Finnish Red Cross Blood Service
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THE IMPACT OF MEMBRANE PHOSPHOLIPID COMPOSITION AND EXTRACELLULAR VESICLES ON THE IMMUNOREGULATIVE PROPERTIES OF HUMAN MESENCHYMAL STROMAL CELLS

Lotta Kilpinen

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, for public examination in Nevanlinna Auditorium of the Finnish Red Cross Blood Service, Kivihaantie 7, Helsinki, on 9th June 2017, at 12 noon.

Helsinki 2017

ACADEMIC DISSERTATION FROM THE FINNISH RED CROSS BLOOD SERVICE NUMBER 62

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ISBN 978-952-5457-43-8 (print)
ISBN 978-952-5457-44-5 (pdf)
ISSN 1236-0341
http://ethesis.helsinki.fi
Unigrafia
Helsinki 2017

It always seems impossible until it's done
-Nelson Mandela

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I [1]Kilpinen L[*], Tigistu-Sahle F[*], Oja S, Greco D, Parmar A, Saavalainen P, Nikkilä J, Lehenkari P, Korhonen M, Käkelä R, Laitinen S (2013) Aging bone marrow mesenchymal stromal cells have altered membrane glycerophospholipid composition and functionality. J Lipid Res 54:622-635

II [2]Kilpinen L, Parmar A, Greco D, Lehenkari P, Saavalainen P, Laitinen S (2016) Expansion induced microRNA changes in bone marrow mesenchymal stromal cells reveals interplay between immune regulation and cell cycle. Aging (Albany NY) 8:2799-2813

III [3]Kilpinen L[*], Impola U[*], Sankkila L, Ritamo I, Aatonen M, Kilpinen S, Tuimala J, Valmu L, Levijoki J, Finckenberg P, Siljander P, Kankuri E, Mervaala E, Laitinen S (2013) Extracellular membrane vesicles from umbilical cord blood-derived MSC protect against ischemic acute kidney injury, a feature that is lost after inflammatory conditioning. J Extracell Vesicles 2: 10.3402/jev.v2i0.21927. eCollection 2013

The publications are referred to in the text by their roman numerals I-III. The original publications are reproduced with the permission of their copyright holders. In addition, some unpublished results are presented.

[*] The authors have equally contributed to the study. The publication has been included in the thesis of Feven Tigistu-Sahle.

1 Author designed the experiments, performed cell culture and functionality experiments, analyzed the data and wrote the manuscript.

2 Author designed the experiments, performed cell culture experiments, analyzed the data and wrote the manuscript

3 Author designed the experiments, performed co-culture assays, analyzed the data and wrote the manuscript

Other publications not included in the thesis:

Tigistu-Sahle F, Lampinen M, **Kilpinen L**, Holopainen M, Lehenkari P, Laitinen S, Käkelä R (2017) Metabolism and phospholipid assembly of polyunsaturated fatty acids in human bone marrow mesenchymal stromal cells. J Lipid Res 58:92-110

Laitinen A, Lampinen M, Liedtke S, **Kilpinen L**, Kerkelä E, Sarkanen JR, Heinonen T, Kögler G, Laitinen S (2016) The effects of culture conditions on the functionality of efficiently obtained mesenchymal stromal cells from human cord blood. Cytotherapy 18:423-437

Laitinen, A, Oja S, **Kilpinen L**, Kaartinen T, Moller J, Laitinen S, Korhonen M, Nystedt J (2016) A robust and reproducible animal serum-free culture method for clinical-grade bone marrow-derived mesenchymal stromal cells. Cytotechnology 68:891-906

Kerkelä E, Hakkarainen T, Mäkelä T, Raki M, Kambur O, **Kilpinen L**, Nikkilä J, Lehtonen Siri, Ritamo I, Pernu R, Pietilä M, Takalo R, Juvonen T, Bergström K, Kalso E, Valmu L, Laitinen S, Lehenkari P, Nystedt J (2013) Transient Proteolytic Modification of Mesenchymal Stromal Cells Decreases Lung Entrapment and Increases Targeting to Injured Tissue. Stem Cells Transl Med 2:510-520

ABBREVIATIONS

AKI	Acute kidney injury
αMEM	Minimum essential medium alpha
AT-MSC	Adipose tissue derived mesenchymal stromal cells
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
BM-MSC	Bone marrow derived mesenchymal stromal cells
CB-MSC	Cord blood derived mesenchymal stromal cells
CFSE	Carboxyfluorescein diacetate N-succinimidyl ester
COX	Cyclooxygenase
DC	Dendritic cell
DHA	Docosahexaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FBS	Fetal bovine serum
GPL	Glycerophospholipid
EPA	Eicosapentaenoic acid
ESI-MS	Electrospray ionization mass spectrometry
EVs	Extracellular vesicles
IDO	Indoleamine 2,3-dioxygenase
IFNγ	Interferon gamma
IRI	Ischemia-reperfusion injury
HLA	Human leukocyte antigen
HO	Hemioxygenase
HSC	Hematopoietic stem cell
MHC	Major Histocompatibility Complex
mRNA	Messenger RNA
miRNA	MicroRNA
MSC	Mesenchymal stromal/stem cells
NTA	Nanoparticle tracking analysis
PBMC	Peripheral blood mononuclear cell
PUFA	Polyunsaturated fatty acid
SDF-1	Stem cell derived factor-1
SASP	Senescence associated secretory phenotype
SPM	Specialized pro-resolving mediators
TNFα	Tumor necrosis factor alpha
Treg	Regulatory T-cell

ABSTRACT

Human mesenchymal stem/stromal cells (hMSCs) are currently used in many advanced cellular therapies. The clinical use of hMSCs requires extensive cell expansion, but the consequences of expansion, especially at the molecular level, are not fully understood. The therapeutic effect of MSCs is mediated at least partially via paracrine interactions with immune cells modulating both innate and adaptive immune response. Membrane glycerophospholipids (GPLs) provide precursors for signaling lipids, which modulate cellular functions, including immunological effects via eicosanoids and docosanoids.

The aim of this study was to investigate the effect of the donor's age and cell doublings on the GPLs, gene expression and microRNA (miRNA) profiles of human bone marrow MSCs (hBM-MSC). In order to gain more insight into the functional mechanisms of MSCs, we investigated the extracellular vesicle (EV) secretion from human umbilical cord blood derived MSCs (hCB-MSC), and evaluated their immunosuppressive capacity *in vitro* as well as their possible immunomodulative and protective effect in kidney ischemia-reperfusion injury (IRI) *in vivo*. We were able to demonstrate that the hBM-MSCs, harvested from 5 young adults and 5 old donors, showed clear compositional changes in their GPL profiles during expansion. Most strikingly, the molar ratio of prostaglandin E2 precursor arachidonic acid (20:4n-6) containing species of phosphatidylcholine and phosphatidylethanolamine accumulated, while the species containing monounsaturated fatty acids decreased during passaging. The lipid changes correlated with the decreased immunosuppressive capacity of hBM-MSCs during expansion, suggesting a connection between lipid signaling and immunomodulatory functions. The existence of such a connection was further supported by gene expression results for several enzymes involved in lipid metabolism and immunomodulation. Our results show that extensive expansion of hBM-MSCs harmfully modulates membrane GPLs, affecting lipid signaling, and eventually impairing functionality.

Although we were able to see clear alterations in gene expression levels and lipid profiles, the miRNA expression was more stable. To summarize, the expression levels of 37 miRNAs were changed in the old donors group and 36 miRNAs were changed in the young donors group. Of these, only 12 were differentially expressed in both young and old donor BM-MSCs and their predicted target mRNAs, the expression of which was changed, were mainly linked to cell proliferation and senescence. Interestingly, members of the well-studied miR-17/92 cluster, which is involved in cell cycle regulation, aging and the development of immune system, were down-regulated especially in the old donors group. The role of this cluster in MSC

functionality is worth future studies since it links expansion, aging and immune system together.

When we studied the immunological effect of EVs derived from CB-MSC, we were able to demonstrate their immunological potential both *in vitro* and *in vivo*. Interestingly, these properties were weakened especially in the animal model when MSCs were preconditioned with interferon gamma. A comparative mass spectrometric analysis revealed a clear distinction in the protein content of the IFNγ stimulated EVs. We discovered that two differently produced EV pools contained specific Rab proteins suggesting that, depending on external signals, the same cells produce vesicles originating from different intracellular locations, which also influences their functional properties.

In conclusion, these studies provide a detailed analysis of molecular changes during MSC expansion. The present study demonstrates that the combination of *in vitro* and *in vivo* models accompanied with a detailed analysis of molecular characteristics is essential to a profound understanding of the complexity of the MSC paracrine regulation.

1 INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) were first described as non-hematopoietic progenitor cells from the bone marrow in the 1960's by Friedenstein and co-workers (Friedenstein et al. 1968). Traditionally minimal criteria of International Society for Cellular Therapies (ISCT) defines MSCs as plastic adherent fibroblast like cells that are capable of differentiating into adipocytes, osteoblasts and chondrocytes and that express certain cell surface markers (CD73,CD90 and CD105) but not hematopoietic markers (CD14, CD19, CD34, CD45, and HLA-DR) (Dominici et al. 2006). One of the most fascinating properties of MSCs is their versatile ability to modulate immune cells and enhance the repair of injured tissue. The immunomodulative capacity has made MSCs one of the most attractive cell types for advanced cellular therapies and therefore ISCT suggests that an immunosuppression assay should be included in the releasing criteria for a clinical-grade MSC product (Galipeau et al. 2016).

After the initial discovery in the bone marrow, MSCs have been isolated from several other adult and fetal tissues. It has been suggested that MSCs reside inside all tissues nearby perivascular space (da Silva Meirelles et al. 2008). However, the identification of MSCs' *in vivo* location and physiological role has been extremely challenging, and little is known about their true identity and role in the tissue homeostasis. Several molecular mechanisms have been described, but many open questions still remain. How do these cells perform their function and how many different types of MSCs are there in the body?

After establishment, MSCs are expanded *in vitro*. MSCs, like other primary cells, are not able to divide infinitely, but reach cellular senescence. In recent years, the use of autologous MSCs in several indications impacting aged patients has increased. Donor age introduces another dimension to the puzzle of MSC therapy. The effects of donor age and extensive expansion on the characteristics and functionality of hBM-MSCs are worth studying, in order to develop more efficient MSC therapies.

2 REVIEW OF THE LITERATURE

2.1 Mesenchymal stromal cell niche

Niches are local tissue microenvironments that maintain and regulate stem cells. Hematopoietic stem cell (HSC) niches are present in diverse tissues throughout the development, beginning with the yolk sac, followed by placenta, fetal liver and finally bone marrow (Mikkola and Orkin 2006). In the adult human body, bone marrow has many crucial functions. Most importantly bone marrow is the scene of hematopoiesis, a process that leads to formation of all blood cells. Hematopoietic stem cells (HSC) are self-renewing and multipotent cells giving rise to all blood cells of the myeloid and lymphoid lineages (**Figure 1**). The HSC pool comprises actively self-renewing HSCs (ST-HSC) and quiescent long-term HSCs (LT-HSC). The bone marrow microenvironment seems to be adapted to support these HSC properties. Using mouse as the animal model, it has been suggested that these two types of HSCs are located in different places: LT-HSCs near the endosteum (endosteal niche) and ST-HSC in connection with sinusoidal endothelium (perivascular niche) (Wilson et al. 2008). HSCs are located in the bone marrow in accordance with their differentiation state, and it is the blood flow rate rather than the actual distance from the endosteum that defines the niche (Winkler et al. 2010a). The development of more specific labels to identify HSCs *in vivo* has shown that most of the HSCs are found near sinusoids in the highly vascular endosteal region (Nombela-Arrieta et al. 2013).

In addition to HSCs, a heterogeneous population of stromal cells is found in the bone marrow. A small proportion of these MSCs exhibit stem cell characteristics, such as self-renewing capacity and differentiation potential into adipocytes, osteoblasts and chondrocytes (**Figure 1**). The identification of MSCs *in vivo* is challenging and therefore MSCs are defined according to their characteristics *in vitro*. In mice, several mesenchymal progenitors, such as CXC chemokine ligand (CXCL) 12 abundant reticular (CAR) cells (Omatsu et al. 2010, Sugiyama et al. 2006), stem cell factor expressing cells (Ding et al. 2012), nestin expressing cells (Mendez-Ferrer et al. 2010) and platelet derived growth factor receptor a (PDGFR-a)$^+$, Sca-1$^+$, CD45$^-$ and Ter119$^-$ cells (Ding and Morrison 2013, Greenbaum et al. 2013), have been described. All these cells possess highly similar properties, indicating that bone marrow may contain several different pools of MSCs with different roles in the regulation of HSCs. In human bone marrow, CD146+ osteoprogenitors with self-renewing capacity have been described, indicating that stromal cells are necessary for maintaining HSC niche also in the human bone marrow (Sacchetti et al. 2007).

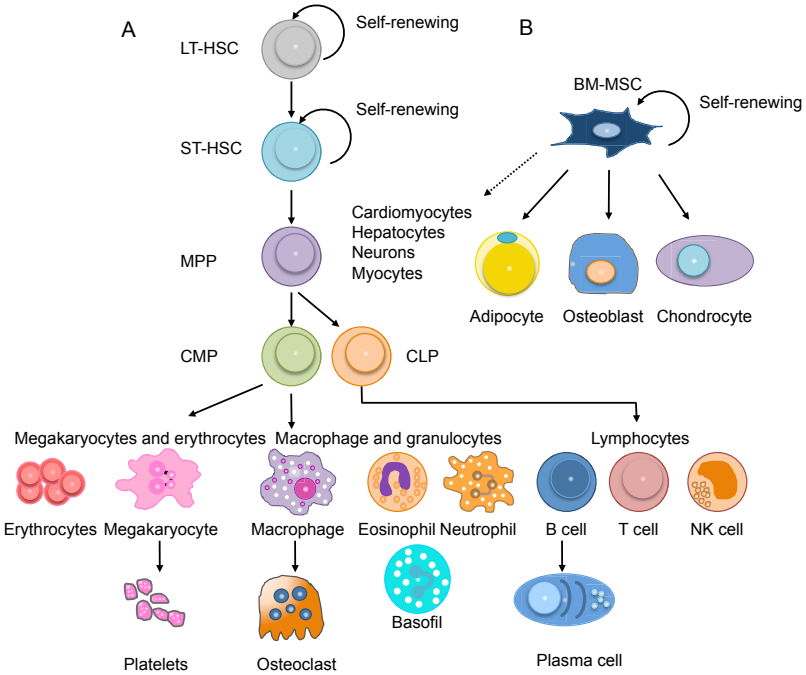

Figure 1 The regenerative cells of the bone marrow niche. A. Hematopoietic stem cells (HSC) are precursors of all mature blood cells. A proportion of these self-renewing cells remain undifferentiated to maintain a pool of long-term reconstituting HSCs (LT-HSC) and short term reconstituting HSCs (ST-HSC). **B.** Bone marrow mesenchymal stromal cells (BM-MSCs) are multipotent, self-renewing progenitor cells that can differentiate into other cell types. Abbreviations: CLP, common lymphoid progenitor cell; CMP, common myeloid progenitor; MPP, multipotent progenitor; NK cell, natural killer cell. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Rheumatology 12:154:168, copyright 2015.

