in accepting tissues with organ-specific "soils" [78]. In this regard we can conclude that metastatic preference is a combination of spreading of tumor cells through vasculature and lymphatic system and also circulating tumor cells (CTCs). In this chapter we will focus more on CSC which share similar features with EMT and CTCs (Kasimir-Bauer et al. 2012; Sun et al. 2011).

3.1 Metastasis to Bone (s12943)

The study done by D'Amico et al. indicates that breast CSCs-like have a tendency to metastasize bone with a mesenchymal and migratory $CD44^+$ $CD24^-$ phenotype suggesting that breast CSCs favors the bone as a soil to metastasize (D'Amico et al. 2013). This migration is supported by vascular endothelial growth factor receptor 1 (VEGFR-1) expressed by the bone marrow-derived hematopoietic progenitor cells to form clusters and fibronectin (Kaplan et al. 2005). Osteogenic environment also induces colonization by adherens junctions like osteogenic N cadherin E-cadherin derived from cancer cells and ultimately initiate mTOR pathway and additional oocyte secretion of factors including CCL5, MMP and extracellular ATP (Sottnik et al. 2015) promote tumor progression (Wang et al. 2015).

Presence of the recognized stem/progenitor cell ($CD44^+CD24^-$) subpopulation is primary found within the disseminated tumor cell (DTC) component in bone marrow by Balic et al. In their study they showed that breast cancer stem cell phenotype was described as CK+ in all of their patients. It has been known that majority of the patients with DTC may have a lifetime risk for relapse (Dearnaley et al. 1991).

3.2 Metastasis to Liver

Usually cancer cells that migrate to liver as a metastatic site are not known as liver cells rather different parts of the body where the primary tumor initiated. Metastatic liver cells are considered to cause the advanced stage of the tumor. As for migration tendency hepatic stellate cells are known to play significant roles in preparing the pro-metastatic environment (Eveno et al. 2015). In a recent study done by Nielson, secreted granulin by macrophages excites hepatic stellate cells to release of periostin so that fibrotic niche in the liver provides metastasis (Nielsen et al. 2016). Studies indicate that subpopulation of $CD26^+$ cells present in the primary and metastatic tumors in colorectal cancer patients cause liver metastasis suggesting that $CD26^+$ CSCs indicate greater potential for invasion and migration (Pang et al. 2010). In a similar study done on colon cancer patients that has $CD133^+/CD44^+$ genotype seem to possess metastatic properties to liver (Bellizzi et al. 2013). Other subpopulations including $CD133^+CXCR4^+$ may increase the tendency to metastasize to liver and cause reduced two-year survival rate in colon cancer patients (Zhang et al. 2012). A expressional correlation between $CD133^+$ (Horst et al. 2009) and Nanog (Ibrahim et al. 2012), which are important cancer stem cell markers, in colorectal cancer cells are found to be involved in the liver metastasis (Xu et al. 2012). Inhibitory factors such as macrophage migration inhibitory factor (MIF) also play roles in stimulating hepatic cells to be migratory, proliferative and apoptotic resistant in colorectal cancer cells (Hu et al. 2015a).

Colonization of colorectal cells in the liver are induced by accumulation of soluble factors like angiopoietin-like 6 protein in hepatic blood vessels (Marchio et al. 2012).

3.3 Metastasis to Brain

Brain metastases were thought be associated strongly with astrocytes (Barros et al. 2014). It has been known that astrocytes are part of the brain microenvironment and do play and important role in facilitating metastasis. Secretion of IL-23 from astrocytes upregulates the MMPs specifically MMP2 to improve the metastasis of melanoma cells to brain (Klein et al. 2015). An important study has been done by Lin Zhang et al. who demonstrated that astrocytes cause loss of PTEN in tumor cells by secreting exosomal miRNA leading a permissive metastatic microenvironment for cancer cells. In their study they showed that signals that from the brain niche are received by cancer cells causing the secretion of chemokines especially one named CCL2 stimulating development mechanistically, cancer cells receive signals from the brain microenvironment that lead to metastatic cells (Zhang et al. 2015).

Besides these extracellular factors generated from brain microenvironment other cellular effects take place in the brain metastatic cascade. Tumor initiating cells share a common mechanism with the metastasis (Croker and Allan 2008). Only very few amount of cells that are shed from the primary tumor can survive in the vasculature system, metastasize and form their colony as the secondary tumor (Kienast et al. 2010; Luzzi et al. 1998). Studies have described CSCs being involved in the increased adhesion, migration, invasion and development of metastases (Croker et al. 2009; Liu et al. 2010; McGowan et al. 2011; Davis et al. 2010). The presence of cancer stem cells and metastasis in lung tumor led idea that metastasis to brain from lung might be involved in cancer stem cells (Nolte et al. 2013). In that study Sara et al. showed that brain metastases from lung presents cells having self-renewal and sphere forming capacities, which are CSC properties.

Cancer stem cells are found to act in an organ specific manner to lead tumor cells for metastasis to brain. Okuda et al. shows that CSCs characterized with a CD24−/CD44+/ESA+ genotype from metastatic breast cell lines are significantly more metastatic than non-CSC populations. They reasoned this by conclusion of lower level of miR-7 which targets and inhibits an induced pluripotent stem cell marker, KLF4 expression causing significantly and inversely correlation to brain but not bone metastasis in animal models (Okuda et al. 2013).

3.4 Association of CSCs in Metastasis Therapy

Heterogenic subpopulation specifically called CSCs is measured based on the ability to seed tumors at limiting dilutions in animal models. Enriched cell populations by CSC also display certain properties in vitro. For instance, CSC-enriched subgroups can be isolated with cell-surface markers as described previous stem cell based studies (Al-Hajj et al. 2003; Li et al. 2007; Ricci-Vitiani et al. 2007; Singh et al. 2003; Zhang et al. 2008a). As an example breast CSCs are enriched in the $CD44^{+}/CD24^{-}$ side populated cells (Al-Hajj et al. 2003). Another property CSCs has is their ability to form sphere or tumor-spheres in CSC-enriched tumor cells (Dontu et al. 2003). Lastly, CSC-enriched populations are highly resistant to conventional therapeutics and ionizing radiation CSC-enriched populations exhibit increased resistance to chemotherapeutic agents (Bao et al. 2006; Dean et al. 2005; Diehn and Clarke 2006; Eyler and Rich 2008; Li et al. 2008; Zhang et al. 2008a) and ionizing radiation (Diehn and Clarke 2006; Woodward et al. 2007).

Available treatment methods could be possibly improved by targeting CSCs to reduce the possibility of recurrence and metastasis. Automated screening technologies are found to be enabling the identification of agents that kill CSCs. Due to its heterogenic structure of tumor bulks one can not selectively kill only CSCs since they only comprise a small portion of the entire population. Therefore

standard cell viability assays should not be applied to tumor as a whole and only CSC-specific toxicity should be identified. As long as highly enriched populations of cancer stem cells are screened then one can surely target cancer stem cells who are known to be responsible for initiation and progression of the tumor. Although selective treatment seems promising it is not applicable for current solid tumors since cancer stem cell enrichment is lost in vitro culture as shown by Fillmore et al. during breast cancer stem cell studies (Fillmore and Kuperwasser 2008).

In 2008 Mani et al. showed that EMT in normal as well as cancer with epithelial origin causes the enrichment of cells with stem-like features (Mani et al. 2008). In their study Gupta et al. sowed that extrinsically induced EMT led increase in drug resistance. They further applied a chemical screening to assess novel therapeutic agents causing toxicity on selected cell populations. They concluded that new agents to target breast CSCs selectively was possible (Gupta et al. 2009).

Loss of differentiation ability, which is a typical stem cell characteristic leads to de-differentiaiton phenotype and ultimately stem cell-like traits associated with metastasis (Cao et al. 2014). In a related study done by Morales et al. Retinoic Acid Receptor Responder 3 (RARRES3) might be potential biomarker and when downregulated it caused a suppression in lung metastasis from breast cancer and considered as an differentiation (as adjuvant) therapy promoting tumor differentiation (Morales et al. 2014). Induction of dedifferentiation and stem cell-like properties aids in promoting lung metastasis so by loss of homeobox domain-containing transcription NKX2-1, a lung lineage-specific transcription factor lung adenocarcinoma, genetically leads an increase in metastatic seeding (Winslow et al. 2011). Li et al. showed that in parallel with other factors like lineage-specific transcription factors (FOXA2 and CDX2), NKX2-1 repressed lung metastasis (Li et al. 2015). It is very crucial to target lineage cell fate related genes since they promote differentiation and inducing stem cell characteristics that promote lung metastasis. In a very similar study done by Cheung et al. two differentiation transcription factors named GATA6 and HOPX synergistically work as inhibitors of metastatic progression (Cheung et al. 2013). Another differentiation factor found to be lost in melanoma is microphthalmia-associated transcription factor (MITF) so targeting this pathway might benefit in the design of new melanoma therapies (Cheli et al. 2011).

4 Future Perspectives

Metastasis related death is the major challenge in cancer therapy. Therapies targeting tumor initiation, progression and finally metastasis were investigated and novel methodologies were developed in years however there is still a long way to go against cancer battle. CSCs play crucial roles in aiding throughout the tumor progression journey beginning from the initiation to the final step, metastasis. Targeted therapies against CSCs require a thorough enrichment in CSC in the tumor bulk. Combinational therapies against genes regulating every step of metastasis and corresponding stem cell markers might be targeted synergistically to improve the these approaches.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 115–141
https://doi.org/10.1007/5584_2018_213

Published online: 17 May 2018

Regenerative Medicine Applications of Mesenchymal Stem Cells

Samaneh Hosseini, Leila Taghiyar, Fatemeh Safari, and Mohamadreza Baghaban Eslaminejad

Abstract

A major research challenge is to develop therapeutics that assist with healing damaged tissues and organs because the human body has limited ability to restore the majority of these tissues and organs to their original state. Tissue engineering (TE) and regenerative medicine (RM) promises to offer efficient therapeutic biological strategies that use mesenchymal stem cells (MSCs). MSCs possess the capability for self-renewal, multilineage differentiation, and immunomodulatory properties that make them attractive for clinical applications. They have been extensively investigated in numerous preclinical and clinical settings in an attempt to overcome their challenges and promote tissue regeneration and repair. This review explores the exciting opportunities afforded by MSCs, their desirable properties as cellular therapeutics in RM, and implicates their potential use in clinical practice. Here, we attempt to identify challenges and issues that determine the clinical efficacy of MSCs as treatment for skeletal and non-skeletal tissues.