Recent advances in imaging techniques have elucidated the location and role of MSCs in the microenvironment of bone marrow. Similar findings in fetal human bone marrow are available, (Pinho et al. 2013) but a more detailed analysis of the HSC niche in humans is required. According to the animal studies, MSCs reside mostly perivascularly together with HSCs, but traffic to the endosteal surface to differentiate into osteoblasts (Morrison and Scadden 2014). Recently, a new pool of progenitor cells was described and called bone lining cells. These cells are located on the endosteal surface and act as precursors for osteoblasts and osteoclasts (Matic et al. 2016). The adipogenic potential of these cells has yet to be investigated, but these results reinforce the possibility of secondary pool of MSCs in the bone marrow. One of the major tasks of MSCs is to create a supportive microenvironment for HSCs self-renewal and quiescence in the bone marrow (Mendez-Ferrer et al. 2010, Omatsu et al. 2010). MSCs produce stem cell factor (SCF) and stromal derived factor (SDF1,) also called CXCL12, and other cytokines that promote the maintenance of HSCs (Sacchetti et al. 2007). In addition to the soluble molecules, miRNAs, delivered in extracellular vesicles, may act as one

mechanism for the maintenance of HSCs (De Luca et al. 2016). The interaction between MSCs and HSCs is far more complex and studies have shown that also HSCs secrete cytokines relevant to the proliferation and differentiation of MSCs as well as to the inhibition of their senescence (Zhou 2015).

Besides HSCs and MSCs, there are several other cell types residing in the niche that are essential for its function. These include osteoblasts, osteoclasts endothelial cells, bone marrow adipocytes, megakaryocytes, tissue resident macrophages, immune cells, and neurons (**Figure 2**). Osteoblasts were the first cell population shown to have an influence on HSCs. Two groups showed with animal models that osteolineage cell activation increased HSC number *in vivo* (Calvi et al. 2003, Zhang et al. 2003). The interaction of angiopoietin-1, expressed by osteoblasts, with its receptor Tie-2, expressed by HSCs, is shown to be essential for HSC quiescence (Arai et al. 2004). Also, local calcium ion concentration has been shown to recruit HSCs to endosteum via calcium sensing receptors (Adams et al. 2006). Osteoclasts are derived from the HSCs in the bone marrow. There is evidence that in addition to participating in the remodeling of the bone, osteoclasts also regulate the HSC niche through the degradation of CXCL12 and SCF (Kollet et al. 2006). Currently, the roles of endosteum, as a regulatory region, and osteoprogenitor cells in the maintenance of HSCs are acknowledged, but mature osteoblasts and osteoclast contribute to the HSC maintenance most likely indirectly through the modulation of the microenvironment (Morrison and Scadden 2014).

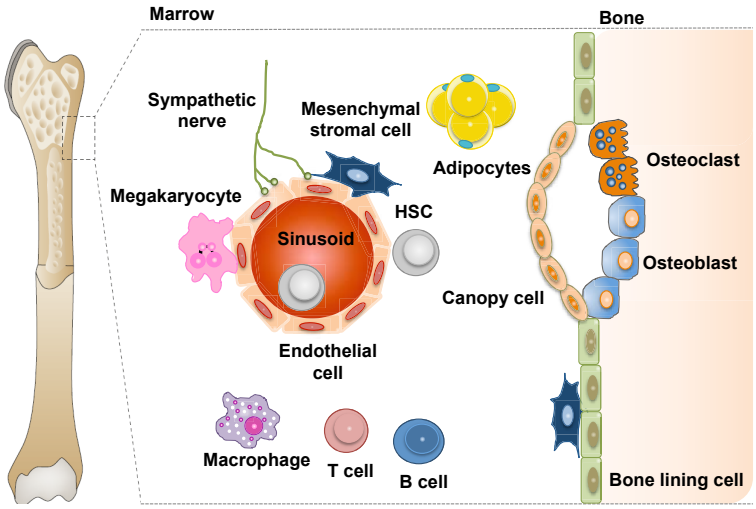

Figure 2 Structure and cellular components of bone marrow niche. Bone marrow is a complex organ containing hematopoietic and non-hematopoietic cell types. The interface of bone marrow is called endosteum, which is covered by bone lining cells, osteoblasts and osteoclasts. Sinusoids are specialized blood vessels that allow trafficking of cells into circulation. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Rheumatology 12:154-168, copyright 2015.

Sinusoidal endothelial cells participate in the maintenance of HSCs by expressing SDF-1 (Greenbaum et al. 2013), SCF (Ding et al. 2012) vascular endothelial growth factor (VEGF) receptor 2 (Hooper et al. 2009) and e-selectin (Winkler et al. 2012). Megakaryocytes contribute to the niche by supporting HSC quiescence through the production of CXCL4 and transforming growth factor (TGF)-β (Bruns et al. 2014, Zhao et al. 2014b). Marrow adipocytes residing near the endosteal surface do not only act as a filler in the bone marrow, but are also necessary for the maintenance of HSC quiescence through endocrine and paracrine effects (Naveiras et al. 2009). Bone marrow resident macrophages are distributed throughout the bone marrow and are necessary for the endosteal bone marrow niche. Macrophages regulate HSC dormancy by keeping them in their stem-cell state while supporting bone formation via the regulation of MSC osteogenic differentiation (Chang et al. 2008, Winkler et al. 2010b). Finally, the sympathetic nervous system regulates CXCL12-expression in the bone marrow and further HSC mobilization to the circulation (Katayama et al. 2006). HSC mobilization follows physiologically regulated circadian rhythms. Cyclic release of HSCs and downregulation of CXCL12 are regulated by molecular clock genes mediated by circadian release of noradrenaline from sympathetic nerves. The signal is mediated through β3 adrenoreceptor that is expressed by MSCs (Mendez-Ferrer et al. 2008).

If the bone marrow is the scene of hematopoiesis in the adult human body, placenta is a unique hematopoietic organ during the development (Gekas et al. 2005). In contrast to the bone marrow, HSCs are not maintained in dormancy during the development but expand actively without differentiation. HSCs are formed in the large vessels of chorionallantoic mesenchyme and expand in the placental vascular labyrinth. In humans, HSCs seems to be present in the placenta throughout the development. Similar to the bone marrow, the placenta contains stromal cells that support HSCs *in vitro* (Robin et al. 2009). In the human placenta, MSCs are located particularly in the chorionic villi of the placenta residing near blood vessels and regulate vessel maturation and stabilization (Castrechini et al. 2010). The regulation of the HSC niche is complex and involves the activity of several cell types including MSCs, trophoblasts and endothelial cells. One of the key mechanisms for regulating HSCs in the placenta is PDGF-B signaling (Chhabra et al. 2012). Compared to the bone marrow, cellular components and molecular signals maintaining the microenvironment in the placenta are not well understood.

2.2 Mesenchymal stromal cells in cell therapy

MSCs have several properties that make them an intriguing tool for cellular therapy. They have been considered immune privileged since they express low levels of major histocompatibility complex class 1 (MHCI) and they do not constitutively express MHC class II or costimulatory molecules such as CD40, CD80, and CD86 (Tse et al. 2003). These features could protect them from alloreactive natural killer (NK) cell lysis and promote their survival and growth in an allogenic environment (Sotiropoulou et al. 2006). Later studies have shown that MSCs are rejected by NK cells and therefore they should be considered immune evasive rather than immune privileged (Ankrum et al. 2014). MSCs have the ability to home to the sites of inflammation (Spaeth et al. 2008) and perform immunomodulatory functions in the injured tissue. MSCs use both direct cell-to-cell contact as well secreted soluble immunosuppressive molecules to interact with a wide range of immune cells, including T cells, B cells, NK cells, dendritic cells, and macrophages.

Although MSCs are able to differentiate into various cell types, many of their favorable effects are mediated through cell-to-cell communication, which inhibits inflammation and stimulates the recovery of injured tissue (**Figure 3**). MSCs communicate with target cells either via paracrine signaling or direct cell-to cell contact. In addition to soluble molecules like bioactive lipids, growth factors and cytokines, paracrine mechanisms of MSCs include secreted extracellular vesicles (EVs). EVs encompass different types of vesicles that differ by their route of origin. Shedding vesicles or microvesicles are produced by outward budding and fission of plasma membrane. Exosomes originate inside cellular multivesicular endosome (MVE) and apoptotic bodies originating from the shedding cells during programmed cell death (Raposo and Stoorvogel 2013). Most studies have not clearly defined the origin of EVs since the development of methods to discriminate between exosomes and microvesicles constitutes a major challenge. Differences in properties such as size, morphology, buoyant density, and protein composition seem insufficient for a definitive distinction (Bobrie et al. 2011). EVs participate in cell-to-cell communication by transferring functionally relevant biomolecules such as proteins, mRNA, and miRNA (Ragni et al. 2017, Tomasoni et al. 2013, Valadi et al. 2007).

MSC mediated immunomodulative and angiogenesis promoting effects are decreased when transwell systems are used, indicating that at least some of the MSC functions are mediated via contact dependent signaling. Besides direct protein-interaction mediated intercellular signaling, MSCs have also been shown to utilize tunneling nanotubes to transfer molecules and even cell organelles such as mitochondria to the target cells such as macrophages and T-cells (Jackson et al. 2016, Liu et al. 2014, Matula et al. 2016).

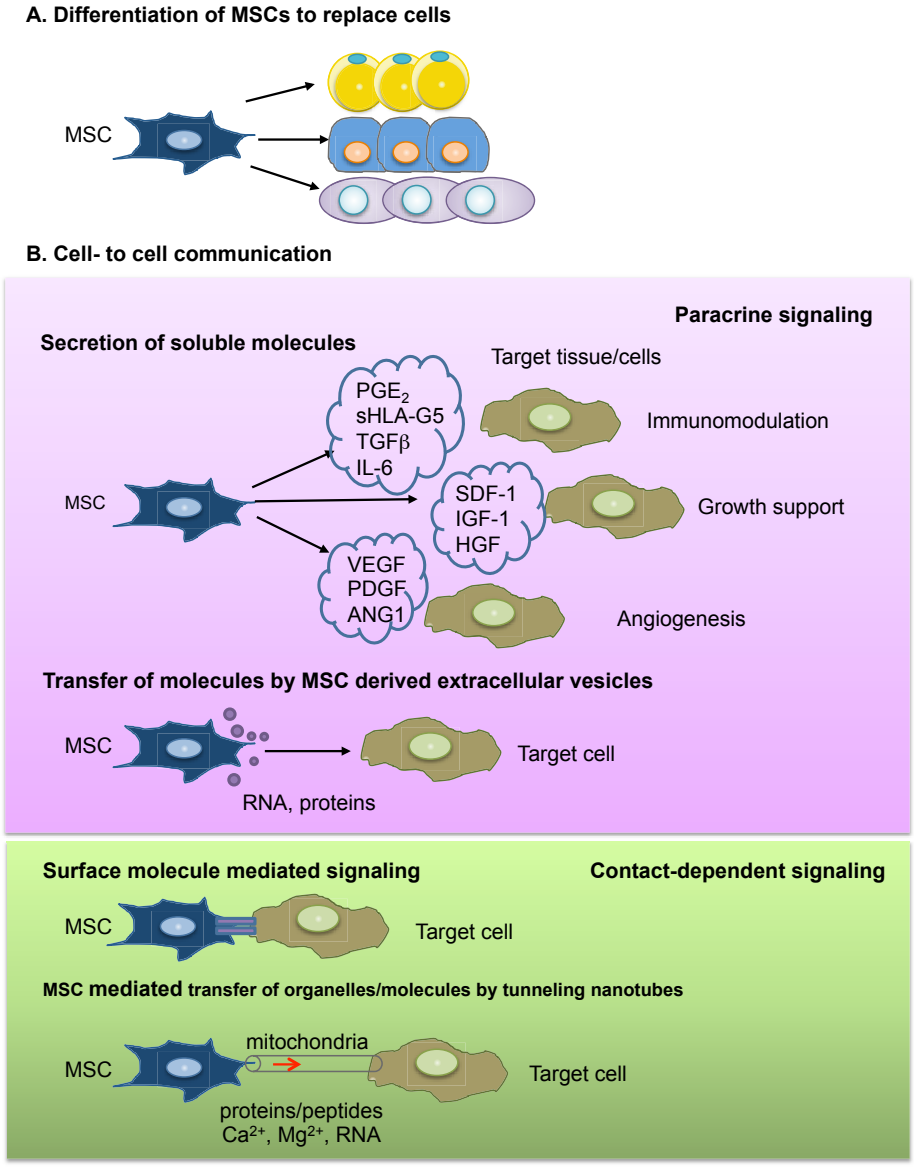

Figure 3 MSCs repair injured or damaged tissues by diverse mechanisms. A. Differentiation into different cell types to replace cells in the damaged tissue. **B**. Intercellular signaling includes paracrine and contact-dependent mechanisms. Paracrine mechanisms include secretion of soluble molecules such as growth factors, hormones and cytokines and secretion of extracellular vesicles to transfer protein/peptides, RNA or other molecules. Contact-dependent signaling is mediated by surface proteins or tunneling nanotubes. Abbreviations: PGE_2, prostaglandin E_2; sHLA-G5, soluble human leukocyte antigen G5; TGFβ, transforming growth factor beta; IL-6, interleukin-6; SDF-1, stromal-derived factor-1; IGF-1, insulin-like growth factor-1; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; ANG1, angiopoietin-1. Figure is adapted from (Spees *et al.* 2016) under license http://creativecommons.org/licenses/by/4.0/

The beneficial properties of MSCs are utilized for various indications in clinical trials and the number of ongoing trial is continuously increasing. As of August 2016, the clinical trials database (http://www.clinicaltrials.gov) showed 418 clinical trials using MSCs for various indications (**Figure 4**). Bone marrow is still the most commonly used source of MSCs (n=204), but other tissues, especially adipose tissue (n=79) and umbilical cord (n=53), are also often used. In particular, interest in adipose tissue as autologous source of MSCs is increasing.

Most of the clinical trials are in Phase I (safety studies, n=147) or in combined Phase I and II (n=151). A total of 84 studies have reached phase II (proof of concept for human patients). Only 18 studies are currently in phase III (comparison of new treatment with standard treatment) and 13 studies are in combined Phase II and Phase III. In general, MSCs appear to be well tolerated and safe. In meta-analysis, a significant association between MSC administration and transient fever was reported (Lalu et al. 2012).

Fetal bovine serum (FBS) has historically been considered essential for obtaining MSCs of high quantity and quality. However, FBS carries the risk of transmitting immunogenic xenoproteins or infectious agents. The development of human supplements, such as platelet rich plasma or platelet lysate, has led supplements to produce high-quality clinical-grade MSCs for therapeutic applications (Bieback et al. 2009, Juhl et al. 2016, Laitinen et al. 2016a). Also, the actual composition of nutrients in FBS has not been optimized and especially the fatty acid composition may not be optimal in terms of the best possible therapeutic outcome (Tigistu-Sahle et al. 2017). Despite the availability of animal serum-free culture methods, FBS is still the most commonly used medium supplement in expansion of MSCs since only seven clinical trials reported the usage of a human medium supplement.

The most significant results on the immunosuppressive effects of MSCs so far have been observed in the treatment of acute graft versus host diseases (GVHD) after allogenic hematopoietic stem cell transplantation (Le Blanc et al. 2008). After the first trials, MSCs have been shown to be a promising new therapy for both acute and chronic steroid resistant GVHD (Chen et al. 2015). Recently, the use of MSCs has been expanded to other hematological disorders (Cle et al. 2015) and co-transplantation in allogenic HSC transplantation to prevent GVHD or improve the engraftment (Liu et al. 2011a). Based on their ability to modulate T-cell proliferation and function, MSCs have also been proposed as a therapeutic tool for Chron's disease (Forbes et al. 2014), autoimmune diseases (Wang et al. 2014), diabetes (Carlsson et al. 2015), renal transplantation rejections (Reinders et al. 2015), and in the treatment of various immune-mediated neurodegenerative disorders such as amyotrophic lateral sclerosis (Oh et al. 2015, Petrou et al. 2016) and multiple sclerosis (Karussis et al. 2010, Llufriu et al. 2014). Osteoarthitis is a disorder that leads to cartilage damage associated with synovial inflammation. The immunomodulatory capacity of AT-MSCs is used to alleviate osteoarthritis in the knee and other sites (Pers et al. 2016).

The regenerative potential and particularly the differentiation capacity into osteoblasts exhibited by MSCs have given rise to studies investigating their therapeutic use in osteogenesis imperfecta (Gotherstrom et al. 2014). Even though the majority of the experimental evidence on differentiation capacity of MSCs comes from *in vitro* experiments, there are also results indicating that engrafted MSCs can promote tissue regeneration by differentiating into tissue-specific cells *in vivo* such as cardiomyocytes needed for repair of injured tissues (Gojo et al. 2003). Later it has been shown that the trophic effects of MSCs are of great significance in tissue regeneration. After engraftment, MSCs produce a number of molecules that can not only reduce inflammation but also stimulate tissue generation by promoting cell-to-cell connections (Plotnikov et al. 2008). The regenerative potential of MSCs is nowadays harnessed to treat various conditions such as, ischemic cardiomyopathy (Hare et al. 2009), liver diseases (Shi et al. 2012, Zhang et al. 2012), spinal cord injury (Mendonca et al. 2014, Satti et al. 2016), and stroke (Honmou et al. 2011, Lee et al. 2010).

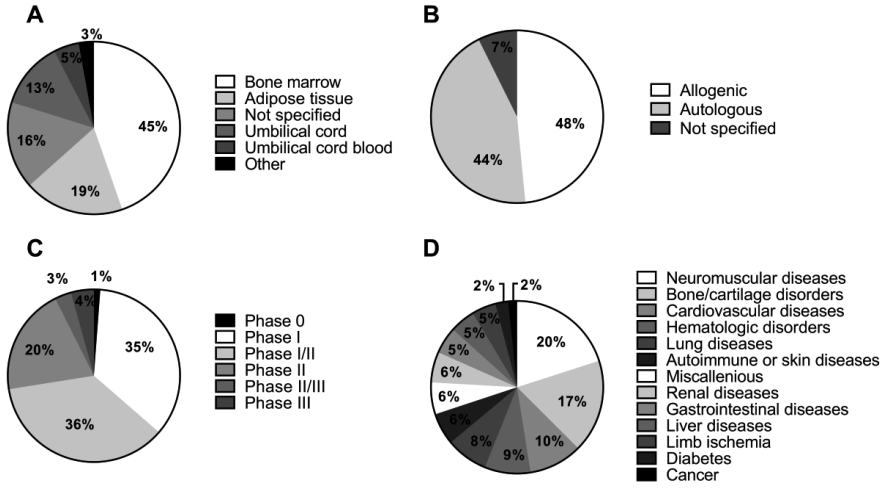

Figure 4 Summary of clinical trials using MSCs. The data were derived from ClinicalTrials.gov (http://www.clinicaltrials.gov) on August 8, 2016. Keywords "mesenchymal stromal cells OR mesenchymal stem cells" were used. Trials with unknown status or terminated before enrolment were excluded from the analysis, n=418 **A**. Tissue sources of MSCs currently used. "Not specified" category includes trials that did not clearly state their cell sources including trademark products. "Other" includes tissue sources such as menstrual blood or skin. **B.** Donor type of MSCs used in clinical trials **C**. Current phases of clinical trials **D**. Clinical indications that are targeted with MSC therapy. Disorders are divided in larger categories.

MSC therapy in acute kidney injury

Acute kidney injury (AKI) is a common clinical syndrome in hospitalized patients, particularly those with chronic kidney disease, diabetes, and vascular diseases. Furthermore, 30-40% of all cases of AKI observed during hospitalization occur in the context of surgical operations and especially after cardiovascular surgery. AKI is currently defined as a rapid decrease in kidney function as measured by an increase in serum creatinine and/or urine output (Erpicum et al. 2014, Tögel and Westenfelder 2012). AKI commonly involves tubular damage called acute tubular necrosis (ATN) and a reduced glomerular filtration rate caused by transient ischemia and reperfusion (I/R) (Schrier et al. 2004). Pathophysiology of I/R consists of diverse metabolic and inflammatory events. A rapid drop in oxygen and nutrition concentrations leads to vasoconstriction, separation of tubular cells and obstruction of tubules. Even though oxygen supply limits oxidative phosphorylation, anaerobic glycolysis allows a residual production of ATP. However, the formation of lactate results in the acidification of cell cytosol, finally leading to the disruption of mitochondrion and cell functions. In addition, the lack of energy delivery induces morphologic changes, including disruption of the cytoskeleton and intercellular tight junctions, loss of cell polarity and the translocation of the Na^+/K^+ATPase transporter from the basolateral membrane to the cytoplasm causing apoptosis of tubular cells (Schrier et al. 2004, Seo-Mayer et al. 2011).

Both innate and adaptive immune responses are important in the pathology of ischemic kidney injury. Injured tubular epithelium releases pro-inflammatory cytokines and chemokines, which aid in recruiting immune cells. Epithelial cells also express adhesion molecules, toll-like receptors (TLRs), complement and complement receptors, and T cell costimulatory molecules, which activate the immune cells (Bonventre and Yang 2011). T cells are key mediators in the renal IRI (Ascon et al. 2006). The recruitment of regulatory T cells is one of the anti-inflammatory mechanisms in the recovery of an injured kidney (Gandolfo et al. 2009, Kinsey et al. 2010). Recently, the importance of TLR9 in Treg recruitment in AKI was demonstrated (Alikhan et al. 2016).

Currently, available treatments are largely supportive, including fluid maintenance, vasoactive drugs, cytoprotective therapy, and dialysis (Thakar 2013). There is a clear need for novel therapeutic approaches to increase the survival rate in AKI. Even when injured, the kidney has a great regeneration potential. MSCs could offer an innovative approach to the promotion of proliferation and differentiation of progenitor cells inside the kidney and inhibition of inflammation. Previous studies based on animal models have shown the effectiveness of MSCs derived from bone marrow (Alfarano et al. 2012, Morigi et al. 2008, Wise et al. 2014), adipose tissue (Furuichi et al. 2012), and umbilical cord blood (Jang et al. 2014) in the treatment of IRI. In addition, other models using glycerol (Herrera et al. 2004, Qian et al. 2008)

or cisplatin (Qi and Wu 2013) to induce kidney injury have been used to study the therapeutic effect of MSCs. In the initial clinical trials, the safety and feasibility of MSC therapy were assessed in patients with a high risk of developing AKI after cardiac surgery (Gooch et al. 2008, Tögel and Westenfelder 2012). Two phase II trials aimed at showing the efficacy of MSC treatment in AKI patients are on-going (NCT01275612 and NCT01602328), but no results have been reported so far for these trials.

The mechanisms of MSCs-mediated renoprotective functions are divided into differentiation-dependent and differentiation-independent mechanisms. Although studies in both animals and humans have shown that MSCs are able to differentiate into tubular epithelial cells (Herrera et al. 2004, Morigi et al. 2004), the primary modes of action are considered to be paracrine and also endocrine since only very limited engraftment and differentiation into target cells have been observed *in vivo* (Morigi et al. 2008). More recently, Zhao *et al.* (2014a) showed that an intrarenal injection of MSCs resulted in differentiation into vascular endothelial cells indicating that at least part of the regenerative potential could after all be differentiation-dependent.

The paracrine action of MSCs involves the delivery of trophic factors and anti-inflammatory soluble molecules to the kidney. AKI causes a significant up-regulation of SDF-1 and hyaluronic acid in the kidney, thus resembling the microenvironment in the bone marrow niche. Infused MSCs are able to home to the injured kidney through the use of homing receptors CXCR4 (SDF-1 receptor) and CD44 (the hyaluronic acid receptor) (Herrera et al. 2007, Tögel et al. 2005b). The expression of CXCR4, however, decreases during *in vitro* cell culture, thus reducing the homing ability of transfused MSCs. There is an elevated expression of transforming growth factor β (TGF β) in I/R injured kidneys, which induces the expression of CXCR4 on cell membranes, resulting in enhanced homing of MSCs (Si et al. 2015). Also, *in vitro* hypoxic preconditioning is shown to increase the expression of CXCR4, further enhancing the homing and therapeutic effects in AKI (Liu et al. 2012a).

MSCs *in situ* adapt their gene expression profile in response to extracellular signals and, via cross-talk, regulate the gene expression of renal epithelial, endothelial, and immune cells. MSCs down-regulate the expression of pro-inflammatory TNF-α, IL-1β, and IFN-γ, and significantly up-regulate gene expression of anti-inflammatory cytokine IL-10, antiapoptotic BCL-2, basic fibroblast growth factor (bFGF), and transforming growth factor α (TGFα) in order to accelerate the repair process in the injured kidney (Tögel et al. 2005a).

MSCs release vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), hepatocyte growth factor (HGF), and SDF-1, which exert anti-apoptotic, mitogenic, and angiogenic functions in AKI (Imberti et al. 2007, Tögel et al. 2009, Zarjou et al. 2011). The secretion of anti-inflammatory factors, such as prostaglandin E2 (PGE2), TGFβ, HLA-G5, and HGF, can down-regulate several types of immune cells, such as T cells (both

CD4+ and CD8+), NK cells, B cells, dendritic cells, and macrophages. The relevance of these factors in IRI has yet to be fully studied. In addition to soluble molecules, MSCs release extracellular vesicles as part of their paracrine mechanisms to transfer mRNA, miRNA, and other molecules to the injured cells to protect against AKI or to induce recovery (Bruno et al. 2009, Collino et al. 2015, Tomasoni et al. 2013). In contrast, Xing *et al.* (2014) used MSC conditioned medium to show no effect in AKI.

In IRI induced AKI, the lack of oxygen causes one of the most immediate effects by depressing the oxidative phosphorylation in mitochondria. Recently, MSCs have been shown to fully restore mitochondrial respiration, resulting in enhanced ATP synthesis in kidney mitochondrion after I/R (Beiral et al. 2014).

2.3 Immunomodulative mechanisms of MSCs

MSCs affect both innate and adaptive immune systems by suppressing the proliferation of T cells, maturation and antigen presentation of dendritic cells, and the proliferation and antibody production of B cells, by inhibiting the cytokine production and cytotoxicity of NK cells, and by promoting the generation of regulatory T cells. The full picture of how MSCs regulate different immune systems remains unclear. A number of mechanisms have been reported, involving cell-to-cell contact, secretion of soluble factors and extracellular vesicles, and induction of anergy, apoptosis, and regulatory cells (**Figure 5**). In the immune system, immune cells and non-immune cells are linked together in a complex network of cytokine production and responses. MSCs are not considered to be immune cells, but should be regarded as coordinators of immune system (Hoogduijn 2015).

When lymphocytes are activated, they proliferate and differentiate to execute their effector functions. Various stimulants can be used to activate T cell proliferation. MSCs are shown to inhibit dose dependently T-cell proliferation induced by alloantigens (Krampera et al. 2006, Meisel et al. 2004, Ryan et al. 2007), polyclonal activators such as PHA (Aggarwal and Pittenger 2005) and antiCD3/CD28 (Castro-Manrreza et al. 2014, Melief et al. 2013). This immunosuppressive capacity has been demonstrated by using both peripheral blood mononuclear cells and populations enriched in $CD3^+$, $CD4^+$, and $CD8^+$ T cells. Besides proliferation, MSCs can modulate T cell differentiation into subtypes characterized by the secretion of a set of cytokines. For instance, MSCs inhibit the production of proinflammatory cytokines IL-17, IL-22, IFNγ, and TNFα and the differentiation of naïve $CD4^+$ lymphocytes to Th17 cells. Additionally, MSCs promote the production of IL10 and the expression of transcription factor Foxp3, indicating differentiation toward Treg phenotype (Ghannam et al. 2010). Recently, it has been suggested that these phenomena are not independent; instead,

MSCs are able to convert Th17 cells into cells with a Treg phenotype (Obermajer et al. 2014).