Keywords

Mesenchymal stem cells · Regenerative medicine · Clinical setting · Skeletal tissues · Non-skeletal tissues

Abbreviations

AKI	Acute kidney injury
ALS	Amyotrophic lateral sclerosis
ABGs	Autologous bone grafts
ACI	Autologous chondrocyte implantation
BM	Bone marrow
BMMC	Bone marrow mononuclear cells
BMT	Bone marrow transplantation
CPCs	Cardiac progenitor cells
CCR	C-C chemokine receptor type
CKD	Chronic kidney disease
CXCR	C-X-C chemokine receptor type
DCM	Dilated cardiomyopathy
DMD	Duchenne muscular dystrophy
ESCs	Embryonic stem cells
EPCs	Endothelial progenitor cells
ECM	Extracellular matrix

S. Hosseini, L. Taghiyar,
and M. Baghaban Eslaminejad (✉)
Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
e-mail: eslami@royaninstitute.org

F. Safari
Bone Biology & Orthopaedic Research, Department for BioMedical Research, University of Bern, Bern, Switzerland

FTSW	Full-thickness skin wounds
GVHD	Graft versus host disease
GFP	Green fluorescence protein
HF	Heart failure
HCELL	Hematopoietic cell E-/L-selectin ligand
HGF	Hepatocyte growth factor
hAD-MSCs	human adipose derived-MSCs
HA	Hydroxyapatite
iPSCs	induced pluripotent stem cells
IGF-1	Insulin-like growth factor 1
ISCT	International Society for Cellular Therapy
IA	Intra-arterial
IC	Intracoronary
IV	Intravenous
MHC	Major histocompatibility complex
MSCs	Mesenchymal stem cells
MMPs	Metalloproteinases
MSC-CM	MSCs-conditioned medium
MS	Multiple sclerosis
MI	Myocardial infarction
NIH	National Institute of Health
NYHA	New York Heart Association
NO	Nitric oxide
OA	Osteoarthritis
OI	Osteogenesis imperfecta
PDGF	Platelet-derived growth factor
PSCs	Pluripotent stem cells
PCL	Poly-ε-caprolactone
ciPTEC	Proximal tubule epithelial cells
RM	Regenerative medicine
SCs	Satellite cells
SECs	Sinusoidal endothelial cells
SDF-1	Stromal derived factor-1
TA	Tibialis anterior
TE	Tissue engineering
TGF-β	Transforming growth factor-beta
UC	Umbilical cord
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor

1 Introduction

Damaged or lost organs, diseased and injured tissues, and tumor resections present urgent circumstances that necessitate the use of therapeutic approaches. The clinical strategies for treatment of tissues and organs to restore them to fully functional structures are basically classified into three main categories – drug therapy; surgery (autograft and allograft); and novel therapeutic approaches such as gene therapy, cell therapy, and tissue engineering (TE). The complicated healing process in most diseases, requires the simultaneous use of two or more approaches to achieve desired outcomes. Drug therapy, a traditional approach for all disease types, is normally used as a co-treatment with other strategies. For example, administration of immunosuppressive drugs following organ/tissue engraftment (e. g., kidneys, lungs, skin, or liver transplant) is necessary throughout the patient's life (van Gelder et al. 2014). In some cases, drug therapy merely results in a promising outcome. Pharmacologics that target mitochondrial-associated protein kinase C and its substrates, such as aldehyde dehydrogenase 2, reduce ischemic damage and induce cardioprotection (Chen et al. 2008). Advances in nanotechnology, bioinformatics, and biology have increased novel drug designs and delivery systems for effective drug therapy; however, additional attempts are needed to address diseases and chronic conditions such as spinal cord and brain injuries.

Surgery is a second approach that attempts to revive and repair damaged tissues. Surgeons frequently perform tissue reconstruction in cases of tumor resection, trauma injuries, and allotransplantations. For example, autologous bone grafts (ABGs) are the current gold-standard for repair and reconstruction of critical-sized bone defects (Roberts and Rosenbaum 2012). Annually, more than 2 million bone grafts are used in orthopedic procedures worldwide in both adolescents and adults (Blank et al. 2017). Although surgical

approaches are being adapted for skeletal tissues (bone and cartilage), the benefits must be carefully weighed against the risks that include lifelong immunosuppressive therapy. In addition, despite the increased success rates of surgical repair and reconstruction of injured tissue along with technological advances and improved modern surgical tools, repair of some injured non-skeletal tissues (brain, kidneys, and liver) remain challenging.

Limitations with traditional therapeutic approaches have urged scientists to develop novel, effortless, efficient strategies for tissue regeneration. Recently, a new branch of medicine, regenerative medicine (RM), has emerged with the intent to restore normal function of damaged tissues and organs by stimulation of endogenous repair processes. RM may use progenitor cells, stem cells, or therapeutic agents such as genes and trophic factors. Among these, extensive attention has focused on stem cells. Stem cells have greatly improved the disciplines of TE, gene therapy, developmental biology, cell therapy, and nanotechnology. The presence of regenerative cells was first hypothesized in the late nineteenth century by Cohnheim (1867). Currently, we know that most adult tissues possess progenitor and stem cells that are employed to repair minor tissue lesions. Stem cells deliver multiple agents in contrast to the single agent delivery of pharmaceutical drugs. They have the ability to respond to local micro-environmental clues or signals by secretion of bioactive factors. Stem cells can be engaged by gene therapy and material science to revolutionize the regenerative potential of each approach (Taghiyar et al. 2017). Insertion of a relevant gene sequence into a target cell and seeding the cells onto an appropriate natural or artificial material may result in the desired biological effect. The regenerative potency of stem cells, particularly mesenchymal stem cells (MSCs), is taken into consideration in this review. We discuss the numerous basic, translational and clinical studies in skeletal and non-skeletal tissues in an attempt to address current advancements and challenges of MSCs used for clinical applications.

2 Stem Cells and Regenerative Medicine (RM)

Cellular therapy has shown great progress in both preclinical research and the clinical setting. The initial cell transplantation attempts involved intravenous (IV) transfusion of whole blood (Giangrande 2000). Cell therapy, particularly stem cell therapy, was predominantly confined to bone marrow (BM) transplantation for hematological diseases as well as epidermis transplantation for massive burns (Atiyeh and Costagliola 2007). Today, various stem cell sources of adult and pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced PSCs (iPSCs) have been introduced for tissue repair. Adult stem cells can only differentiate into a limited number of cell types, whereas ESCs and artificially generated iPSCs develop into all three germ layers and are referred to as PSCs (Hosseini and Baghaban Eslaminejad 2017). ESCs derived from the inner-cell mass of the blastocyst provide potent cell sources for clinical applications (Thomson et al. 1998). Pluripotency and the ability for self-renewal make ESCs appropriate for treatment of diseases whereas adult stem cells or progenitor cells have not been clearly identified or are difficult to expand in culture. However, ethical issues exist with harvesting the cells from embryos. In addition, the possibility exists for immunogenicity and tumorigenicity, both of which have delayed clinical translation of ESC research. iPSCs preserve the pluripotency and self-renewal ability of ESCs, yet overcome the ethical concerns associated with ESCs. They can be maintained in culture where they self-renew indefinitely and produce an infinite number of progeny (Takahashi and Yamanaka 2006). Autologous cells can be served even from patients with specific mutations to create iPSCs (Wiley et al. 2015). Kawamata's group evaluated the safety and regenerative capacity of iPSCs in preclinical setting and performed the first clinical trial for treatment of age-related macular degeneration (Kanemura et al. 2014; Souied et al. 2017). Nevertheless, the tumorigenicity risk remains unsolved.

MSCs, a promising cell source, can be harvested from various sources such as BM, adipose tissue, umbilical cord (UC), and dental tissues (Baghaban Eslaminejad et al. 2011; Eslaminejad et al. 2010). MSCs have been studied in clinical trials and there is accumulating evidence regarding their robust potential to treat numerous diseases (Tables 1 and 2). By June 15, 2015, there were 493 MSC-based clinical trials for a wide range of therapeutic applications. Despite clinical success in MSC cell therapy, the long-term safety of MSC-based therapies is poorly established (phase III clinical trials) and continues to pose a major limitation to translating MSCs into clinical practice. Of note, the majority of cell therapy clinical trials have used non-ESCs (postnatal stem cells that included cord blood and MSCs) that were isolated from patients or donor tissues. Most were phase I and phase II, or a mixture of phase I/II studies to explore the safety and efficacy of stem cells in human being. Only a small number were phase III or phase II/III trials.

3 Properties of Mesenchymal Stem Cell (MSC) Related to Their Therapeutic Effects in Regenerative Medicine (RM)

The biological properties of MSCs were unknown when initially isolated from BM by Friedenstein et al. in 1970 (Friedenstein et al. 1970). Numerous attempts have been made to isolate MSCs from various sources to determine their molecular and cellular properties. Understanding the biological characteristics of MSCs would provide clear insight for their prospective clinical applications. In 2006, the International Society for Cellular Therapy (ISCT) defined MSCs on the basis of the following criteria: adherence to plastic substrate under standard tissue culture conditions; ability to express cell surface markers CD73, CD90, and CD105; do not express CD45, CD34, CD14, or CD11b, CD79 alpha or CD19 and HLA-DR surface molecules; and have the capability to differentiate into osteoblast, adipocyte, and chondroblast lineages under external stimuli (Dominici et al. 2006).

Currently, four paramount features of MSCs make them promising for RM, including their self-renewal and multi-lineage differentiation potential. In addition, intravenously injected MSCs have the capability to migrate and home to the sites of injury in response to inflammatory factors. They exert anti-inflammatory effects through secretion of multiple bioactive molecules, which in turn stimulates the recovery of injured cells. Finally, MSCs lack immunogenicity and exhibit immunomodulatory properties (Fig. 1). Here, we provide a brief description of each property.