B lymphocytes are involved in the adaptive immune response. These cells are responsible for humoral immunity and are specialized in antibody production. MSCs from healthy human donors can inhibit normal B cell proliferation, differentiation, and antibody secretion (Corcione et al. 2006). When purified mouse B cells were used, MSCs seemed to induce the proliferation and differentiation of B cells (Traggiai et al. 2008). This phenomenon can be explained by cross-talk between T and B cells. The signals arising from T cells are required for MSCs to apply their immunomodulatory effects on B cells (Rosado et al. 2015).

Dendritic cells (DC) are the most important antigen presenting cells in the body. DCs must undergo maturation to initiate an appropriate immune response and during this process, DCs increase their expression of HLA class II molecules and costimulatory molecules CD80 and CD86. MSCs have an effect on the recruitment, maturation, and function of DCs. MSCs can significantly reduce monocyte differentiation into DCs, affecting the upregulation of costimulatory molecules and other dendritic cell markers (Spaggiari et al. 2009, Jiang et al. 2005).

Within the innate immune system, macrophages are key players in initiating and controlling inflammation. Monocytes arriving at the site of inflammation can differentiate into activated pro-inflammatory M1 macrophages or convert into alternatively activated anti-inflammatory M2 macrophages (Mantovani et al. 2013). The production of proinflammatory M1 macrophages or T cells may activate MSCs and trigger the release of mediators that skew the differentiation of monocyte toward M2 profile (Le Blanc and Mougiakakos 2012). Together with macrophages, NK cells are important in innate immunity and participate in the body's defenses against infections and cancer. MSCs affect the phenotype, proliferation, cytotoxicity, and cytokine production of NK cells (Sotiropoulou et al. 2006, Spaggiari et al. 2008). Neutrophils act as first line defense in the innate immunity against pathogens. MSCs' interaction with macrophages and NK cells has been described in detail, but neutrophils have been studied to a lesser extent. Recently, Jiang *et al.* (2016) showed that MSCs suppress respiratory burst, apoptosis, peroxidase, and protease release in neutrophils *in vitro*.

Currently, several mechanisms involving both cell-cell contact-dependent and contact-independent mechanisms for MSC immunoregulation have been described. However, the results are derived form co-culture *in vitro* assays, and only little is known about MSCs' immunoregulative capacity and functions *in vivo* (Consentius et al. 2015).

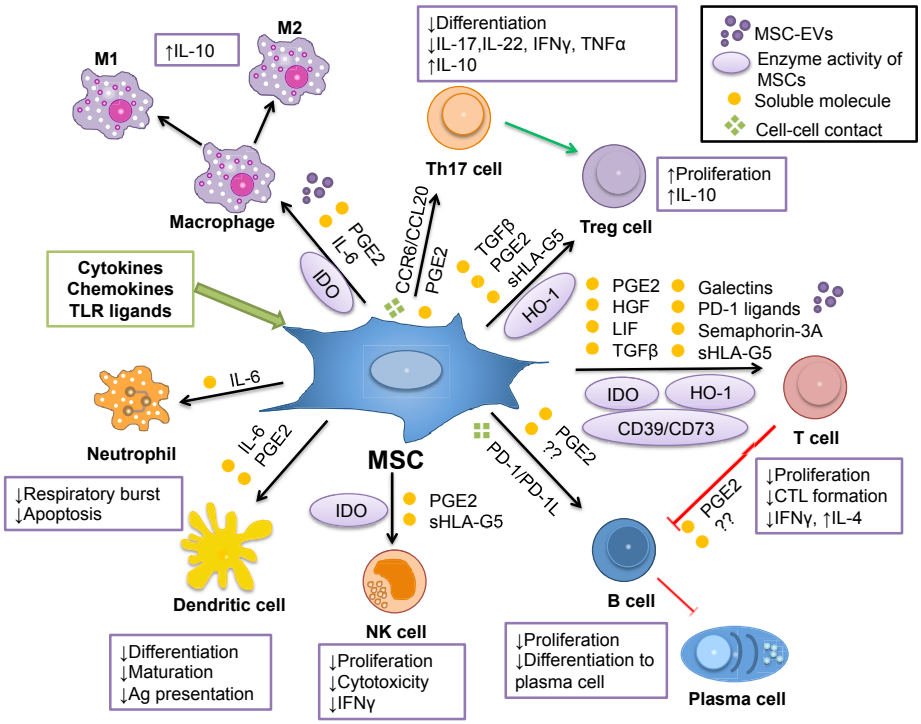

Figure 5 Immunomodulatory effects of MSCs and potential mechanisms in response to inflammatory signals. CTL, Cytotoxic T lymphocyte; HGF, hepatocyte growth factor; HLA, HO, hemioxygenase; human leukocyte antigen; IFN; interferon, IL, interleukin; IDO, indoleamine 2,3-dioxygenase; LIF, Leukocyte inhibitory factor; NK, natural killer; PGE2, prostaglandin E2; PD-1, Programmed death-1; TLR, Toll-like receptor

2.3.1 Soluble factors

MSCs regulate immune cells via both cellular contact-dependent and independent mechanisms. The first molecules described in MSC-mediated immunoregulation of alloantigen-activated T-lymphocytes were TGFβ1 and HGF (Di Nicola et al. 2002). MSCs constitutively express TGFβ1 and HGF, which seem to act synergistically (Ryan et al. 2007). TGFβ1 is involved in MSC-mediated generation of CD4+, CD25+Foxp3+ Tregs (English et al. 2009) and in decreased proliferation of NK cells (Spaggiari et al. 2008).

HLAG molecules are non-classic HLA molecules that have a limited number of allelic polymorphisms and are expressed in specific tissues. MSCs express the soluble isoform sHLA-G5, which expression is increased by IL-10. The secretion of HLA-G5, which requires direct cell- to- cell contact, has been demonstrated to suppress T-cell proliferation, probably via Treg induction. In addition to adaptive immunity, HLA-G5 regulates innate immunity by decreasing the cytotoxicity and IFNγ production of NK cells (Selmani et al. 2008).

Several other soluble molecules are identified as modulators of MSC-mediated T-cell proliferation including Galectins 1, -3, -9, (Gieseke et al. 2013, Lepelletier et al. 2010, Sioud et al. 2011), Semaphorin 3A, (Lepelletier et al. 2010), LIF1 (Nasef et al. 2008), and PD1 ligands (Davies et al. 2017). Despite several molecules and factors having been described so far, MSCs' immunodulative mechanisms are not yet fully understood. Most of these molecules were identified in *in vitro* blocking assays, and their relevance *in vivo* remains to be investigated.

Prostaglandin E2

Membrane lipids not only are structural components of cell membranes but also mediate biological signals through G-protein activators, second messengers, and nuclear receptors. The structures of glycerophospholipids (GPL), an important group of membrane lipids, are extensively varied. As a consequence of the combination of different polar head groups assembled together with various acyl, alkyl, or alkenyl chains, a single cell can contain more than a thousand different GPL molecular species. Cellular mediators such as inositol phosphates, diacylglycerols, lysophospholipids, ceramides, cleaved fatty acids, or their derivates are released from membrane lipids. These mediators have been shown to have a crucial role in many physiological processes, including the regulation of immune system and inflammation. The essential polyunsaturated fatty acid (PUFA) arachidonic acid (20:4n-6, AA) is found primarily at the sn-2 position of most membrane phospholipids. It is a precursor for the synthesis of prostaglandins, thromboxanes, and leukotrienes (Funk 2001). PGE2 is one of the lipid mediators produced from 20:4n-6 in a reaction catalyzed by COX1 and COX2 enzymes. These enzymes are constitutively expressed in MSCs, although COX2 expression increases dramatically in an inflammatory environment. Similarly, the production of PGE2 is induced by IFNγ and TNFα (Aggarwal and Pittenger 2005). PGE2 has been shown to have pleiotropic effects on immune cells. PGE2 inhibits the proliferation of T lymphocytes (Aggarwal and Pittenger 2005) and B lymphocytes (Hermankova et al. 2016) and promotes Treg differentiation (English et al. 2009). PGE2 is involved in a decrease in differentiation of DCs from monocytes (Spaggiari et al. 2009) and a decrease in proliferation and cytotoxic activity of IL2 activated NK cells (Spaggiari et al. 2008).

Extracellular vesicles

Besides soluble molecules, MSCs secrete EVs that have been demonstrated to possess immunoregulative properties. EVs collected from MSC-conditioned medium suppressed the production of pro-inflammatory cytokines TNFα and IL1β but increased the concentration of TGFβ during *in vitro* culture. In addition, EVs reduced the differentiation of Th17 cells while promoting Treg

differentiation (Chen et al. 2016). Del Fattore *et al.* (2015) reported similar results showing that MSC-EVs promote Treg induction and IL-10 production but do not inhibit the proliferation of CD3+ T-cells. As IDO seems to be one of the main mechanisms in MSC mediated immunoregulation, MSC-EVs mediate their functions through IDO-independent mechanisms (Chen et al. 2016, Del Fattore et al. 2015). One possible mechanism might involve CD73 and CD39 enzymatic activity. Kerkelä *et al.* (2016) showed that both MSCs and MSC-derived EVs produce adenosine from adenosine monophosphate. Recently, Di Trapani *et al.* (2016) elegantly demonstrated the uptake of MSC-derived EVs into T, B and NK cells and that the uptake on the effector cells was enhanced by cytokine priming. Most interestingly, only the inhibition of endocytic vesicle production pathway influenced immunomodulation. In addition to regulation of lymphocytes, MSC-EVs have been shown to promote the macrophage polarization toward M2 phenotype (Lo Sicco et al. 2017).

2.3.2 Contact dependent mechanisms

The immunosuppressive capacity of MSCs can only partially be explained by soluble factors. However, in human MSCs only few contact-dependent mechanisms have been identified so far. MSCs express notch ligand Jagged-1, and with the addition of Jagged-1 neutralizing antibody, MSCs recovered their ability to suppress the proliferation of CD4+ T cells (Liotta et al. 2008). In inflammatory conditions, MSCs inhibit the differentiation and function of Th17 cells through cell-cell contact, mediated by joint participation of CCR6 and CD11a/CD18, expressed by T-cells, and their respective ligands CCL20 and CD54 (Ghannam et al. 2010).

Instead, in mouse models contact dependent mechanisms have been studied more extensively. In addition to PD1/PD-L1 pathway, which participates in the inhibition of T and B cell activation (Augello et al. 2005, Hermankova et al. 2016), other membrane proteins, such as Fas-FasL and adhesion molecules intercellular adhesion molecule 1 (ICAM1), and vascular cell adhesion molecule 1 (VCAM1), have been suggested to mediate MSC immunoregulation (Akiyama et al. 2012, Ren et al. 2010).

2.3.3 Enzymatic activity of MSCs

In human MSCs, tryptophan degrading enzyme indoleamine 2,3-dioxygenase (IDO) represents the main pathway of immunosuppression. Tryptophan starvation is the primary reason for T-cell inactivation, but tryptophan metabolites, such as kynurenine, also regulate the proliferation and survival of T-cells (Mellor and Munn 2004). IDO has been reported to have an immunosuppressive role in many other settings including cancer (Katz et al. 2008). Meisel *et al.* (2004) were the first to show that MSCs express IDO in an IFNγ-dependent manner and thus identified IDO-mediated tryptophan

catabolism as a novel T-cell inhibitory mechanism of MSCs. Recently IDO-independent mechanisms induced by IFNγ using P7H1 and B7DC/PD1 pathways were demonstrated (Chinnadurai et al. 2014).

Extracellular adenosine triphosphate (ATP) and adenosine (Ado) are involved in inflammatory processes. ATP is a mostly pro-inflammatory molecule and is released during hypoxic conditions and by necrotic cells, as well as by activated immune cells and endothelial cells. Extracellular ATP can be hydrolyzed into Ado by a two-step enzymatic process catalyzed by two ectonucleotidases, CD39 and CD73, which are expressed in many tissues. Regulatory T-cells (Tregs) are shown to utilize CD39 CD73 machinery to inhibit T-cell proliferation and cytokine production (Deaglio et al. 2007, Schuler et al. 2014). Human MSCs express constitutively CD73, but CD39 expression levels vary according to the tissue source (Kerkelä et al. 2016). It has been demonstrated that this pathway is at least partially responsible for T-cell suppression of MSCs (Huang et al. 2017, Huang et al. 2017, Kerkelä et al. 2016, Saldanha-Araujo et al. 2011).

Hemioxygenases (HOs) are intercellular enzymes that catabolize heme into biliverdin, CO, and divalent iron. HO-1 is constitutively expressed in MSCs and has been described as immunoregulative molecule (Najar et al. 2012). Later studies have demonstrated that HO-1 may act as one mediator of suppression of alloactivated T-cells (Chabannes et al. 2007) and induction of Tregs (Mougiakakos et al. 2011).

2.3.4 Extracellular signals and immunomodulation

In any site of inflammation, there are several cytokines in abundant quantities. It has been suggested that MSCs monitor the inflammatory environment and switch between pro-inflammatory and anti-inflammatory modes of action (Bernardo and Fibbe 2013). There is extensive evidence showing that MSCs need to be activated by cytokines produced by T cells, macrophages, and NK cells, indicating that communication between MSCs and immune cells is reciprocal. Atleast IFNγ on its own is needed for MSC activation but it can be accompanied by TNFα, IL-1α, or IL-1β (Aggarwal and Pittenger 2005, Ryan et al. 2007, Ryan et al. 2007). Pro-inflammatory cytokines induce the expression of HLA II molecules as well as the expression or production of several other molecules, such as IDO, galectin-9, HO-1, and PGE2 mediating the immunomodulative functions of MSCs (Le Blanc et al. 2003, Aggarwal and Pittenger 2005, Gieseke et al. 2013, Mougiakakos et al. 2011). Pro-inflammatory preconditioning affects also the functionality of MSC-derived EVs (Di Trapani et al. 2016). Interestingly, pro-inflammatory cytokines alter the phospholipid profile of MSCs, indicating that the composition of their membrane lipids is relevant to their function (Campos et al. 2016). MSCs express several TLRs, including TLR3 and TLR4, and it has been shown that the *in vitro* activation of specific TLRs affects the immunoregulative properties of MSCs. For instance, MSCs induce the

formation of Tregs after TLR3 and TLR4 activation in a contact dependent manner (Rashedi et al. 2017). Interestingly, it has been suggested that MSCs may polarize into either pro-inflammatory or immunosuppressive phenotypes depending on the received signals. MSCs primed with TLR3 ligand poly (I:C) suppressed T-cell proliferation, while the expression of IDO and PGE2 production was enhanced. TLR4 activation with lipopolysaccharide on the other hand did not induce the production of immunosuppressive factors, but TLR3-primed MSCs were in fact pro-inflammatory (Waterman et al. 2010). Recently, it was demonstrated that direct cell-to-cell contact with M1 or M2 macrophages through CD54 also changes the immunosuppressive capacity of MSCs (Espagnolle et al. 2017).