3.1 Differentiation Potential

The multi-lineage differentiation capability of MSCs has been extensively studied in vitro and in vivo (Nadri et al. 2013a, b). MSCs have the potential to give rise to myogenic, adipogenic, osteogenic, and chondrogenic mesodermal lineages (Galli et al. 2014). It has also reported that MSCs can commit to ectodermal and endodermal cell fates. Our group succeeded in differentiation of MSCs to photoreceptor cells on nanofibrous scaffolds (Nadri et al. 2013a, b). Kopen et al., for the first time, have demonstrated the ability of MSCs to commit to astrocytes and neuron-like cells after they were injected into the central nervous systems of newborn mice (Kopen et al. 1999). In a clinical study, human MSCs (hMSCs) were transplanted into the spinal cord of amyotrophic lateral sclerosis (ALS) patients. This study showed that transplantation of hMSCs were safe and well-tolerated by ALS patients (Mazzini et al. 2003). Recently, several research groups used MSCs in combination with nanomaterials as a promising therapeutic strategy for skin TE both in vitro and in the clinical setting. Wu et al. injected green fluorescence protein (GFP^+) allogeneic BM-derived MSCs (BM-MSCs) around a wound in normal and diabetic mice. They observed significantly enhanced wound healing in both experimental groups compared to control mice (Wu et al. 2007). Another study used biomimetic nanofiber scaffolds (NFSs) seeded with BM-MSCs to treat acute

Table 1 Clinical trials related to mesenchymal stem cell (MSC)-based therapy of skeletal tissues

No.	Disease	Type of cells	Type of injection	Result	References
1	Limb ischemia	AD-MSCs	Multiple intramuscular	At 6 months, a significant improvement was observed in pain rating scales and claudication walking distance. Numerous vascular collateral networks was formed across affected arteries as evidenced by digital subtraction angiography 6 months post MSC implantation.	Lee et al. (2012)
2	Femoral head osteonecrosis	AD-MSCs	Local	The results showed the long-term reduction in hip pain and improvement in MRI scan.	Pak (2012)
3	Long bone non-union	BM-MSCs	Local	The results confirmed the safety of MSC implantation combined with platelet lysate during 12 months and bony union had occurred in four patients.	Labibzadeh et al. (2016)
4	Femoral head osteonecrosis	BM-MSCs	Implantation	Increased Harris hip score along with the reduced volume of the necrotic lesion was observed in group treated by BMMSC.	Zhao et al. (2012)
5	Femoral head osteonecrosis	BM-MSCs	Perfusion via medial circumflex femoral artery	92.31% of hips showed a satisfactory clinical outcome. Only 6 hips (7.69%) progressed to clinical failure.	Mao et al. (2013)
6	OA	AD-MSCs	Intra-articular	An improved knee function and reduced knee pain was observed in cell treated groups particularly high-dose group.	Jo et al. (2017)
7	OA	AD-MSCs	Intra-articular	During 2 years follow-up, none of the patients underwent total knee arthroplasty. But 87.5 % of elderly patients (14/16) improved or maintained cartilage status at least 2 years postoperatively	Koh et al. (2015)
8	OA	BM-MSCs	Intra-articular	Therapeutic benefits such as increased walking distance and decreased visual analog scale (VAS) with no evidence of tumor or neoplastic changes in the patients observed during the 30-month follow-up.	Emadedin et al. (2015)
9	OA	AD-MSCs	Intra-articular	All clinical outcome parameters that include pain, function, and mobility were improved, particularly in low-dose AD-MSCs. Four patients experienced transient knee joint pain and swelling after local injection	Pers et al. (2016)
10	OA	BM-MSCs	Intra-articular	No local or systemic adverse events detected after 1 year. MRI confirmed an increase in cartilage thickness. Pain, knee function, and walking distance were getting improved up to 6 months post-injection.	Emadedin et al. (2012)
11	OA	AD-MSCs	Intra-articular	AD-MSCs treated group showed significant improvement in four clinical scores. Radiography showed neither improvement, no further joint degeneration.	Spasovski et al. (2018)
12	Osteoarthritic knees	BM-MSCs	Intra-articular	MRI revealed better Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores in the patients received BM-MSCs.	Wong et al. (2013)
13	Osteogenesis imperfecta (OI)	BM-MSCs	Transplantation	Total body bone mineral content of all cell-recipient patients increased. And frequencies of bone fracture. Reduced.	Horwitz et al. (1999)

Table 2 Clinical trials related to mesenchymal stem cell (MSC) based therapy of non-skeletal tissues

No.	Disease	Type of cells	Type of injection	Result	References
1	Grade II to IV graft-versus-host disease (GVHD)	BM-MSCs	Intravenous	86 adverse events and serious adverse events most of which (72.1%) were of infectious nature are reported. Overall survival at 1 and 2 years from the first MSC administration was 50.0% and 38.6%, with a median survival time of 1.1 years.	Introna et al. (2014)
2	Chronic GVHD (cGVHD)	BM-MSCs	Infusion	Patients experienced no adverse effects post MSC infusion. The 2-year survival rate was 77.7%. Clinical improvement was accompanied by the increasing ratio of CD5+CD19+/CD5-CD19+ B cells and CD8+CD28-/CD8+CD28+ T cells.	Weng et al. (2010)
3	Myocardiopathy	UC-MSCs	Left ventricular	Patients treated with UC-MSCs showed improvements in left ventricular function, functional status, and quality of life.	Bartolucci et al. (2017)
4	Autosomal dominant polycystic kidney disease (ADPKD)	BM-MSCs	Cubital vein	No adverse and serious adverse events observed in cell- treated patients and the mean serum creatinine level increased after a 12-month follow-up.	Makhlough et al. (2017)
5	Acute-on-chronic liver failure (ACLF)	UC-MSC	Intravenously through the cubital vein of the arm	The UC-MSC treatment resulted in increased survival rates in ACLF patients; and reduced the end-stage liver disease scores. Liver function was improved as indicated by increased serum albumin, cholinesterase, and prothrombin activity; and increased platelet counts. Serum total bilirubin and alanine aminotransferase levels were significantly decreased in the UC-MSC group.	Shi et al. (2012)
6	Decompensated hepatitis B cirrhosis	hUC-MSCs	Intravenous infusion	The results indicated significant reductions in the serum levels of inflammatory cytokines (IL-6 and TNFα) while the level of immunosuppressive cytokines (IL-10 and TGFβ) increased. Moreover, percentages of T4 cells and Treg cells were increased and T8 cells and B significantly reduced.	Fang et al. (2016)
7	Spinal cord injury (SCI)	AD-MSCs	Intrathecal	There was no sign of tumorous conditions or calcification as evidenced by MRI. Motor recovery was observed in 5 patients at 8 months follow-up. Voluntary anal contraction improvement was seen in 2 patients. ASIA sensory score recovery was seen in 10, although degeneration was seen in one. In somatosensory evoked potential test, one patient showed median nerve improvement.	Hur et al. (2016)
8	Spinal cord injury (SCI)	BM-MSCs	Direct injection into lesion sites	It confirmed the safety of allogenic hMSCs in patients with SCI, however, it might not be efficacious; especially in patients with chronic SCI.	Bhanot et al. (2011)

(continued)

Table 2 (continued)

No.	Disease	Type of cells	Type of injection	Result	References
9	Spinal cord injury (SCI)	BM-MSCs	Direct injection into lesion sites	Total of 75% patients improved with grade A SCI, three with grade B injury and eight patients (100%) with grade C injury, 1 month post transplantation.	Jiang et al. (2013)
10	Secondary progressive multiple sclerosis (SPMS)	BM-MSCs	Intravenous infusion	No serious adverse events were detected. An increase in optic nerve area was observed after treatment in visual acuity. They found no substantial effects on color vision, visual fields, macular volume, retinal nerve fiber layer thickness, or optic nerve magnetization transfer ratio. Bacterial infection was observed in 20% of patients.	Connick et al. (2012)
11	Multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS)	BM-MSCs	Intrathecal and intravenous	MRI of the brain and whole spine did not reveal any significant unexpected pathology and confirmed the existence of MSCs in the occipital horns of the ventricles. Immunological analysis showed a 72% increase in the proportion of $CD4^+$ $CD25^+$ regulatory T cells and a reduction in expression of $CD40^+$, $CD83^+$, $CD86^+$, and HLA-DR on myeloid dendritic cells.	Karussis et al. (2010)
12	Severe emphysema	BM-MSCs	Intravenous	There was no evidence of induction of fibrotic responses in the lung by MSCs. Expression of the endothelial cell marker CD31 in the alveolar septa of emphysematous lung tissue increased after lung volume reduction surgery (LVRS) and MSC infusions.	Stolk et al. (2016)
13	Idiopathic pulmonary fibrosis (IPF)	BM-MSCs	Infusion	The results confirmed the safety of a single infusion of hMSCs in patients with mild-moderate IPF.	Glassberg et al. (2017)
14	Acute respiratory distress syndrome (ARDS)	BM-MSCs	Infusion	The safety of a single dose of allogeneic BM-MSCs in patients with moderate-to-severe ARDS was observed.	Wilson et al. (2015)

full-thickness skin wounds (FTSW) in a rat model. This construct resulted in epithelialization and normal skin formation with hair follicles and sebaceous glands, as well as collagen deposition over 10 days (Ma et al. 2011). Regarding the differentiation potential of MSCs to endodermal lineages, it has been shown that hBM-MSCs and human adipose derived-MSCs (hAD-MSCs) have the ability to transdifferentiate into lung epithelial cells (Mendez et al. 2014). Likewise, MSCs that were systemically injected into C57BL/6 mice after a radiation-induced injury immediately gave rise to functional (epithelial and endothelial) lung cells (Yan et al. 2007). Various in vivo and in vitro experiments reported similar findings (Wang et al. 2018). Transplantation of BM-MSCs into chimeric mice that expressed GFP with ischemically injured renal tubules resulted in differentiation toward renal tubular epidermal cells (Duffield et al. 2005). Although there has been an increase in the therapeutic use of MSCs, direct differentiation and paracrine effects of MSCs used to treat diseases are completely unknown.

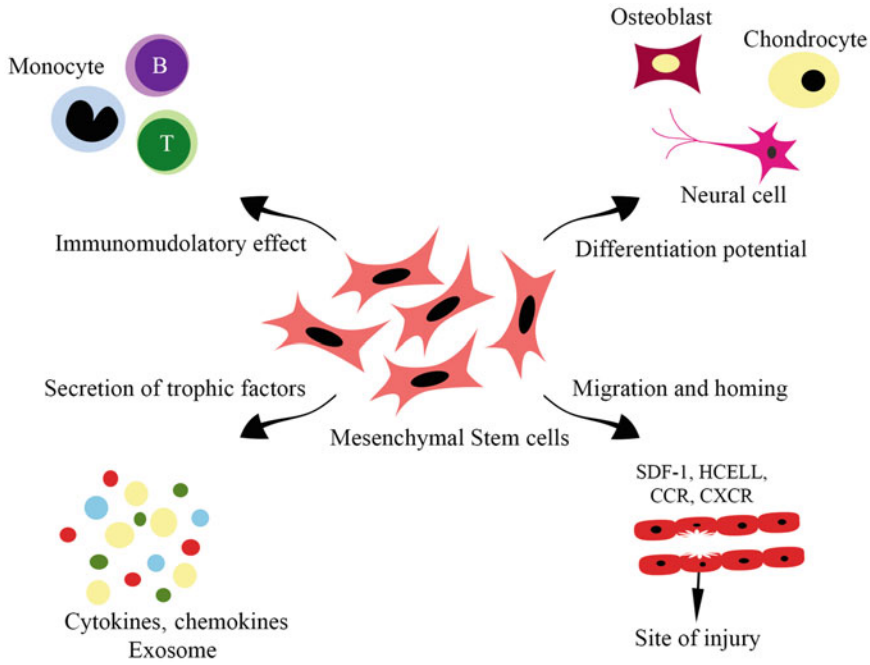

Fig. 1 The paramount features of MSCs related to their therapeutic effects in regenerative medicine (RM) including their multi-lineage differentiation potential, homing and migration capacity, secretion of trophic factors and immunomodulatory effects

3.2 Migration and Homing Capacity

MSCs are therapeutically capable of homing to inflammation sites via systemic infusion routes, such as IV infusions, intra-arterial (IA) injections, and intracoronary (IC) local administration. They exert their functional effects locally in the resident tissue. Regardless of the tissue, MSCs migrate to the injury sites under a variety of pathologic conditions. Ortiz et al. have shown that MSCs attenuated inflammation in lung tissues of bleomycin-challenged mice following homing to the lung in response to an injury (Ortiz et al. 2003). Similarly, transplanted MSCs migrated towards injured muscle tissues in mdx mice (Liu et al. 2007). Agematsu et al. conducted a study to address the origin of MSCs following allogeneic BM transplantation (BMT). They demonstrated that stromal fibroblasts cells in long-term cultures originated from the recipients as evidenced by in situ hybridization using a Y-chromosome specific cDNA probe (PHY10) (Agematsu and Nakahori 1991). However, the number of MSCs in injection site differed in various systemic infusions. Various in vitro and in vivo studies have reported that MSC selectively migrate to the injured site by mediation of numerous cytokines such as receptor tyrosine kinase-dependent growth factors [e.g., platelet-derived growth factor (PDGF) and insulin-like growth factor 1 (IGF-1)] and chemokines (e.g., CCR2, CCR3, CCR4 or CCL5) (Ponte et al. 2007). These homing signals are secreted by injured cells and/or respondent immune cells. Baek et al. have reported that C-C chemokine receptor type 1 (CCR1), CCR7, C-X-C chemokine receptor type 4 (CXCR4), CXCR5, CXCR6, EGF receptor, fibroblast growth factor receptor 1, transforming growth factor-beta (TGF-β) receptor 2, TNF receptor superfamily member 1A, PDGF receptor A, and PDGF receptor B regulate the migration capacity of hAD-MSCs (Baek et al. 2011). Besides these homing signals, other molecules are implicated in different steps of the homing process. For example, CXCR4-stromal derived factor-1 (SDF-1) is of crucial

importance for BM homing (Wynn et al. 2004). Hematopoietic cell E-/L-selectin ligand (HCELL), a specialized glycoform of CD44, is involved in cell migration (Sackstein 2011). G-protein coupled receptors, integrins as adherent molecules such as integrin β1 and integrin α4, which interact with vascular cell adhesion molecule 1 (VCAM-1) are functionally involved in MSC homing. Since efficient cell delivery is the major challenge in RM, the presence of these factors would be a promising strategy to facilitate therapeutic delivery of MSCs and target the injured tissue. Yun et al. showed that prostaglandin E_2 (PGE_2) stimulation facilitated MSCs migration to the injured tissue (Yun et al. 2011) .

3.3 Secreting Multiple Bioactive Molecules

Therapeutic applications of MSCs are associated with direct differentiation of MSCs at the injury site and largely related to an indirect capacity in suppressing immune and inflammatory reactions, activation of normal tissue repair processes, fibrosis and apoptosis inhibition, and enhancement of angiogenesis. MSCs exert these roles by secretion of trophic factors – a variety of paracrine and autocrine factors as well as extracellular vesicles such as exosomes and microvesicles. Various studies have demonstrated that cytokines secreted by MSCs contributed to functional improvement of an infarcted heart (Timmers et al. 2011), spinal cord injury (Cantinieaux et al. 2013), and ischemic limb regeneration (Bhang et al. 2014) models. Ulivi et al. reported that MSCs turned the pro-inflammatory phenotype of macrophages into a phenotype with the ability to inhibit production of inflammatory cytokines (Ulivi et al. 2014). Moghadasali et al. showed the MSCs-conditioned medium (MSC-CM) recovered cell viability and migration of human proximal tubule epithelial cells (ciPTEC) after drug-induced nephrotoxicity (Moghadasali et al. 2013). Systemic infusion of MSCs-CM reduced the expression levels of pro-inflammatory cytokines, which resulted in enhanced survival of hepatocytes and sinusoidal endothelial cells (SECs) in reduced-size liver transplantation (RSLT) in a rat model. (Du et al. 2013). A comprehensive expression profile of BM-MSCs that used an antibody array revealed 120 cytokines and chemokines with 6 highly secreted cytokines (IL-6, IL-8, TIMP-2, MCP-1, VEGF, and OPG) (Park et al. 2009). However, the functional roles of these cytokines have yet to be determined.

3.4 Immunomodulatory Functions of Mesenchymal Stem Cells (MSCs)

The immunosuppressive feature of MSCs was first reported in the early 2000s (Bartholomew et al. 2002). Since then, MSCs have attracted great attention for therapeutic applications. Liechty et al. designed a xenogeneic system to address the fate of MSCs after cell injection/transplantation. They transplanted hBM-MSCs into fetal sheep in the early phase of pregnancy and observed that hBM-MSCs gave rise to multiple tissues (cartilage, heart, adipose tissue, muscle, BM, and thymic stroma). MSCs existed in a xenogeneic environment due to unique immunologic characteristics along with preservation of their multipotential capacity post-transplantation (Liechty et al. 2000). Various studies have shown that MSCs have the ability to affect almost all cells of both the innate and adaptive immune systems and induce an anti-inflammatory phenotype. MSCs modulate the immune response by soluble factors (e.g., IL-6, M-CSF, IL-10, TGF-β, HGF, and PGE2) and cell-cell contact (Xu et al. 2007). Adhesion molecules that include VCAM-1, ICAM-1, and LFA-3 are involved in T-cell interaction and play an important role in MSC-mediated immunosuppression (Xu et al. 2007). Nicola et al. have shown that co-culture of BM-MSCs and T cells led to a significant, dose-dependent reduction of T-cell proliferation (Di Nicola et al. 2002). Apparently, MSCs suppress subpopulations of T-cells such as CD8+ (Chen et al. 2002). It has been demonstrated MSCs have naturally low immunogenic properties due to low expression level of major histocompatibility complex (MHC) class I

antigens and lack of MHC class II and co-stimulatory molecules such as CD80, CD86, and CD40 (Krampera et al. 2003). Recent studies revealed that MSCs-CM exhibited a similar immunomodulatory effect as MSCs. Hashemi et al. compared AD-MSCs-CM derived from BALB/c, C57BL/6, and DBA mouse strains. The immunological assays showed some variation among the strains in the cytokines, nitric oxide (NO), and indoleamine 2,3-dioxygenase production as well as immunomodulatory effects on splenocyte functions. There was suppression of splenocyte proliferation in the presence of ADMSC-CM in the three inbred mouse strains, though, BALB/c CM caused a stronger immunosuppressive effect (Hashemi et al. 2013). Determining MSCs suppressive immune response mediatory role would improve prospective clinical applications of MSCs.

4 Mesenchymal Stem Cells (MSCs) in Skeletal Tissues

Advances in MSC therapy for bone, cartilage, tendons, and muscles will be reviewed in this section. Table 1 lists the clinical studies that employed MSCs as treatment of skeletal diseases.

4.1 Bones

Bones have self-healing capability for small, non-intensive and uncomplicated injuries. Bone healing is a complicated process that consists of overlapping phases – inflammation, repair, and remodeling. Numerous intracellular signaling pathways play a role in bone healing. Newly formed bone is indistinguishable from the surrounding native bone in both its micro structure and macro structure. However, this ability for self-healing is unable to repair large-sized bone defects, which lead to formation of malunions, delayed unions, nonunions, osteomyelitis, necrosis, and tumors. Therefore, an efficient therapeutic approach is of crucial importance for treatment of bone lesions. ABG are the current gold-standard procedure. Annually, more than 2 million ABG are performed as orthopedic procedures worldwide (Blank et al. 2017). Allografts and xenografts are considered to be alternative strategies for bone treatment. Despite the satisfactory results of the aforementioned methods, a number of shortcomings and complications limit their availability and application. Administration of MSCs alone or in combination with biomaterials has emerged as a promising strategy for bone repair and is currently under intensive investigation.