2.3.5 Tissue source

Bone marrow was the first established source of MSCs. Since this discovery, it has remained the most widely investigated and used MSC source in therapeutic applications. After being initially found in the bone marrow, MSCs have been isolated from various adult and neonatal tissues, of which adipose tissue (AT) and birth associated tissues (placenta, umbilical cord UC and cord blood CB) are well characterized and commonly used. The collection of bone marrow is always an invasive procedure containing a risk of infection, thus making alternative sources very attractive. Both birth associated tissues, as well as excess adipose tissue removed by liposuction can be regarded as biological waste and available for MSC isolation and production.

According to the minimal criteria defining MSCs issued by the ISCT, all MSCs should be plastic-adherent spindle-shaped cells with certain immunophenotype and tri-lineage differentiation potential (Dominici et al. 2006). Although MSCs from different sources in general fulfill these criteria, there is some variation in the expression of cell surface antigens, differentiation potential, and especially in the functional properties of MSCs. When initial capacity for colony formation was compared, AT turned out to be superior in comparison to CB or BM (Kern et al. 2006). Even though CB contains a low number of MSCs and the success rate for isolating MSCs is lower, the proliferation rate and capacity of CB and other birth associated tissues-derived MSCs are higher (Barlow et al. 2008, Jin et al. 2013, Kern et al. 2006). The expression of cell surface antigens seems relatively invariable since in most comparative studies no differences in immunophenotype were reported. The only observed deviation to the minimal criteria is moderate expression of hematopoietic marker CD34 in AT-derived MSCs at least at the beginning of *in vitro* culture (Gronthos et al. 2001, Maumus et al. 2011, Mitchell et al. 2006, Pachon-Pena et al. 2011, Traktuev et al. 2008). In contrast to immunophenotype, differentiation potential shows more variation among MSCs from different sources. In several studies MSCs derived from CB and other birth associated tissues show poor or decreased

adipogenic potential (Barlow et al. 2008, Castro-Manrreza et al. 2014, Kern et al. 2006).

The origin of MSCs seems to affect more dramatically the epigenetics (Reinisch et al. 2015), gene expression (Al-Nbaheen et al. 2013, Kang et al. 2016, Roson-Burgo et al. 2014, Wagner et al. 2005, Wegmeyer et al. 2013), miRNA expression (Ragni et al. 2013), proteomics (Jeon et al. 2016), secreted molecules (Pires et al. 2016), and even the content of extracellular vesicles (Baglio et al. 2015), but the functional consequences of these differences are not yet known. The ability to modulate immune responses is one of the most important properties of MSCs. In many comparative studies, MSCs collected from different tissues showed very similar immunomodulatory properties when a T-cell proliferation assay or a mixed lymphocyte reaction was used (**Table 1**). When cytokine secretion, TLR expression, or MSCs' responses for the inflammatory milieu were studied, some differences between MSC from different sources were observed (Prasanna et al. 2010, Raicevic et al. 2011b, Roemeling-van Rhijn et al. 2013b). This indicates that MSCs of different origins may have slightly different roles *in vivo* and more importantly their mechanisms of action may be different.

The comparison of functional properties of MSCs from different sources is challenging as the same culture conditions may not be optimal for all the MSC types, which may explain some of the differences observed in the standardized culture conditions (Fazzina et al. 2016). Furthermore, MSC donors show high heterogeneity, which has an impact on MSC functionality (Ketterl et al. 2015). When BM-MSCs and AT-MSCs derived from the same donor were compared, there was no difference in the inhibition of T-cell or NK cell proliferation. However, only BM-derived MSCs were able to inhibit NK cell cytotoxicity, and correspondingly AT-MSCs were more potent in inhibiting dendritic cell differentiation (Valencia et al. 2016). On the other hand, when placenta-derived MSCs were compared with umbilical cord-derived MSCs from the same donor, placenta-derived MSCs were shown to be more efficient in the T cell suppression and also in supporting the growth of Treg population (Talwadekar et al. 2015).

Table 1 *Summary of comparative studies of hMSCs from different tissue sources*

Cell types	*Parameters*	*Outcome*	*Reference*
AT, BM, PL, UC	Angiogenesis	BM, PL>AT, UC	(Du et al. 2016)
AT, BM, UC	Proliferation	CB=AT>BM	(Fazzina et al. 2016)
	Differentiation	BM=AT>CB	
	T-cell proliferation	BM>UC>AT	
AT, BM, CB, PL	Proliferation	PL>AT=BM=CB	(Heo et al. 2016)
	Differentiation	BM=AT (CB,PL none)	
	T-cell proliferation	BM>AT>CB>PL	
BM, CB	Intestinal ischemic injury model	BM=CB	(Jensen et al. 2016)
BM, WJ, UC	T-cell proliferation	No differences	(Mennan et al. 2016)
AT, BM	T/NK proliferation	BM=AT	(Valencia et al. 2016)
	NK cytotoxicity	BM>AT	
	Dendritic cell differentiation	AT>BM	
BM, UC	Immunogenicity	BM>UC	(Barcia et al. 2015)
	Lymphocyte proliferation	BM<UC	
PL, UC	T-cell proliferation	PL>UC	(Talwadekar et al. 2015)
BM, CB, PL	Adipogenic differentiation	BM=PL (CB none)	(Castro-Manrreza et al. 2014)
	Osteogenic differentiation	BM=CB=PL	
	T-cell proliferation	BM=CB>PL	
AT, BM, PL, WJ	Proliferation	WJ>AT>PL>BM	(Li et al. 2014)
	Adipogenic differentiation	AT>WJ>BM>PL	
	Osteogenic differentiation	WJ>PL>AT>BM	
	T-cell proliferation	WJ>PL>AT>BM	
AT, BM	Lymphocyte proliferation	AT>BM (at low doses)	(Montespan et al. 2014)
AT, BM, CB	Proliferation	CB>BM>AT	(Jin et al. 2013)
	Senescence	CB<BM=AT	
	Immunosuppression	CB>BM=AT	
BM, PL	T-cell proliferation	BM=PL	(Luan et al. 2013)
AT, BM	Lymphocyte proliferation	AT>BM	(Melief et al. 2013)
	Dendritic cell differentiation	AT>BM	
AT, BM	T-cell proliferation	AT>BM	(Menard 2013)
AT, BM, WJ	T-cell proliferation	AT>BM=WJ	(Najar et al. 2013)
AT, BM, UC	T/NK cell proliferation	AT>BM=UC	(Ribeiro et al. 2013)
	B cell proliferation	AT=BM (UC none)	
AT, BM	T-cell proliferation	AT=BM	(Roemeling-van Rhijn et al. 2013a)
CB, PL, CL, WJ	Proliferation	CL, CB>PL, WJ	(Stubbendorff et al. 2013)
	Migration	CL, CB, PL, WJ	
	Adipogenic differentiation	only CL	
	Immunogenicity	CB, WJ>CL,PL	
	T-cell proliferation	CL>CB=PL=WJ	
AT, BM	Proliferation	AT>BM	(Xishan et al. 2013)

	T-cell proliferation	BM>AT	
AT, BM, PL	T-cell proliferation	AT=BM=PL	(Lee et al. 2012)
AT, BM, WJ	TLR expression	BM=AT, WJ lack	(Raicevic et al. 2011a)
	T-cell proliferation	TLR4 BM=AT=WT,	
BM, WJ	Lymphocyte proliferation	WJ>BM	(Prasanna et al. 2010)
AT, BM	Dendritic cell differentiation	AT>BM	(Ivanova-Todorova et al. 2009)
AT, BM, CB, WJ	T-cell proliferation	no differences	(Yoo et al. 2009)
BM, PL	Proliferation	PL>BM	(Barlow et al. 2008)
	Adipogenic differentiation	BM>PL	
	Osteogenic differentiation	BM=PL	
	in vivo toxicity	BM=PL	
AT, BM, CB	Isolation success rate	AT=BM>CB	(Kern et al. 2006)
	Proliferation	UCB>AT>BM	
	Osteogenic differentiation	No differences	
	Adipogenic differentiation	BM=AT (CB none)	
AT, BM	Immunogenicity	BM=AT	(Puissant et al. 2005)
	Lymphocyte proliferation	BM=AT	

Abbreviations: AT, adipose tissue; BM, bone marrow, CB, cord blood; CL, umbilical cord lining cells; PL, placenta; UC, umbilical cord; WJ, Wharton's jelly

2.4 Replicative senescence of MSCs

Although MSCs are present in almost all tissues, they are rare in the human body. The frequency of MSCs is approximately 0.01-0.001% and their number has been shown to decline with age (Caplan 2009). The decline in the numbers or potential of stem cell population in adult organs may contribute to human aging and even affect the life span of an individual (Van Zant and Liang 2003). The effect of donor age on the characteristics of hMSCs has yet to be thoroughly studied. Zhou *et al.* (2008) showed an age-dependent decrease in proliferation and osteoblast differentiation, and an increase in senescence-associated β -galactosidase-positive cells and apoptosis in hMSCs. Wagner *et al.* (2009) show that donor age influences the gene expression of MSCs. In rodents, donor age is shown to have a negative effect on the growth kinetics as well as capacity to suppress the proliferation of CD3+CD4+ T cells (Wu et al. 2014).

MSCs, like other cultured primary cells, do not grow infinetily, instead the cells can undergo a limited number of divisions before reaching senescence (Hayflick 1965). At a molecular level, telomere-dependent replicative senescence or stress-induced premature senescence is triggered by retinoblastoma protein (Rb) or p53 pathways, which activate cyclin-dependent kinase inhibitors p16 and p21, respectively. These pathways can induce each other and work cooperatively (Collado et al. 2007). Senescent

cells undergo irreversible growth arrest, but stay metabolically active and develop large, flat morphology and typically exhibit a senescence-associated β-galactosidase (β-gal) activity. In addition, senescent cells secrete growth factors, proteases, and cytokines called senescence associated secreted phenotype (SASP) (**Figure 6**).

Cell therapy protocols usually require hundreds of thousands hMSCs per treatment and because they are so rare in the human body, extensive *in vitro* expansion is required to obtain sufficient numbers of MSCs for cell therapy. Long-term expansion may cause continuous changes in gene expression, miRNA expression, and DNA metylation patterns of MSCs (Bork et al. 2010, Hackl et al. 2010, Schellenberg et al. 2011, Wagner et al. 2008, Wagner et al. 2009, Yoo et al. 2014). However, these studies were not able to identify specific markers for senescent MSCs. Cellular senescence induced by gamma radiation affects hMSCs' immunoregulatory properties both *in vitro* and *in vivo* (Sepulveda et al. 2014).

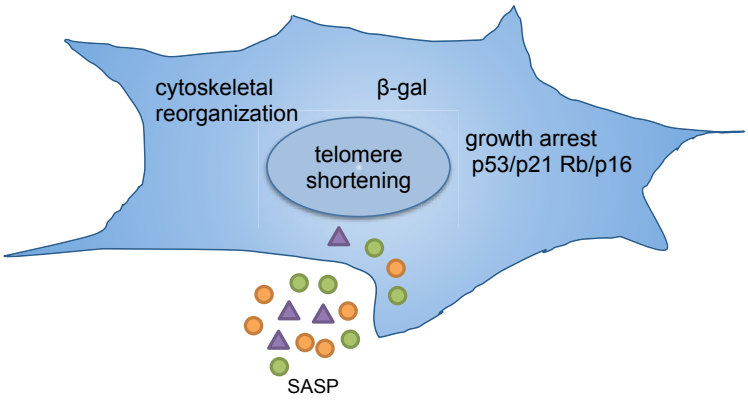

Figure 6 The phenotype of senescent MSCs Abbreviation: SASP, Senescence associated secretory phenotype. Figure is adapted from (Turinetto et al. 2016) under license http://creativecommons.org/licenses/by/4.0/.

2.5 MicroRNA regulation in MSCs

MiRNAs are small non-coding RNAs (20-22 nucleotides) targeting messenger RNA (mRNA) for degradation or direct translational repression, thus suppressing protein synthesis. To date 2588 distinct miRNA molecules have been identified (Griffiths-Jones et al. 2006) (http://www.mirbase.org/index.shtml, accessed February 25, 2017), and it was estimated that miRNAs regulate 30% of protein coding genes (Lewis et al. 2005). One miRNA can target several dozen or even hundreds of mRNAs and conversely a single gene has binding sites for several miRNAs in their 3' untranslated regions (Selbach et al. 2008). Development of computational and bioinformatics prediction methods has accelerated the reseach on

miRNA function and possible targets, revealing the full complexity of the miRNA regulation network (Bartel 2009, Lu et al. 2012, Maragkakis et al. 2009). Currently, there are numerous different prediction tools and softwares available, but the high rate of false positive interactions hampers the research (Pinzon et al. 2017). MiRNAs have been shown to have a crucial role in regulating almost all cellular processes, including cell growth, proliferation, and differentiation (Ameres and Zamore 2013, Bartel 2009). The role of miRNA regulation in both embryonic and somatic stem cell fate and differentiation has been studied extensively during the past few years (Ivey et al. 2008, Ong et al. 2015). Most studies investigating the role of miRNA regulation in MSCs have focused on osteogenic or adipogenic differentiation (**Table 2**). In hBM-MSCs silencing, Dicer or Drosha inhibits both adipogenic and osteogenic differentiation, indicating that miRNAs are important regulators of the MSC differentiation (Oskowitz et al. 2008).