The capability of MSCs to undergo osteogenic differentiation was identified in 1976 (Friedenstein et al. 1976). This finding encouraged scientists to exploit this new technology in the preclinical and clinical settings. The successful outcome of intravascular injection of complete BM and/or BM-MSCs has been reported for regeneration of maxillofacial defects, osteonecrosis, and distraction osteogenesis (Zamiri et al. 2013). According to the official database, the most reported translational use of cell therapy is related to non-union bone defects (Ballini et al. 2017). Healey et al., in 1990, have reported desirable outcomes in 8 patients with delayed union who were treated by percutaneous engraft of autologous BM (Healey et al. 1990). Percutaneous BM grafting in patients with tibial non-union resulted in union treatment in 15 of 20 patients at 4 months after treatment (Goel et al. 2005). Another group injected concentrated autologous BM in patients with tibia nonunion and observed good clinical outcomes (Hernigou et al. 2005).

Direct injection of MSCs is an ineffective delivery method in large bone defects where a significant amount of the bony tissue is lost. Acceleration of the bone healing process of critical size defects fails due to lack of angiogenesis, which would enhance repair capacity. Recently, it has been suggested that the controlled delivery of MSCs and growth factors within biomaterial substrates (hydrogel, scaffold) promotes healing and accelerates functional new bone formation (Khojasteh et al. 2013). Khojasteh et al. delivered MSCs and endothelial progenitor cells (EPCs) in β-tricalcium phosphate scaffolds that contained vascular endothelial growth factor (VEGF)-

loaded microspheres and implanted them in bilateral mandibular bone defects in dogs. Their results showed the most bone formation in the VEGF/MSC scaffold compared with the other groups. The amount of new bone regeneration was highest in the MSCs/EPC/VEGF group (Khojasteh et al. 2017). In clinical settings, Quarto successfully treated a 4 cm tibial critical size defect with autologous BM-MSCs in combination with hydroxyapatite (HA). The injury healed within 6 months (Quarto et al. 2001). Subsequently, Bajada et al. used BM-MSCs combined with calcium sulfate carriers to treat a non-union tibial fracture and the fracture healed 2 months after surgery (Bajada et al. 2007).

Osteonecrosis (avascular necrosis) is a bone and cartilage distraction caused by disease or severe trauma, such as a fracture or dislocation that affects the blood flow to a bone. The National Institute of Health (NIH) considered surgical core decompression technique as the only treatment option for early stage osteonecrosis (Helbig et al. 2012). However, concurrent cell therapy and core decompression approaches have successfully prevented progression of osteonecrosis. In a pilot clinical study, patients with ostenecrotic hips simultaneously underwent treatment with implantation of an autologous BM concentrate and core decompression that resulted in pain reduction and joint symptoms 24 months after the procedure. Only one out of 10 patients in this group progressed to the final stage. In contrast, 62% of the control hips that only received core decompression had evidence of end-stage avascular necrosis (Gangji et al. 2004). Similarly, Sen et al. observed better clinical outcome and hip survival following MSCs transplantation and core decompression (Sen et al. 2012). Numerous studies have incorporated BM aspirated-MSCs or expanded-MSCs into tissue-engineered scaffolds for treatment of non-traumatic osteonecrosis (Centeno et al. 2011). Long-term follow-up of autologous BM-engrafted patients revealed slow (rare) deterioration to the fracture stage over 60 months (Gangji et al. 2011). Use of the same therapeutic approach confirmed decreased pain in all the patients postoperatively, and delayed the progression of the disease to collapse during 17–20 years of follow-up (Hernigou et al. 2003). A recent study reported autologous BM grafting for advanced osteonecrosis of the humeral head and observed disparate outcomes among patients. This finding was most probably related to differences in the amount of BM and varied number of transplanted MSCs (Makihara et al. 2017).

A number of scientists assessed allograft MSCs after reports of their safety and immunosuppressive properties. Horwitz et al., for the first time, reported the feasibility of simultaneous allogeneic BM and MSC transplantations in children with severe osteogenesis imperfecta (OI) (Horwitz et al. 1999). In 2005, Le Blanc et al. conducted a novel clinical trial that used in utero transplantation of allogeneic MSCs into a female fetus with severe OI. After birth, the infant showed no immunoreactivity against the donor and only three fractures occurred during the first 2 years. Both normal psychomotor development and correct growth tendencies were observed in long-term follow-up (Le Blanc et al. 2005). In another study, allogeneic AD-MSCs healed cranial critical-sized defects in a canine model without inducing an immune response by the host (Liu et al. 2013). Similarly, implantation of allogeneic BM-MSCs with hydroxyapatite-tricalcium phosphate (HA-TCP) scaffolds has resulted in bone regeneration of femoral diaphysis defects with no adverse immune response (Arinzeh et al. 2003). However, there is still insufficient data to argue that allogeneic MSCs are safe for clinical applications.

Despite the numerous reports of successful bone healing with BM-MSCs, determining the proper cell sources is a challenge for cell therapy of bone disorders. Numerous in vitro and preclinical studies have been conducted to examine the potential of MSCs derived from various sources such as periosteum, muscle, adipose, and UC on osteogenesis and bone regeneration (Hosseini and Baghaban Eslaminejad 2017). Linero et al. showed that AD-MSCs induced bone regeneration in critical size jaw defects in rabbits. They observed similar results between AD-MSCs and BM-MSCs in terms of amount and quality of neo-formed bone, bone thickness, collagen fiber structure, maturation, and mineral matrix

calcification. For the first time, they have demonstrated that ADSCs have a paracrine effect in bone regeneration and can be a therapeutic alternative for MSCs therapy (Linero and Chaparro 2014). Tawonsawatruk et al. evaluated the ability of human ADSCs (hADSCs) to prevent fracture nonunion in rat models. Cells were injected percutaneously at the fracture site. At 8 weeks, 80% of the animals in the hAD-MSCs treatment group showed evidence of bone healing with substantial improvement in bone mineralization and maturity of bone tissues at the fracture gap compared to only 14% of those in the control group (Tawonsawatruk et al. 2016). Stockmann et al compared the ability of various cell populations for bone regeneration in a pig calvaria defect and observed no significant differences among implanted collagen scaffold seeded with AD-MSCs, PMSCs, and BM-MSCs (Stockmann et al. 2012). In a recent work, Cell tracing or mapping strategies showed that neural-crest stem cells were recruited to the jaw and skull bone defects during the healing process (Lombard et al. 2016). These findings showed the value of NCSCs and/or stem cells from the head and neck area such as dental pulp-derived MSCs as new, relevant cell sources for therapeutic applications. Giuliani et al. seeded human dental pulp-derived MSCs onto collagen I scaffolds to treat human mandible defects. A fully compact bone that had higher matrix density was observed compared to the control, human alveolar spongy bone. The regenerated bone, being entirely compact, completely differed from normal alveolar bone. Long-term follow up showed regeneration of the mandible (Giuliani et al. 2013).

A literature search and database have shown that most clinical trials of bone regeneration administered BM-MSCs; a few have used MSCs from other sources. Of note, these trials are mostly phase I or II. These studies show that MSCs are a prosperous treatment, even in long-term follow-up. However, the mechanisms underlying stem cell therapy are still largely unknown and should be addressed.

4.2 Cartilage

Cartilage is as an avascular, aneural tissue present in the joints, intervertebral disks, and nose, among other locations. There are three different types of cartilage – hyaline cartilage, elastic cartilage, and fibrocartilage. Each has its own chemical and mechanical properties. Articular cartilage is a hyaline cartilage mainly composed of water, extracellular matrix (ECM) components, and chondrocytes (2% of total volume). Chondrocytes are responsible for the synthesis of ECM as well as repair of cartilage defects. Collagen type II is the major constituent of the ECM, which provides high strength and low friction in joints (Camarero-Espinosa et al. 2016). Owing to the absence of a vascular network and low cellularity, cartilage displays a limited intrinsic regeneration capacity when injured. The absence of pain in the damaged aneural cartilage leads to continued loading of the joint, which eventually results in an osteochondral defect and osteoarthritis (Camarero-Espinosa et al. 2016).

Osteoarthritis, a debilitating disease, is the most common chronic joint disorder, which frequently occurs in elderly individuals and athletes as a result of overuse or stress on the joints (Ruiz et al. 2016). Various surgical and non-surgical methods have been employed to treat osteoarthritis, though these approaches are unable to regenerate articular cartilage. These techniques mostly relieve pain and reduce inflammation in the damaged joint (Jo et al. 2014). Total joint replacement is the only definitive therapeutic option for patients with severe arthritis, though this method is invasive and may result in infection and thrombosis (Ruban et al. 2000).

Cellular therapies and TE have been developed to overcome these limitations and promote cartilage regeneration. Given the essential role of chondrocytes in ECM synthesis, autologous chondrocyte implantation (ACI) has been considered to repair cartilage lesions. However, chondrocyte dedifferentiation and two-stage surgical procedure may result in further cartilage damage and degeneration (Knutsen et al. 2007). RM thus offered MSCs as a powerful approach for cartilage repair.

As mentioned above, MSCs can be simply isolated from various tissues and expanded to provide off-the-shelf products for therapeutic applications. Given the fact that epigenetic memory has a significant impact on chondrogenic potential of MSCs, the source of MSCs plays an important role in treatment efficacy. According to recent researches, synovium-derived MSCs and BM-MSCs have exhibited the highest chondrogenic differentiation potential compared to other sources (Li et al. 2011). In contrast, AD-MSCs have a very low chondrogenic capacity (Barry and Murphy 2013). Thus, 68% of clinical trials have used BM-MSCs in cartilage TE and RM (Goldberg et al. 2017).

Numerous studies have proven the crucial role of the TGF-β superfamily to achieve efficient chondrogenic differentiation in vitro (Kim et al. 2014). Nevertheless, multiple factors control the process of chondrogenesis in vivo such as growth factors, mechanical loads, and cell interactions (Sekiya et al. 2002). Providing MSCs with these factors under in vitro conditions would more likely result in fully functional chondrocytes. However, important issues such as hypertrophy and ossification following differentiation are controversial in terms of in vitro chondrogenesis.

MSC transplantation for cartilage repair by direct injection or within different scaffolds at the site of injury have been widely reported and showed promising results (Giannini et al. 2010). In most animal models, MSCs were transplanted into joints and followed up to 6 months after the surgery or injection. Most likely, MSCs' immunomodulatory effects at the damaged site led to improvements rather than their differentiation into chondrocytes. Local MSCs and resident articular chondrocytes have the capability to migrate into the defect site and synthesize a reparative matrix (Davatchi et al. 2016).

Various scaffolds have been developed to promote chondrogenic potential of MSCs after transplantation. Use of collagen gel in some clinical trials improved cartilage regeneration; two separate studies – one reported benefit after 6 months and the other after 5 years after transplantation in osteoarthritis (OA) patients (Davatchi et al. 2016; Wakitani et al. 2004). Other studies reported promising results after 12 months when they used hydroxyapatite ceramic and platelet fibrin glue scaffolds for transplantation of MSCs (Adachi et al. 2005)

The first clinical trial that used an intra-articular injection of autologous BM-MSCs in a patient with OA was reported in 2008. At the 6-month follow-up, the patient reported pain relief, improvement in walking distance and other physical activities (Centeno et al. 2008). After this study, other trials that had 1–2 year follow-up periods reported that the improvements were only limited to the first 6 months after cell treatment. In the second 6 months, patients noticed an increase in symptoms (Davatchi et al. 2011). After these contradictory results, a 5-year follow-up of MSCs therapy for OA was reported in 2016 (Davatchi et al. 2016). It was noted that while symptoms deteriorated after 1 year of treatment, the treated knees were still better compared to untreated knees after 5 years. Although it was unclear why the improvements declined after 1 year, MSCs' behavior supposedly would change in response to the new microenvironment. Inflammation in the defect area causes local MSCs to produce metalloproteinases (MMPs) instead of ECM, which leads to further cartilage degeneration (Richardson et al. 2016).

Over past 16 years, autologous MSCs have been frequently used in trials rather than allogeneic MSCs to eliminate immunogenic responses. However, allogeneic MSCs combined with autologous chondrocytes revealed more promising results than the group without MSCs. Although the therapeutic mechanism of MSCs has yet to be identified, it seemed that suppression of inflammation was more likely to be responsible for the major healing efficacy of MSCs at the transplantation site (Davatchi et al. 2016). Given the short life span of transplanted MSCs, chondrogenic differentiation would hardly be a decisive factor in the healing process.

An important issue in using MSCs for cartilage regeneration is the formation of fibrocartilage

instead of hyaline cartilage, which has an inferior therapeutic outcome. Additionally, hypertrophy occurs upon chondrogenic differentiation. The biomechanical properties of the chondrocytes change after production of collagen type II. Although different techniques have been used to address this issue, there is a need to develop more effective methods to generate hyaline cartilage at the defect site.

4.3 Muscles

Skeletal muscle is a highly organized tissue composed of numerous myofibers as basic structural units, in addition to blood vessels, nerves, and extracellular connective tissue. It attaches to the bone via tendons and generates forces for voluntary movement and locomotion. In adulthood, skeletal muscle has an inherent ability to regenerate minor injuries. This ability is mainly allocated to the existence of a population of undifferentiated mononuclear cells, known a satellite cells (SCs) (Yin et al. 2013). During postnatal muscle development, these cells reside in a quiescent state and activate in response to environmental cues such as injury and inflammatory factors (cytokines). They subsequently proliferate, undergo terminal differentiation and form myofibers, and eventually integrate into the muscle tissue (Collins et al. 2005). On the other hand, muscle does not have the capability to regenerate severe injuries, such as myopathies, large traumatic injuries, muscle tumors, and chronic denervation. Lack of regeneration frequently leads to fibrous scar tissue formation and fatty degeneration of muscle causes volumetric muscle loss.

Current treatments for severe muscle trauma and myopathy include engraftment of intact, vascularized, and innervated autologous muscle and injection of myoblasts. Although myoblasts are the first natural cell sources for cell therapy of skeletal muscle, they have not shown a favorable outcome (Mendell et al. 1995). Myoblasts derived from patients who suffer from Duchenne muscular dystrophy (DMD) poorly expand under in vitro conditions and rapidly undergo senescence (Gussoni et al. 1997). Muscle SCs are an alternative cell sources for muscle treatment. However, various clinical and preclinical studies have reported the shortcomings of SCs transplantation (Boldrin et al. 2015). In addition to the need for a large numbers of injected SCs to treat a complete muscle, they provoke immune responses in the host body and most die during the early hours after the injection.

Muscle-derived stem cells were examined to determine if they could contribute to muscle repair. BM-MSCs were the first that underwent myogenic differentiation and participated in muscle repair (Bossolasco et al. 2004). However, Gang et al. reported contradictory results and observed that BM-MSCs could not regenerate muscle in dystrophin-deficient mice (Gang et al. 2009). Overexpression of PAX3, the master regulator of the embryonic myogenic program, in BM-MSCs was performed to evaluate the ability of these cells to restore dystrophin expression in immunodeficient mice. Transplantation of PAX3-transduced MSCs resulted in more clusters of dystrophin+ myofibers, but there was no functional improvement observed compared to untransduced MSCs (Gang et al. 2009). Dezawa efficiently induced BM-MSCs to differentiate into mature myotubes that were PAX7+ and caused muscle regeneration in mdx-nude mice (Dezawa et al. 2005). To achieve the best outcome, a number of research groups examined the potential cells from adipose, UC, and synovial membrane sources (De Bari et al. 2003; Fukada et al. 2002; Goudenege et al. 2009). The safety and efficacy of muscle-derived CD133+ stem cells in patients with DMD was also assessed in a phase I clinical trial which resulted in positive outcomes with no observed adverse effects (Torrente et al. 2007). Another study compared the regenerative capacity of intramuscular injection of human muscle–derived CD133+ cells and myoblasts to cryoinjured tibialis anterior (TA) muscle in a mouse model. There was efficient muscle regeneration in the group that received muscle-derived CD133+ cells in terms of the numbers of fibers that expressed human proteins and the numbers of human cells in a SC position compared to the myoblast group (Negroni et al. 2009). Despite the apparent

success of MSCs, generation of functional, large-scale muscle tissues is a tremendous clinical challenge. Cells, as therapeutic agents, combined with TE approaches would provide an integrated system whereby the cells could interact with their environment to have a fully functional, mature skeletal muscle. Vandenburgh et al., for the first time, have described the use of tissue engineered constructs for muscle regeneration (Vandenburgh et al. 1988). They cultured myotubes in collagen matrix in vitro and observed that the cells highly preserved their contractile state during expansion Another group developed a poly-ε-caprolactone (PCL)/collagen based nanofiber scaffold to guide morphogenesis of skeletal muscle cells (Choi et al. 2008). Recent attempts have been undertaken to create functional, engineered skeletal muscle with enhanced vascularization, increased innervation, and morphology similar to native muscle (Chan et al. 2006). Witt et al. co-cultured MSCs with myoblasts. Under stimulation with hepatocyte growth factor (HGF) and IGF-1, the three-dimensional (3D) cultivation in fibrin-collagen I gels induced higher levels of myogenic differentiation compared with the two-dimensional experiments (Witt et al. 2017). For the most part, the in vivo applications of muscle TE technologies are in the early stage of pre-clinical development.

4.4 Tendons

Tendons, as specialized connective tissues, are joint stabilizers that curb skeletal muscle damages by transmitting mechanical forces from muscle to bone. Tendons are mainly composed of collagen type I (approximately 80%-95%) and small amounts of other types of collagen (III,V,VI,XII, XIV), glycosaminoglycans, and proteoglycans. Collagen fibers are longitudinally aligned along the tendon axis that causes high mechanical strength and elasticity of the tendon (Spanoudes et al. 2014). Tenocytes and tenoblasts, two major cell types within the tendons, produce the complex tissue-specific extracellular environment. Tenocytes are fibroblast-like cells with an elongated morphology, which are located between the collagen fibers (Spanoudes et al. 2014). Tendons or surrounding tissue contribute to the healing process of an injured tendon by producing a new ECM. There are two healing mechanisms in tendons, intrinsic and extrinsic. Tenocytes and tenoblasts are actively involved in the intrinsic healing mechanism, whereas other cell types, such as BM-MSCs from surrounding tissues, are implicated in the extrinsic healing mechanism. Nevertheless, the healing mechanisms cannot effectively deal with rehabilitation of injured tissue because of the tendon's limited vascularity and low cellularity.

Among the available therapeutic strategies known to promote regeneration of injured tendons, cell-based TE appear to be the most promising. Studies have demonstrated that tenocytes cultured in vitro encounter numerous difficulties that include dedifferentiation, morphology deformation to spindle shape, and trans-differentiation (Yao et al. 2006). The phenotypic drift in tenocytes affects its function and makes it inappropriate for cell based therapy approaches. To overcome this issue, numerous studies have demonstrated the potential of transplanted MSCs for tendon repairs (Awad et al. 1999). Nevertheless, providing suitable mechanical and chemical cues for fully tenogenic differentiation of MSCs in vitro and in vivo is of significant importance. Due to the longitudinal alignment of collagen fibrils within the tendon units, it has been proven that aligned scaffolds promote tenogenic differentiation of MSCs as they imitate the tendon's architecture (Erisken et al. 2013). Despite promising preliminary outcomes, some reports indicated formation of ectopic bone after injection of MSCs in the defect site. In order to address this issue, pre-treatment of MSCs in vitro should be taken into consideration. Recent researches have shown that utilizing growth factors (GDF-5, BMP12) and mechanical stimulation upregulated collagen type I and other tendon specific markers in MSCs (Lee et al. 2011).

There are limited numbers of clinical trials in which the efficacy of MSCs for tendon repair is unclear. The lack of control groups and a defined protocol guideline make it difficult to show

successful tendon regeneration after MSCs transplantation (Veronesi et al. 2017). Despite the promising results from in vitro and in vivo studies, the optimal scaffold and cell population for tendon repair and regeneration has yet to be addressed to avoid ectopic bone formation after cell therapy.