Several miRNAs have been shown to either promote or inhibit the osteogenic differentiation of hBM-MSCs. MiR-20a, miR-21, miR-29, miR-489b, and miR-196 have been shown to promote the osteogenic differentiation, whereas miR-27a, miR-31, miR-34a, miR-138, miR-204, miR-489, and miR-541 have been shown to have an inhibitory effect (Baglio et al. 2013, Candini et al. 2015, Chen et al. 2014, Eguchi et al. 2013, Huang et al. 2010, Kapinas et al. 2010, Schoolmeesters et al. 2009, Yang et al. 2013b, Zhang et al. 2011). Adipogenesis and osteogenesis are usually presented as reciprocally regulated events. Several miRNAs regulate the balance between osteogenesis and adipogenesis. MiRNAs, including miR-17-5p and miR-106, have been shown to promote adipogenesis and inhibit osteogenesis (Li et al. 2013a), whereas miR-22 inhibits adipogenesis and stimulates osteogenesis in human adipose tissue derived MSCs by repressing its target *HDAC6* (Huang et al. 2012). MiRNA-mediated regulation of adipogenic differentiation of hBM-MSCs is less studied, but it has been shown that miR-369-5p inhibits adipogenic differentiation, whereas miR-371 has a strong enhancing effect (Bork et al. 2011).

MiR-21, miR-155, and miRNA family 17~92 are called immunomirs and they regulate T-cell differentiation, function, and aging (Kroesen et al. 2015). Of these miRNAs, miR-21 is highly expressed in MSCs and its expression has been shown to be induced by an anti-inflammatory lipid molecule, resolvin D1, which participates in the attenuation of the inflammatory response (Recchiuti et al. 2011). MiR-155, on the other hand, inhibits the immunosuppressive capacity of MSCs by reducing iNOS expression in mice (Xu et al. 2013). MiR-181 is not included in immunomirs, but it has been shown to be involved in T- and B-cell development (Ebert et al. 2009). In MSCs, elevated miR-181 expression enhances IL-6 and IDO expression, and the capability of MSCs to suppress mitogen activated T-cell proliferation was also attenuated by miR-181 in, at least partly, IL-6-dependent manner (Liu et al. 2012b).

MSCs can be isolated from virtually any tissue (Meirelles et al. 2006). In therapeutic applications, BM, AT and CB are the most relevant sources. The different origins have been shown to produce MSCs with different immunomodulatory properties (Di Trapani et al. 2013), but the results from different studies are contradictory (Mattar and Bieback 2015). Since miRNA regulation is thought to be one of the key players in MSC differentiation and functionality, MSCs have been proposed to have a typical miRNA expression pattern, which varies only slightly according to the tissue origin (Ragni et al. 2013).

The age of MSC donors and the required *in vitro* culture of the therapeutic MSCs are considered to be a potential factor that may affect the properties and the functionality of MSCs and eventually deteriorate the therapeutic outcome. The age-dependent changes in miRNA expression may be one possible explanation for the observed differences between the aged and younger donors. Despite miRNA expression being relatively stable in MSCs, some age-dependent miRNA expression changes have been previously identified (Candini et al. 2015). Interestingly, the miRNA expression of hBM-MSCs and hAT-MSCs is differently affected by the donor age (Pandey et al. 2011). Replicative senescence has also been described to be at least partly driven by miRNA regulation (Wagner et al. 2008, Yoo et al. 2014). The results are, however, contradictory and the influence of donor age on senescence has not yet been thoroughly studied.

Table 2 *MiRNAs in regulation of hMSCs*

miRNA	*MSC source*	*Target gene*	*Effects*	*Reference*
Differentiation				
miR-10a	BM	KLF4	Promote differentiation	(Li et al. 2013b)
miR-22	AT	HDAC6	Inhibit adipogenesis Promote osteogenesis	(Huang et al. 2012)
miR-21	AT	SPRY2, TGFBR2	Promote adipogenesis	(Kim et al. 2009a, Mei et al. 2013)
miR-30	AT	RUNX2	Promote adipogenesis	(Zaragosi et al. 2011)
let-7	BM	IL-6	Inhibit adipogenesis	(Sung et al. 2013)
miR-27	AT	PPARγ, PHB	Inhibit adipogenesis	(Kang et al. 2013, Karbiener et al. 2009)
miR-23b	BM	PRKACB	Promote chondrogenesis	(Ham et al. 2012)
let-7, miR-24, miR-125b, miR-138	BM	n.d	Promote osteogenesis through PDGF pathway	(Goff et al. 2008)
miR-148b	BM	n.d	Promote osteogenesis	(Schoolmeesters et

				al. 2009)
miR-17	PO	SMURF-1	Promote osteogenesis	(Liu et al. 2011b)
miR-196a	AT	HOXC8	Promote osteogenesis	(Kim et al. 2009b)
miR-20a	BM	PPARγ, BAMBI, CRIM1	Promote osteogenesis	(Zhang et al. 2011)
miR-21	AT, BM	SPRY1/2, SPRY2	Promote osteogenesis	(Mei et al. 2013, Yang et al. 2013a)
miR-218	AT	SFRP2, DKK2	Promote osteogenesis	(Zhang et al. 2014)
miR-29a	BM	DKK1, KREMEN2, SFRP2	Promote osteogenesis	(Kapinas et al. 2010)
miR-346	BM	GSK-3β	Promote osteogenesis	(Wang et al. 2013)
miR-26a	AT	SMAD1	Inhibit osteogenesis	(Luzi et al. 2008)
miR-27a	BM	SATB2	Inhibit osteogenesis	(Schoolmeesters et al. 2009)
miR-31	BM	OSTERIX	Inhibit osteogenesis	(Baglio et al. 2013, Gao et al. 2011)
miR-100	AT	BMPR2	Inhibit osteogenesis	(Zeng et al. 2012)
miR-138	BM	FAK	Inhibit osteogenesis	(Eskildsen et al. 2011)
Proliferation/senescence				
miR-10a	BM	KLF4	Inhibit senescence	(Li et al. 2013b)
miR-16	PL	CCNE1	Inhibit proliferation	(Wang et al. 2012)
miR-196a	BM	HOXB7	Inhibit proliferation	(Candini et al. 2015)
Immune regulation				
miR-181	BM	TGFBR1, TGFBRAP1	Inhibit immune suppression	(Liu et al. 2012b)
Angiogenesis				
miR-16	PL	CCNE1	Inhibit angiogenic potential	(Wang et al. 2012)

3 AIMS OF THE STUDY

In general, the aim of this study was to investigate the regulatory network between cell cycle, immunosuppression, and membrane lipids of MSCs by analyzing immunosuppressive capacity, phospholipid composition, and gene expression changes related to expansion and donor age.

The specific aims of this study were:

1. To investigate the immunosuppressive capacity of BM-MSCs and CB-MSCs
2. To investigate the role of membrane lipid composition in the functionality of MSCs
3. To investigate the effect of IFNγ stimulus on the secretion and function of CB-MSC derived extracellular vesicles
4. To analyze age- and expansion-induced changes in phospholipid composition, mRNA, or miRNA expression

4 MATERIALS AND METHODS

Materials and methods used in this study are summarized in **Table 3**. More detailed information can be found in the original publications.

Table 3 *Summary of methods used in the study. The original publications are referred to using their Roman numerals I-III*

Experimental procedure	Publication
Cell culture	I, II and III
Co-culture assays	I and III
Isolation and size determination of extracellular vesicles	III
Experimental animal procedures	III
Gas chromatographic analysis of fatty acids	I
Mass spectrometric lipidomis	I
Mass spectrometric proteomics	III
Gene expression analysis	I and II
Senescence indicators	I
Bioinformatics and Statistical analysis	I, II and III

4.1 Cell culture

Bone marrow derived MSCs (I,II)

Human bone marrow derived MSCs (hBM-MSCs) were obtained from bone marrow aspirates taken from the iliac crest or upper femur metaphysis of adult patients after written informed consent. All patient protocols were approved by the Ethical Committee of Northern Ostrobothnia Hospital District or the Ethical Committee of Hospital District of Helsinki and Uusimaa. The BM-MSCs collected from donors of different ages (anonymous coding) used in this thesis are summarized in **Table 4**.

Cells were cultured in minimum essential medium alpha (αMEM), supplemented with 20mM HEPES, 10% heat inactivated fetal bovine serum (FBS), 2mM L-glutamine, and 100 U/mL penicillin and 100 µg/mL streptomycin. The same serum lot was used throughout the study. The cells were plated at a density of 1000 cells/cm^2, medium was renewed twice a week, and the cells were harvested when 70-80% confluent.

Cord blood derived MSCs (III)

Cord blood units were collected at the Helsinki University Central Hospital, Department of Obstetrics and Gynaecology, and Helsinki Maternity Hospital. All donors gave informed consent and the study protocol was approved by

the ethical review board of the Helsinki University Central Hospital and the Finnish Red Cross Blood Service.

Cord blood mononuclear cells were isolated by gradient centrifugation and 1 x 10^6/ cm^2 mononuclear cells were plated on fibronectin-coated tissue culture plates. The initial CB-MSC establishment was performed under hypoxic conditions (5% CO_2, 3% O_2 at 37°C) in medium containing α-MEM with Glutamax™, 10% FBS, 10ng/mL epidermal growth factor, 10ng/mL recombinant platelet-derived growth factor, 50nM dexamethasone, 100U/mL penicillin, and 100µg/mL streptomycin. The cells were allowed to adhere overnight, and non-adherent cells were washed with medium changes. For further experiments, CB-MSCs were cultured in normoxic conditions (5% CO_2 and 20% O_2 at 37°C) and media was renewed twice a week. For flow cytometric analysis, CB-MSCs were trypsinized and 1x10^5 cells were suspended in a staining buffer (0.3% bovine serum albumin in 2 mM EDTA-PBS). Fluorscein isothiocyanate (FITC) and allophycocyanin (APC) conjugated antibodies against HLA-DR and HLA-ABC were used for direct labeling. Appropriate isotype controls were used.

Table 4 *Age and gender of BM-MSCs from young and old donors used in the thesis.*

BM-MSC donor	*Age (year)*	*Gender*
young donors	*mean age 22.2*	
081	20	male
088	21	male
089	23	female
091	23	female
092	23	male
old donors	*mean age 74.6*	
164	75	male
172	72	female
194	82	male
271	75	male

Co-culture assays (I, III)

PBMCs were isolated from buffy coats from healthy anonymous blood donors (Finnish Red Cross Blood Service) by density gradient centrifugation (Ficoll-Pague plus, GE Healthcare) and cryo-preserved for later use. PBMCs were chosen over purified T-cells for practical reasons. Isolation of PBMCs is less time-consuming and they provide co-stimulation for T-cells. MSCs or EVs were co-cultured with CFSE (5(6)-Carboxyfluorescein diacetate N-succinimidyl ester) labeled PBMCs. To activate the T-cell proliferation, 100ng/ml of the anti CD3 antibody clone Hit3a was added to the co-culture. T-cell proliferation was recorded after four days of incubation as a dilution of

fluorescent dye by flow cytometry. The proliferation of PBMCs without MSCs was designated as 100%.

PBMCs were co-cultured with EVs or CB-MSCs in order to analyze the impact on Treg induction. After 7 days of incubation, non-adherent PBMCs were harvested and labeled with fluorogenic antibodies, CD4-APC-Cy7, CD25-AlexaFluor®647, FOXP3-PerCP5.5) to evaluate the proportion of CD4+CD25+FOXP3+ Tregs. Cells were fixed and permeabilized with FOXP3 Staining Buffer Set (eBioscience) according to the manufacturer's instructions and including the blocking step with 2% rat serum. PBMCs cultured without MSCs or EVs were used as a control. Appropriate isotype controls were used.

Isolation and size determination of extracellular vesicles (III)

Cell culture medium (80-160 ml) was centrifuged at 2,000g for 20 minutes in +4°C, succeeded by a further ultracentrifugation step at 100,000g for 1-2 h at +4°C. The pellet was washed with phosphate buffered saline (PBS) and submitted to a second 1-2h ultracentrifugation in the same conditions. EVs were suspended in 50-150µl of PBS and stored at -80°C.

After centrifugation, EVs were resuspended with PBS, and analyzed with the nanoparticle tracking analysis (NTA) instrument LM14C with blue laser (405 nm, 60 mW) and a CMOS camera to determine the vesicle size distribution and concentration. The data were analyzed with NTA 2.3 software.

4.2 Experimental animal procedures

Eighteen 6- to 7-week-old male Sprague Dawley rats were purchased from Charles River Laboratories (Research Models and Services, Sulzfeld, Germany). The protocols were approved by the Animal Experimentation Committee of the University of Helsinki, Finland, and the Provincial State Office of Southern Finland (approval number STH059A), whose standards correspond to those of the American Physiological Society. The rats were divided into three groups: 1) I/R group (n=8), 2) I/R group + control EV (n=5), 3) I/R group + IFNγ-treated EV (n=5). An established model of kidney I/R injury was used (Lempiäinen et al. 2012). Twenty-four hours after the operation, blood samples were collected from the tail vein under short isoflurane anesthesia. Terminal samples were harvested 48 hours after the operation; the rats were anesthetized with isoflurane, and blood samples were collected from inferior vena cava with a 5ml syringe and 22G needle for biochemical measurements. The kidneys were excised, washed with ice-cold saline, blotted dry, and weighed. The left kidney was used for histological examinations. Tissue samples for histology were fixed in 10% formaline and processed to paraffin with routine methodology.

4.3 Gas chromatographic analysis of fatty acids

Total lipids of the BM-MSCs or FBS were extracted according to Folch *et al.* (1957) and converted into FA methyl esters (FAME) by heating with methylation reagent (1% H_2SO_4 in methanol). The dried and concentrated FAME solutions were analyzed by gas-liquid chromatography (6890N network GC) using flame ionization detection and DB-wax capillary columns (30 m, ID 0.25 mm, film 0.25 μm). Agilent Chemstation software was used for peak area integration. The identification of the FAMEs was based on retention time, mass spectra acquired earlier for similar samples, and comparisons with standards of known composition. The FA composition was calculated as mol%, and the FAs were marked by using the abbreviations: [carbon number]:[number of double bonds] n-[position of the first double bond calculated from the methyl end] (e.g. 22:6n-3).

4.4 Mass spectrometric analysis

ESI-MS analysis of cellular phospholipids (I)

Total lipids of BM-MSCs were dissolved in chloroform/methanol 1:2 for a direct infusion experiment. Several internal standards were used to correct for the effects of the polar head group and acyl chain length on the instrument response according to previously reported procedures (Hermansson et al. 2005, Käkelä et al. 2003, Koivusalo et al. 2001). Immediately prior to the mass spectrometry, 1% NH_4OH was added and the lipid extracts, with the internal standards, were infused to the electrospray source of a Quattro Micro triple quadrupole mass spectrometer) at the flow rate of 8 μl/min. The collision energy of the instrument was set to 25–65 eV, depending on the detection mode, and both negative and positive ion modes were used. Argon was used as the collision gas. The PC and lysoPC (precursor of 184), PE (neutral loss of 141), PS (neutral loss of 87), and PI (precursor of 241) species were selectively detected using head-group specific MS/MS scanning modes (Brügger et al. 1997, Sullards and Merrill 2001). The acyl chain assembly of the major lipid species was confirmed with MS/MS techniques. The mass spectra were processed by MassLynx software and the individual lipid species quantified by using the internal standards and LIMSA software (Haimi et al. 2006). The lipid species were abbreviated: [total carbon number in the chains]:[total number of double bonds in the chains]. The relative concentrations of the lipid classes were obtained by summing up the concentrations of the individual molecular species in a class.