5 MSCs in Non-skeletal Tissues

According to the official website of the NIH, most MSC-based clinical trials evaluated the biomedical potential of hMSCs to treat hematological, inflammatory, and graft versus host disease (GVHD) conditions. Bone and cartilage injuries, heart disease, diabetes, gastrointestinal conditions, diseases of the liver and kidneys, as well as neurological disorders are the targets of MSCs-based therapy (Table 1). Other diseases constitute 12% of total clinical studies (Squillaro et al. 2016). The least number of clinical trials of MSCs therapies belong to lung and related diseases (Liu et al. 2016). Use of MSCs for treatment of neurological disorders is relatively common, despite the scant evidence for their conversion to neural cells in vivo. Autologous MSCs isolated from BM and injected intrathecally into spinal cord cerebrospinal fluid, allowing access to the brain and spinal column, can be accomplished safely in patients with multiple sclerosis (MS) and ALS (Rushkevich et al. 2015). The combination of cell therapy and TE provide an efficient therapeutic approach in RM, particularly in complicated organs and limbs. Advances in tissue engineered materials are of crucial importance as they are the main tools in cell therapy used to rebuild damaged tissues. Material carriers designed to spatially and temporally mimic the tissue cell niche may be of particular importance for the complete regeneration of severely damaged organs. Hence, in complicated organs such as the limbs, a lack of tissue engineered proper composite and proper cell sources (Taghiyar et al. 2017) cause limitations in RM in this field. Here, we discuss MSC-based therapy in three organs.

5.1 Liver

The liver is the largest organ of the body. It displays a multicellular architecture. The liver performs a variety of functions that include detoxification, synthetic and metabolic processes. Liver diseases are caused by different factors such as viral infections, alcoholism, genetic syndromes, and autoimmune attacks. These diseases often lead to liver failure, which results in multiple organ dysfunctions and eventually death. The liver has a unique self-regenerative capacity to restore its function after massive injuries. However, in acute liver failure it is unable to repair the damage. Thus, the liver loses its function. In this cases, orthotropic liver transplantation is the only current treatment to save patients (Bhatia et al. 2014). Lack of a live human liver donor and the increasing demands for transplantation have urged scientists to find an alternative treatment to organ transplantation. TE and cell-based therapy have been recently offered as a promising method to treat end stage liver failures (Lee et al. 2015).

Hepatocytes constitute the main cell type in the liver and are responsible for hepatic regeneration. Transplantation of either hepatocytes or stem cells have been explored in a number of preclinical and clinical studies (Piscaglia et al. 2010). Although hepatocytes are the priority for cell based therapy approaches, they lose their function and proliferative capacity in vitro (Hu and Li 2015). Additionally, an insufficient supply of human hepatocytes is a challenge for therapeutic applications. In the quest for an alternative, since 2004, MSCs have been considered as appropriate cell sources that have the ability to give rise to functional hepatocytes (Ohkoshi et al. 2017). BM-MSCs, UCB-MSCs, and AD-MSCs have hepatogenic capabilities, but AD-MSCs are more likely to be an excellent source for liver regeneration (Alizadeh et al. 2016; Berardis et al. 2015). Interestingly, it has been proven that MSCs secrete anti-fibrotic, anti-inflammatory, and anti-apoptotic molecules, which enables them to treat acute and chronic liver injuries (Christ et al. 2015). Therefore, the idea of

utilizing MSC-CM culture seems to be as effective as using MSCs. Numerous in vivo studies have shown that MSC secretomes stimulate hepatic regeneration after transplantation (Fouraschen et al. 2012).

Since 2007, several clinical trials reported promising results of systemic injections of MSCs to treat liver disorders (Mohamadnejad et al. 2007). Nonetheless, the exact mechanism of MSCs in healing liver diseases has yet to be completely understood. In addition, some studies reported the formation of myofibroblasts upon MSCs transplantation, which must be addressed in future research.

5.2 Heart

Cardiovascular diseases account for the highest mortality worldwide and more than half are allocated to myocardial infarction (MI) (Go et al. 2014). The heart has a limited capacity to naturally regenerate; hence, cardiac diseases may lead to heart failure (HF) and death. Although there are various medical and surgical treatments, cardiac transplantation is the only current options for patients with end-stage myocardial failure. However, the limited numbers of donors preclude its extensive use. One of the leading treatments under investigation for HF is MSCs.

Numerous studies have been published about the therapeutic potential of autologous and allogeneic MSCs from various sources for treatment of cardiovascular diseases. In a pioneering study, Toma et al. injected hMSCs into murine hearts, which gave rise to a cardiac lineage (Toma et al. 2002). These researches showed that MSCs mediate the migration and differentiation of cardiac progenitor cells (CPCs) through paracrine signaling by secreting growth factors, cytokines, and angiogenic factors (Nakanishi et al. 2008; Zhao et al. 2016). Zhao et al. specifically showed that overexpression of HGF in UC derived MSCs reduced cardiomyocytes apoptosis, enhanced angiogenesis, and cardiomyocyte proliferation (Zhao et al. 2016). A recent work suggested that the chemotactic effect of MSCs on proliferation, migration, and differentiation of endogenous CSCs was regulated via the SDF1/CXCR4 and SDF1/CXCR4 signaling pathways (Hatzistergos et al. 2016).

A number of published or ongoing clinical trials have demonstrated beneficial effects of MSC-based therapy in cardiovascular settings. The findings of clinical trials on patients with MI treated with MSCs revealed the beneficial effects of MSCs on improving heart function (Jeong et al. 2018). IV infusion of allogeneic UC-MSC in patients with chronic heart failure considerably upregulated the expression of HGF involved in myogenesis, and improved left ventricular function (Bartolucci et al. 2017). In another clinical study, 53 patients with dilated cardiomyopathy (DCM) were randomized to IC infusion with either autologous MSCs, BM mononuclear cells (BMMC), or normal saline. Improved left ventricular ejection fraction, New York Heart Association (NYHA) classification, and myocardial perfusion were reported after 12 months of follow-up (Xiao et al. 2017). A recent work stated the importance of cell dose for achieving efficient clinical outcome. A total of 30 patients with ischemic cardiomyopathy randomly received 20 million (n=15) or 100 million (n=15) allogeneic MSCs. Only patients who received the high cell dose had an increased ejection fraction (Florea et al. 2017). A phase II clinical trial confirmed the safety and efficacy of ischemia-tolerant MSCs (itMSCs) in 22 patients with nonischemic cardiomyopathy (Butler et al. 2017). Despite the relative success of clinical trials, further studies are required to improve the efficacy of MSC therapy.

5.3 Kidneys

The kidney is a highly complicated organ that consists of millions of functional units, termed nephrons. Nephron production or nephrogenesis in mammals only occurs during gestation. Hence, no new nephrons are generated after birth. There are various specialized cell types in the kidneys –

podocytes, endothelial cells, and tubular epithelial cells. Depending on the type of disease, one or more cell types may be affected and lose their function (Humphreys et al. 2008).

The kidneys have limited regeneration capacity; therefore, an injury may more likely cause tubular necrosis, apoptosis and, eventually, acute kidney injury (AKI) (Liu and Brakeman 2008). AKI in turn, leads to chronic kidney disease (CKD) as a result of fibrosis, scarring, and organ failure (Moon et al. 2016). Dialysis and kidney transplantation are the current therapies for end stage kidney diseases. Nevertheless, long-term follow-up shows a high mortality rate in patients, which highlights the necessity of an alternative treatment. First attempts to administer MSCs in the kidneys has led to partial renal regeneration and opened up a new horizon towards treatment of renal diseases (Morigi et al. 2004). Recent studies have demonstrated the dedifferentiation of tubular epithelial cells and trans-differentiation of interstitial cells after injuries in the kidneys, which facilitated the regeneration process (Chawla and Kimmel 2012). According to these data, MSC-based therapy supposedly renders an efficient alternative to the current therapeutic approaches. In vitro studies have shown MSCs differentiation potential into the renal-specific lineage (Singaravelu and Padanilam 2009). However, the results of in vivo studies are not as promising in the regeneration of renal cells. Given the complexity of the kidney structure, TE might overcome numerous difficulties related to the regeneration of this organ. Combinations of various MSCs and scaffolds have been used to improve MSCs differentiation potential for renal lineages. BM-MSCs and AD-MSCs are two potential candidates for a cell-based therapy approach in kidney regeneration (Prodromidi et al. 2006). In terms of 3D structure for the cells, different scaffolds have been evaluated in which collagen and HA had some levels of similarity to the renal microenvironment (Rosines et al. 2007). However, the mechanical properties of hydrogels have been always an issue. More recently, decellularized kidney has been suggested as a unique microenvironment for seeded cells for kidney TE. In vivo experiments in a rat model showed promising results in a short term study (Song et al. 2013).

Preclinical studies showed some degrees of kidney regeneration which were attributed to the differentiation potential and paracrine properties of MSCs, yet more efforts are needed to develop a fully functional organ. Imitating this complex microenvironment is very challenging and necessitates increased basic knowledge regarding the kidney development and regeneration.

6 Future Trends and Concluding Remarks

Over the past decades, tremendous efforts have been made to disclose the unknown biological and functional characteristics of MSCs to pave the way for their perspective clinical applications. There are several major issues that remain controversial about the use of MSCs in RM. Completed and on-going clinical trials have shown that MSCs are a powerful therapeutics tool. However, these trials are inadequate to assure their safety. Subsequent to a recent report that intravitreal injection of AD-MSCs in patients with macular degeneration led to complete blindness, it increased the certainty to use the MSCs with caution (Kuriyan et al. 2017). Indeed, all beneficial characteristics of MSCs might cause adverse effects. Multilineage potential of MSCs might create unwanted tissue after transplantation. Intramyocardial calcification was observed as a consequence of BM cells injected into zones of acute myocardial ischemia (Yoon et al. 2004). Therefore, discovery of regulatory factors and signaling pathways in the MSC niche that determine the cell fate to a distinct lineage would be a breakthrough in RM. Risk of tumorigenicity is the major concern related to clinical administration of MSCs. Occurrence of increased immune-suppressive factors and prohibition of immune cells (B-cells and NK cells) as a result of the immunomodulatory properties of MSCs also increases the possibility of tumor progression. It has been indicated that MSCs preferentially migrate to a tumor site due to an inflammatory microenvironment and may contribute to growth

of cancer cells (Lee and Hong 2017). The potential for MSCs in new-blood vessel formation and angiogenesis could promote tumor growth and metastasis. These issues necessitate further effective clinical and preclinical studies to clearly address the safety of MSCs, particularly with long-term follow-up.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 143–147
DOI 10.1007/5584_2018_180

Published online: 1 March 2018

Stem Cells Application in Thoracic Surgery: Current Perspective and Future Directions

Francesco Petrella and Lorenzo Spaggiari

Abstract

Two main fields of clinical applications of stem cells in thoracic surgery have been explored: (a) regenerative medicine, that is a branch of translational research in tissue engineering and molecular biology dealing with the replacement, engineering or regeneration of cells, tissues and organs to restore normal function; (b) drug loading and delivery, that is an emerging field proposing stem cells as vectors to deliver anti-cancer agents for targeted therapies.

Bronchopleural fistula is a pathological connection between the bronchus and the pleural cavity that may develop after lung resection, thus causing pleural empyema due to colonization by resident airway bacteria; stem cells and regenerative medicine approach can effectively contribute to impaired bronchial healing, thus preventive a septic and ventilator catastrophe.

In the field of thoracic oncology, MSC are probably one of the best choice for anticancer drug delivery, emerging as potential experimental approach to malignant mesothelioma treatment.

The goal of this review is to focus on clinical applications of stem cell technologies in thoracic surgery, emphasizing regenerative medicine aspects as well as drug loading and delivery in thoracic oncology.

Keywords

Drug loading and delivery · Regenerative medicine · Stem cells

Abbreviations

MSC	mesenchymal stromal cells
BM-MSC	bone marrow mesenchymal stromal cells
SPECT	single photon emission computed tomography
MR	magnetic resonance
PET	positron emission tomography
SPIO	super paramagnetic iron oxide
USPIO	ultrasmall superparamagnetic iron oxide
BPF	broncho pleural fistula
MPM	malignant pleural mesotehlioma
PD-1	programmed cell death protein 1
PTX	paclitaxel

F. Petrella (✉) and L. Spaggiari
Department of Thoracic Surgery, European Institute of Oncology, Milan, Italy

Department of Oncology and Hemato-oncology, University of Milan, Milan, Italy
e-mail: francesco.petrella@ieo.it; francesco.petrella@unimi.it

1 Introduction

Stem cells are undifferentiated cells possessing extensive self-renewal properties and potential to differentiate *in vivo* and *in vitro* into a variety of lineage cells, like osteogenic, chondrogenic, and adipogenic lineages when cultured in specific inducing media (Pittenger et al. 1999; Siegel et al. 2009). There are two main types of stem cells: embryonic stem cells, deriving from the inner cell mass of blastocysts, and adult stem cells, that can be isolated from many tissues, like bone marrow, adipose tissue, blood and – as recently described – from several other tissues (Kern et al. 2006).

Many studies have focused on the potential clinical applications of stem cell technologies in many fields of medicine – including cardiothoracic surgery – but with controversial results (Petrella et al. 2015a, b, 2017).

Two main fields of clinical applications of stem cells in thoracic surgery have been explored: (a) regenerative medicine, that is a branch of translational research in tissue engineering and molecular biology dealing with the replacement, engineering or regeneration of cells, tissues and organs to restore normal function (Mason and Dunnill 2008); (b) drug loading and delivery, that is an emerging field proposing stem cells as vectors to deliver anti-cancer agents for targeted therapies (Li et al. 2015).

2 Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSC) – previously defined as mesenchymal stem cells – are undifferentiated multipotent adult cells possessing extensive self-renewal properties and potential to differentiate *in vivo* and *in vitro* into a variety of mesenchymal lineage cells (Pittenger et al. 1999).

Due to their immune phenotype, MSC can evade the host immune system, thus allowing allogenic transplantation without any immunosuppression (Igura et al. 2004): after implantation, in fact, MSC interact with the surrounding microenvironment, stimulating tissue healing, restoration and regeneration by cross-talking with other cells present within damaged tissues or structures (Baiguera et al. 2012); moreover they express a deep anti-inflammatory and immunoregulatory effect on the immune system by several mediators, in particular cytokines and chemokines (Kyurkvhiev et al. 2014).

They were first discovered in bone marrow and subsequently isolated and characterized from a wide spectrum of different adult and fetal tissues like umbilical cord, placenta, adipose tissue, tendon, dental pulp, cornea, thymus, liver, spleen, periosteum, brain and synovial and amniotic fluids (Petrella et al. 2015a, b); although MSC isolated by different sources are different, they express the same profile of secreted cytokines (Park et al. 2009).

MSC are able to migrate and engraft at sites of tissue damage and injury and at a wound site exerting local reparative actions by the paracrine secretion of factors with anti-inflammatory and wound-healing properties rather than through a transdifferentiation process into tissue-specific cell types (Wu et al. 2007).

3 Molecular Imaging of Stem Cells

As MSC have emerged as one of the most interesting technology with many potential clinical applications among the various stem cell populations used for cell therapy (Rizzo et al. 2017), there is a specific need of tracking after transplantation to evaluate several different methods of implantation and to monitor cell migration within the human body, thus quantifying cell accumulation at the target site (Ribot et al. 2014).

Optical methods – based on retroviral vectors and fluorescent proteins – have been widely used, but they allow cells tracking and homing only after sacrifice of the animal, moreover with quite poor results, being the tissue penetrability of fluorescence limited (Sohni and Verfaillie 2013).

For this reason, other methods – able to track injected MSC *in vivo* – such as single photon emission computed tomography (SPECT),

magnetic resonance (MR), and positron emission tomography (PET), have being employed.

SPECT uses the radioactive decay of radionuclides and gamma rays to provide 3D information on cell location using tomographic reconstruction (Bindslev et al. 2006); PET imaging is based on direct approach by labeling stem cells with radioactive compounds such as [18F]-fluorodeoxyglucose (Kircher et al. 2011) or on indirect approach relying on the activation of a tracer dye by a protein transduced by a recombinant viral vector into the cells (Deroose et al. 2012).

MR has emerged as an excellent method for tracking cells both *in vivo* and *in vitro*, thanks to its capacity for high spatial resolution; cells can be labeled either with positive contrast agents – such as gadolinium – or with negative contrast agents, such as superparamagnetic iron oxide (SPIO) and ultra small superparamagnetic iron oxide (USPIO) particles (Rizzo et al. 2017).

4 Clinical Applications in Thoracic Surgery

4.1 Bronchopleural Fistula

Bronchopleural fistula (BPF) is a pathological connection between the bronchus and the pleural cavity that may develop after lung resection, thus causing pleural empyema due to colonization by resident airway bacteria. The incidence of BPF – following pulmonary resection for malignant lung cancers treatments – ranges from 1% to 4%, and its mortality is very high, ranging from 12.5% to 71.2% .

Several different causes con contribute to post resectional BPF development: incomplete or technically poor bronchial closure, alteration of bronchial stump wound healing process or stump destruction by residual bronchial tumor. The clinical effect of BPF, complicated by pleural empyema, may hesitate in a life-threatening septic and ventilator condition and the persistence or the resolution of bronchial fistula makes the difference among recovery, chronicity or death (Petrella et al. 2014).

At present, endoscopic treatment of BPF is based on the delivery of a sclerosing agent, biological glue, coils, covered stents, or sealants to the BPF site by flexible or rigid bronchoscope (Cardillo et al. 2015); the success rate of minimally invasive endoscopic approach mainly depends on the underlying disease and the size of the fistulas, with larger fistulas having poor closure rates (Asamura et al. 1992). After a preclinical experience on a large animal model, disclosing that autologous MSC endoscopic transplantation effectively occludes experimental BPF, we reported a case of successful closure of BPF – developing after right extrapleural pneumonectomy – by autologous bone marrow derived MSC by bronchoscopic transplantation (Petrella et al. 2014, 2015a, b).Further encouraging results have been later reported by other teams, disclosing similar successful results obtained by adipose derived MSC, platelet rich plasma and MSC seeded matrix graft (Díaz-Agero Álvarez et al. 2016; Aho et al. 2016; Dua et al. 2016).

4.2 Drug Loading and Delivery for Malignant Mesothelioma Treatment

Malignant Pleural Mesothelioma (MPM) is an highly aggressive neoplasm resistant to chemotherapy, with a low response rate (20% of treated patients). Platinum-based chemotherapy plus a third-generation antifolate is the front-line standard of treatment whereas there are no effective second-line treatments (Facchetti et al. 2017).

Emerging immunotherapy based on an altered expression of genetic pool, makes some genes an excellent target for antibody-based therapy (Ceresoli et al. 2016); however tremelimumab – a fully human IgG2 monoclonal antibody against cytotoxic T lymphocyte associated protein 4 (CTLA-4) – presents several severe side effects (Maio et al. 2017) while pembrolizumab – an inhibitor of programmed cell death protein 1 (PD-1) – is currently under investigation, demonstrating to be safe and tolerable (Alley et al. 2017).

MSC have been shown the property to accumulate intracellularly and deliver several antineoplastic drugs, without any genetic modifications, thereby decreasing tumor proliferation (Pessina et al. 2011). Among the many different methods of drug delivery described in the last decade, non-modified MSC are probably one of the best choice for anticancer drug delivery as they easily adapt to culture conditions and home to neoplastic tissues when injected in vivo (Pessina et al. 2011).

MSC release active soluble factors and play an effective immunomodulatory rolemoreover they can cross the blood brain barrier, thus representing a potential resource for adult and pediatric brain tumors treatment on the other hand, the issue of whether MSC cross-talk with the tumor microenvironment boosts tumor suppression or instead favors tumor growth remains unsettled (Pacioni et al. 2017). We have demonstrated that bone marrow mesenchymal stromal cells (BM-MSC) loaded with paclitaxel (PTX) – an antineoplastic drug targeting tubulin and stabilizing the microtubule polymer protecting it from disassembly – successfully inhibit *in vitro* proliferation of MPM cells however further studies and *in vivo* testing are required to confirm our preliminary in vitro results as a potential new mesothelioma therapy based on cell drug delivery (Petrella and Spaggiari 2017)

5 Concluding Remarks

Clinical application of regenerative medicine principles and stem cell technologies to thoracic surgery is an intriguing and promising field; however, several drawbacks still exist and should be clearly emphasized before shifting preclinical findings in clinical day practice.

Clear warnings are needed against sensational, anecdotal or enthusiastic reports that jeopardize the complex field of regenerative medicine making it even more dangerous and controversial.

Acknowledgments The Authors thank Anne Collins for editing the English text.

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval The authors declare that this article does not contain any studies with human participants or animals.

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Adv Exp Med Biol – Cell Biology and Translational Medicine (2018) 2: 149–155
https://doi.org/10.1007/978-3-030-04170-0

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