Proteomic analysis of EVs (III)

Gel-based proteome analysis of EVs was performed with the liquid chromatography-mass spectrometry (LC-MS) of tryptic peptides. Proteins from collected EVs were run in a 4-20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. The gel was silver stained and sliced into pieces. Each gel piece was processed using an in-gel reduction, alkylation and trypsin digestion protocol as previously described (Shevchenko et al. 2006). Peptides were loaded to precolumn (Protecol Guard C18, 150µm, 10mm, 3µm) and separated in a reversed-phase analytical column (Acclaim PepMap100 C18, 75µm, 150mm, 3µm) with a linear gradient of acetonitrile. Ultimate 3000 LC instrument was operated in nano scale with the flow rate of 0.3 µl/min. Eluted peptides were introduced to LTQ Orbitrap XL mass spectrometer via ESI Chip interface (in a positive ion mode). Data files from MS were processed with Mascot Distiller. The processed data was searched with Mascot Server against human proteins in UniProtKBdatabase (release 2012_08).

4.5 Gene expression analysis

RNA was extracted using Qiagen AllPrep DNA/RNA Mini Kit (Cat. no. 80204, Qiagen, CA, USA) and a Qiagen supplementary protocol "Purification of total RNA containing miRNA from animal cells using the RNAeasy Plus Mini Kit "according to the vendor's instructions.

Labeled RNAs (800 ng/sample) were hybridized onto Agilent SurePrint G3 Human GE 8x60K or Agilent Human miRNA 8x60K microarray (release 16.0) and then the slides were washed and scanned according to the manufacturer's recommendations. The raw data files (.txt files) were imported into the R v. 2.13 software (R Core Team 2015) and preprocessed by the BioConductor package limma v.3.4.5 (Smyth 2005). After quality control of the data, the median probe intensities were log2 transformed and normalized according to the method of the quantiles (Bolstad et al. 2003). The probes for the same Entrez Genes or lincRNAs (as of 1st January 2012) were averaged. A linear model including the AGE*PASSAGE + SUBJECT + DYE terms followed by a moderated t-test was utilized for finding the differentially expressed genes (log2 Fold Change >0.58) in the comparisons of interest (nominal p-value < 0.01).

For miRNA expression analyzes, 100 ng of total RNA was reverse-transcribed and amplified with real-time PCR using miScript-System including miScript II RT-Kit, miScript SYBR-Green PCR-Kit, and miScript Primer Assay according to the manufacturer's protocol. For endogenous control, RNU6 was used. All PCR reactions were performed in triplicate in 384-well plates and measured by ABI 7900HT detection system. Mean

values and standard deviations were calculated and fold changes were determined using the ΔΔCT method.

4.6 Senescense indicators

Telomere length analysis

Telomere lengths were analyzed by the southern blot analysis of terminal restriction fragment (TRF) lengths (Kimura et al. 2010). Genomic DNA from snap-freezed cell pellets was purified using the Qiagen DNeasy Blood and Tissue Kit and extracted with ethanol. Quality of purified DNA was evaluated with 1% agarose gel electrophoresis. Telomere length analysis was performed using TeloTAGGG Telomere Length Assay Kit. DNA was digested using *RsaI* and *HinfI* enzymes and electrophoresed on a 0.8% agarose gel 5V/cm. Southern blotting was performed using 20X salium sodium citrate buffer. The blot was hybridized overnight using a digoxigenin (DIG)-labeled telomere specific probe (TTAGGG) and incubated with an alkaline phosphatase-labeled anti-DIG antibody. The blot was then incubated with CDP-Star chemiluminescent substrate and exposed to an autoradiography film. The autoradiogram was scanned by densitometry and TRF length was calculated using ImageJ analysis software (Schneider et al. 2012) according to TRF= $\Sigma OD/\Sigma (OD_i/L_i)$, where OD_i is optical density and L_i is the length of the TRF at position i. TRF signals between 3 and 20 kb were used for telomere length measurements (Kimura et al. 2010).

Western blotting

Snap-freezed cell pellets were lysed using RIPA–buffer (containing Protease Inhibitor Cocktail. 20 μg of total protein was run on a 12% SDS-PAGE gel and electrotransferred to Hybond ECL Nitrocellulose membrane. The membrane was then blocked with 5% milk in PBS containing 0.1% Tween-20 and immunoblotted using anti-$p16^{INK4A}$ (1: 800, clone DCS-50) and anti-p21 (1:250, Clone SXM30). β-actin (1: 8000, monoclonal anti-β-actin, clone AC-74) was used as a loading control. Polyclonal anti-mouse horseradish peroxidase (HRP) conjugated antibody was used as a secondary antibody (1:1000). Detection was performed using an enhanced chemiluminescent detection system. Quantification of band intensities was performed using GS 800 densitometer and Quantity One software.

4.7 Bioinformatic and statistical analysis

In study I, the differentially expressed genes were analyzed for significant enrichments of GO-BP classes by using R library GOSim v. 1.2.5 using default parameters (Frohlich et al. 2007). Enrichments with a p-value <0.01 were considered significant.

In study II, the prediction and functional analysis of putative targets for selected miRNAs were performed through the use of QIAGEN's Ingenuity Pathway Analysis (IPA QIAGEN Redwood City, www.qiagen.com/ingenuity).

In study III, subcellular locations for identified EV proteins were collected from UniProt database (as is 23.11.2016). Gene ontology (GO) enrichment analysis of identified proteins was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.7) (Huang da et al. 2009) using default settings.

In all studies results are represented as mean+standard deviation or standard error of the mean. In study I statistical significances for the differences between BM-MSCs from the young and old donors or early and late passages were calculated using student's paired t-test (* p-value <0.05, ** p-value< 0.01, *** p-value <0.001). In study II, non-parametric Spearman correlation was used to calculate correlation between miRNA expression and proliferation of MSCs. In study III, statistically significant differences in mean values were tested by one-way analysis of variance (ANOVA) and the Tukey's post-hoc test. Analyses were performed with GraphPad Prism 7.00 software or using statistical programming software R.

5 RESULTS

5.1 Immunosuppressive properties of MSCs

In this study, the immunosuppressive properties of both BM-MSCs and CB-MSC were assessed. MSCs were characterized and they fulfilled the established minimal criteria for MSCs (data not shown). One of the key functions of MSCs is their capability to suppress T-cell proliferation. The immunosuppressive capacity of both MSC types was ascertained by T-cell proliferation assay. MSCs were co-cultured with CFSE-labeled PBMCs and T-cells were activated by CD3 antibody. Proliferation of CFSE labeled cells was seen as dilution of CFSE dye measured by flow cytometry. We were able to demonstrate that both CB-MSCs and BM-MSCs have strong capacity to suppress T-cell proliferation in a dose-dependent manner (**Figure 7**).

Umbilical cord blood is an attractive MSC source since it is easily available and the collection is non-invasive procedure. The establishment and culturing of CB-MSCs are more complicated than that of BM-MSCs. In our laboratory, CB-MSC culture medium is supplemented with growth factors (EGF, PDGF and dexamethasone (DX)), whereas BM-MSCs are cultured with FBS. Since the different culture conditions have modulating effects on cell functions, it is impossible to separate the effect of the cell source on cell functionality from that of the culture conditions (Laitinen et al. 2016b). Therefore, direct comparison of the immunosuppressive capacity of CB-MSCs and BM-MSCs was not possible. However, the immunomodulative properties of MSCs derived from different tissue sources have been compared in previous studies (summarized in **Table 1**), although with contradictory results. Heo *et al. (*2016) reported that BM-MSCs are more immunosuppressive than CB-MSCs. On the other hand, Castro-Manreza *et al.* (2014) were not able to see any difference in the immunosuppressive capacities. In contrast, Jin *et al.* (2013) showed that CB-MSCs are actually more immunosuppressive than BM-MSCs. While CB-MSCs and BM-MSCs were cultured in similar culture conditions in these studies, T-cell proliferation was assessed by different methods, rendering a comparison of the results difficult.

In addition to CB, AT, umbilical cord, and placenta are commonly used sources of MSCs. Similarly, the results are contradictory and it is impossible to prioritize different tissue sources according to their immunosuppressive capacity. Notably, in all studies MSCs showed clear capacity to suppress T-cell proliferation regardless of tissue source and it is therefore other characteristics such as availability and proliferative capacity that are more important to consider when tissue source is selected.

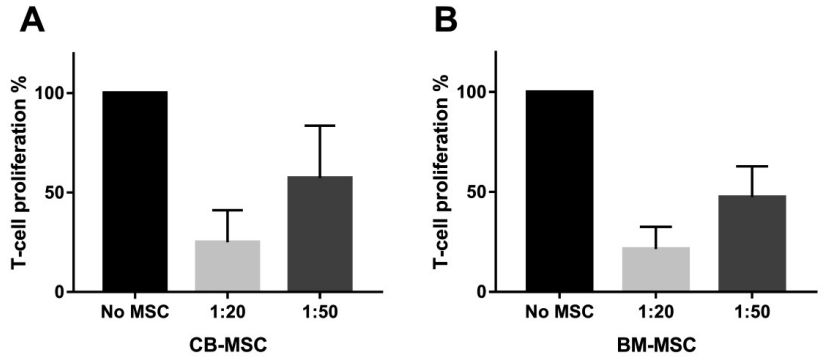

Figure 7 The immunosuppressive capacity of MSCs. A. CB-MSC (n=12) B. BM-MSC (n=16). MSCs between passages 4-5 were used. The proliferation of activated PBMCs in absence of MSCs was set as 100%. PBMC:MSC ratios 1:20 and 1:50 were used. Results are expressed as mean+SD. Unpublished data.

5.1.1 MSCs have dual role in the regulation of T-cell proliferation

The possible immunogenicity of MSCs has been one of the concerns in MSC therapy. According to our results, MSCs do not activate T cell proliferation in co-culture. Since MSCs lack the expression of MHCII molecules as well as costimulatory molecules, CD80 and CD86, on their surface, they are considered to be non-immunogenic, which makes them an excellent candidate for cell therapy (Tse et al. 2003).

In our studies of the immunosuppressive capacity of MSCs, we have used PBMCs as responders and activated them with CD3 antibody alone without the costimulatory CD28 antibody. We have observed inter-experimental variation in the response, and in some experiments T cells do not proliferate at all. When T cells are activated to proliferate, MSCs are highly suppressive. However, in the absence of T-cell response MSCs have the opposite, stimulatory effect on proliferation (**Figure 8**). It is noteworthy that in the absence of CD3 antibody MSCs do not induce T-cell proliferation. This may be due to the inadequate number of antigen presenting cells with co-stimulatory molecules. Further, this leads to inadequate proliferation and cytokine production by T-cells. In these situations, MSCs may act as pro-inflammatory factors, stimulating T-cell proliferation as previously described in mice (Li et al 2012).

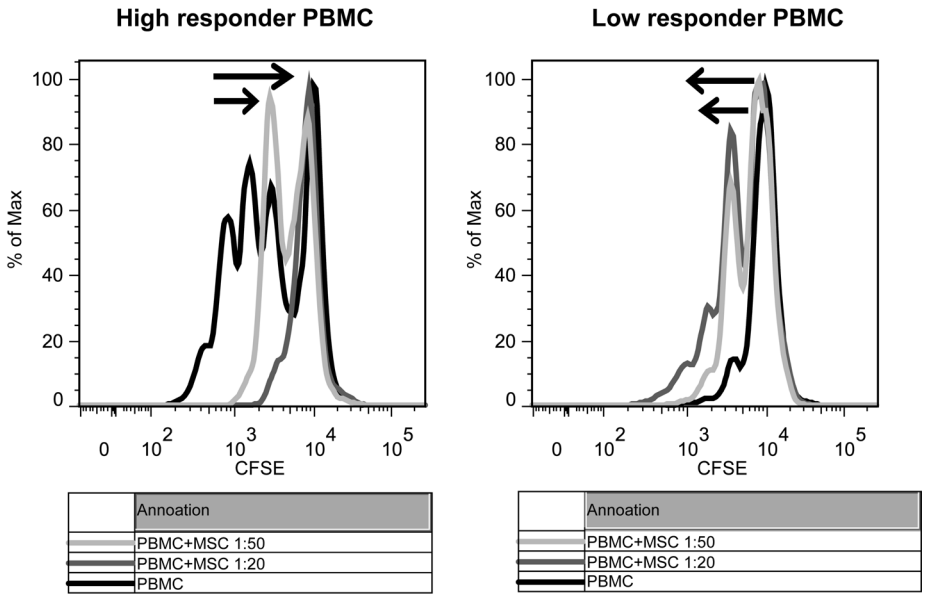

Figure 8 MSCs have an inhibitory and stimulatory effect. BM-MSCs were co-cultured together with either high responder PBMCs (left) or low responder (right). PBMCs were labeled with CFSE and stimulated with antiCD3 antibody. If PBMCs responded poorly to the stimulant (low responder), MSCs boosted the T-cell proliferation. When PBMSCs responded efficiently (high responder), MSCs had an inhibitory effect on the T-cell proliferation. Unpublished data.

5.1.2 Effect of IFNγ preconditioning on MSC immunsuppressive potential

The surrounding microenvironment is one of the key regulators of MSC functionality. MSCs have been shown to have the ability to react differently according to the external stimuli. On the site of inflammation in the injured tissue, there are large numbers of different cytokine molecules in concentrations that fluctuate depending on the phase of the on-going inflammation. Several studies have demonstrated that cytokines, such as IFNγ and TNFα, regulate the mRNA expression of IDO and production of MSC-derived soluble molecules such as PGE_2 (Aggarwal and Pittenger 2005). For this reason, it has been suggested that priming of MSCs may enhance their immunosuppressive capacity and improve the therapeutic outcome in clinical application. In our study, we were not able to see any influence of IFNγ priming on the immune suppressive capacity of BM-MSCs (**Figure 9**). In the literature, the stimulation times, cytokine concentrations, and cytokine cocktails vary greatly. We were able to show that even though the immunosuppressive capacity measured by T-cell proliferation was not affected, the IFNγ stimulation did increase the expression of HLA I and HLA II molecules after 48 hours of stimulation with 100ng/ml IFNγ. This effect was not seen after 24 hours of stimulation (Publication III, Fig. S2). Stubbendorf *et al.* (2013) showed that the same effect could be obtained

using only 25ng/ml IFNγ. We also used a combination of IFNγ and TNFα to stimulate CB-MSC and BM-MSCs. We were not able to see any difference in the immunosuppressive capacity of stimulated or control MSCs (data not shown).

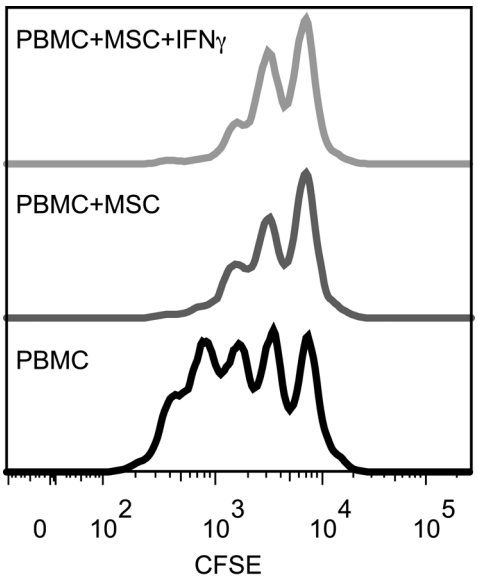

Figure 9 Interferon gamma priming does not improve the immunosuppressive capacity of BM-MSCs. Representative result using BM-MSC: MNC ratio 1:50 is shown.

5.1.3 Membrane lipid composition of MSCs is related to functionality

Instead of being passive structural components of the cell membrane, lipids are actively involved in cellular signaling events employed in immune functions. Membrane lipids act as precursors for lipid mediators that regulate immunological events. PGE2, which is produced from 20:4n-6, has been shown to have a significant role in MSC-mediated immunosuppression (Aggarwal and Pittenger 2005, Chen et al. 2010, Duffy et al. 2011, English et al. 2009). The other important FAs, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), serve as precursor for specialized proresolving mediators (SPM). SPMs have been shown to modulate the innate immune system and their role in regulating the resolution phase of inflammation has recently been described (Serhan et al. 2015). SPMs, such as 22:6n-3-derived resolvins, not only regulate the innate immunity, as previously described, but are also important regulators of adaptive immunity. These mediators reduce cytokine production of activated CD4+ and CD8+ T cells and inhibit the differentiation of Th17 cells (Chiurchiu et al. 2016). The immunomodulatory function of MSCs resembles the active resolution of inflammation (English 2013) and there is a possibility that

SPMs have a role in MSC immunoregulation, which remains to be elucidated in future.

Modern mass spectrometric methods enable researchers to use a lipidomic approach to the analysis of cellular lipids as a whole to get a broader view of the cellular functions. The lipidomic approach has been used to study membrane lipidomes of hematopoietic progenitors and embryonic stem cells (Fuchs et al. 2007, Park et al. 2010), but lipidomics studies on MSCs are lacking. In this study, we report correlation between membrane lipid composition and the immunosuppressive capacity of MSCs. Molecular percentage of 20:4n-6 containing phosphatidylcholine (PC) species PC38:4 and PC38:5 and 20:4n-6 itself correlates negatively (correlation coefficients PC38:4, R=-0.91; PC38:5, R=-0.82 and 20:4n-6, R=-0.71) with the immunosuppressive capacity of BM-MSCs. (Publication I, Table II) Interestingly, a particular n-3 PUFA, 22:6n-3 correlated positively with immunosuppressive capacity (R=+0.75). Our result indicates that variation in the membrane lipid composition and especially changes in the ratio of certain molecular species may have consequences on the MSC functionality. The role of composition of lipid mediator precursors in the cell membrane in the actual production of the novel SPM mediators needs to be confirmed and elucidated in more detail.

5.1.4 MSC-EVs are immunosuppressive

To investigate the potential immunomodulative properties of MSC-EVs, we co-cultured PBMCs together with isolated CB-MSC-EVs. We showed that CB-MSC-EVs suppress T-cell proliferation, but the effect is milder (Publication III, Fig. 1). In addition to direct suppression of T-cell proliferation, MSCs induce the production of regulatory T-cells. When T-cells were co-cultured with MSCs or MSC-EVs, the proportion of $CD4^+CD25^+FOXP3^+$ increased (Publication III, Fig. 1). These results indicate that the immunosuppression of MSCs may be at least partially mediated by secreted EVs. Gouveia de Andrade *et al.* (2015) showed that BM-MSC and AT-MSC derived EVs failed to suppress lymphocyte proliferation. These contradictory results emphasize the need for standardization of EV collection and purification methods.

Di Trapani *et al.* (2016) have shown that the inhibition of exosomes restores T- and B- cell proliferation in the presence of MSCs. This phenomenon is more evident in primed MSCs compared to resting MSCs. Inhibition of microvesicle shedding and apoptotic body release had no effects on effector cell proliferation. In the proteomic analysis, we observed that primed MSCs produce EVs that contain different set of Rab proteins compared to resting MSCs, indicating that priming induces exosome production through MVEs (Publication III, Fig. 7). Taken together, these results suggest that MSCs produce immunosuppressive EVs that are transferred to effector cells, a phenomenon that is enhanced by priming MSCs with TNFα and IFNγ.

One of the proposed mechanisms of MSCs is mediated through purinergic singaling. CB-MSCs and also CB-MSC-EVs contain CD73 (Publication III, Fig. 4). Previously, it has been shown that MSC-EVs exhibit similar activity to MSCs in the production of adenosine from adenosine monophosphate (Kerkelä et al. 2016).

5.1.5 Interferon gamma stimulation deteriorates the *in vivo* functionality of MSC-EVs

MSC-derived EVs have been shown to have a therapeutic effect in AKI. In this study, we used an IRI rat model to investigate the protective capacity of CB-MSC-EVs. In line with previous studies using glycerol or cisplatin induced AKI models, we were able to show that EV-treated rats developed less severe AKI. Kidney damage was measured as elevated serum creatinine and urea levels at time points 24h and 48h after operation. In rats that were treated with MSC-EVs, the dramatic elevation of serum creatinine and urea was significantly reduced (Publication III, Fig. 5). The protective effect of MSC-EVs was also seen in histopathological analysis of the kidney after 48 hours. The signs of injured kidney, vast necrosis of tubular cells, tubular dilatation, and cast formation, were almost absent in the MSC-EV treated rats in comparison to control rats (Publication III, Fig. 6). Our results are thus in line with previous studies, demonstrating that MSC-EVs have renoprotective effects during the early phase of AKI. In our AKI model, EVs were injected to the rats immediately after reperfusion, thus protecting kidneys from injury.

There is convincing evidence that MSCs require extracellular cytokine stimulus for complete immunomodulation. MSCs have been shown to produce EVs constitutively, but we hypothesized that their quantities and/or functionality could be improved with external IFNγ stimulus. We were not able to see any difference in the morphology, particle number, or size distribution between stimulated and nonstimulated MSCs. (Publication III, Fig. 1) Surprisingly, stimulated CB-MSC-EVs did not prevent the development of AKI (Publication III, Fig. 5 and 6).

5.1.6 Proteomic analysis of EVs

Mass spectrometric proteomic analysis was applied to the analysis of the protein content of IFNγ stimulated and nonstimulated CB-MSC-EVs. The identified proteins originated from different cellular compartments as illustrated in **Figure 10**. The distributions of stimulated and nonstimulated EVs were highly similar, while the protein compositions were different. First of all, we were able to identify almost 700 different proteins from stimulated MSC-EVs, whereas from control EVs, only 446 proteins were identified (Publication III, Fig. 2).

According to MS data, only EVstim contained exosome markers CD9, CD63, and CD81. MSC markers CD73 and CD90 were found in both EVs. Of immunomodulative factors (proteins) shown to mediate MSCs immunomodulative functions (see **Figure 5**), only galectins 1 and 3 were identified from the EVs (both stimulated and nonstimulated). On a protein family level, we noticed that members of Rab proteins were present in both stimulated and nonstimulated EVs. However, the individual proteins were mostly different. EVs secreted from stimulated CB-MSCs were linked to exocytosis and deeper endosomal pathway (Rab 1, 2, 6, 8). In contrast, EVs from nonstimulated MSCs contained Rabs (3, 5, 14, 34) that were more related to the early endosomal recycling, near plasma membrane (Publication III, Fig. 7).

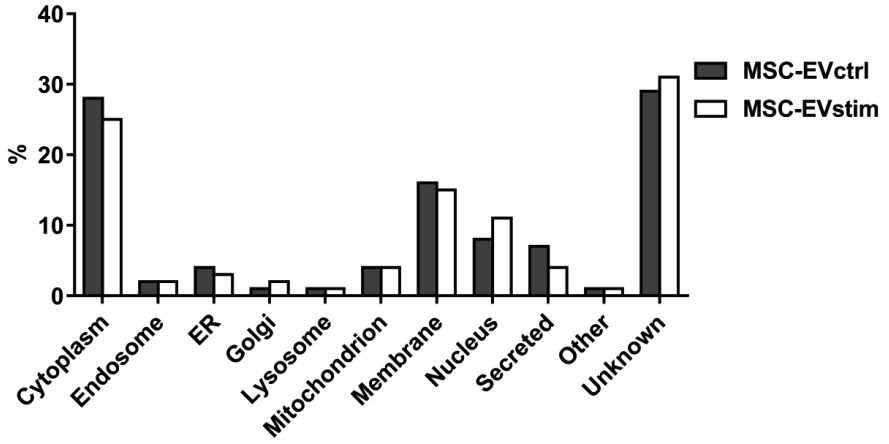

Figure 10 Subcellular locations of MSC-EV proteins. CB-MSC proteome was used as reference. Retrieved from Uniprot as is 23.11.2016. Obsolete protein entries were removed from the analysis.

5.2 Age-induced changes in MSCs

Prior to clinical use, MSCs need extensive *in vitro* expansion. In order to investigate the effects of long-term cell culture on the functional properties of MSCs, we isolated BM-MSCs from five young (20-24 years of age, mean 22.2 years) and five old donors (62-82 years of age, mean 74.6 years) (see **Table 4** for details). BM-MSCs were cultured until senescence was reached and samples for further analysis were collected from every other passage. The proliferation capacity of young and old donors was similar, but considerably more interindividual variation was seen in the old donors group (**Figure 11A** and Publication I, Fig. 1).

Replicative senescence was studied from selected cells and samples. We used morphology, telomere lengths, and protein expression of cell cycle components as indicators for replicative senescence. Late passage BM-MSCs gradually adopted the typical flattened morphology accompanied by increased cell size. Telomere length measurements showed that in passage 11 cells telomeres were 1.0±0.5 kbp shorter than in passage 4 cells **(Figure 11B)**.

The expression of cell cycle components $p16^{INK4A}$ and $p21^{CIP/WAF1}$ were also increased in passage 11 cells in comparison to passage 4 cells as analyzed by western blot **(Figure 11C)**. These results indicate that among the three selected cell donors, the degree of senescence and the proportion of senescent cells were not equally high.

The immunosuppressive capacity of early and late passage cells was measured using CFSE staining and anti CD3 stimulated PBMCs. The early passage cells suppressed T-cell proliferation almost completely, but later passage cells were less immunosuppressive (Publication I, Fig. 1).

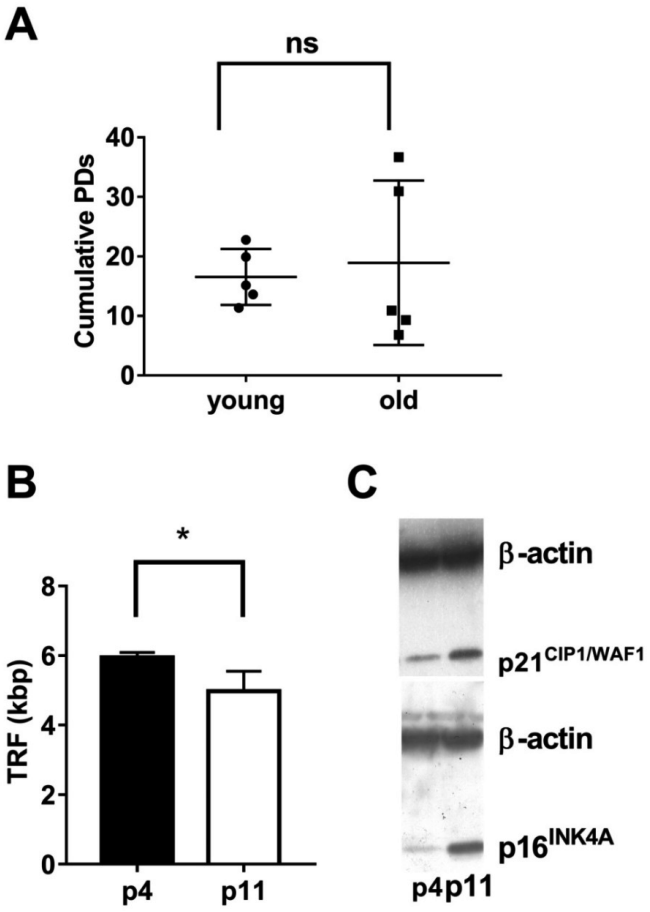

Figure 11 Proliferation and senescence of BM-MSCs. A. Cumulative population doublings at the end of the cell culture. The proliferation capacity between young (n=5) and old donors (n=5) is similar, but there is more individual variation among the old donors group. Non-parametric Mann-Whitney test was used to test the difference between groups (ns= non significant) **B.** Telomere lengths (TRF, Terminal restriction factor) were determined in early and late passage cells. Results are mean+SD of three BM-MSC donors. Paired one-tailed t-test was used to test the difference between the means. * $P<0.05$. **C.** Representative result of western blot analysis of cell cycle components p16 and p21. β-actin was used as a loading control.

5.2.1 MSC lipid profile changes during *in vitro* expansion

Prior to use in therapy, MSCs need to be extensively expanded. While the effect of expansion on the DNA integrity, miRNA profiles, and gene expression has been previously studied, other molecular level changes are less well-studied. We had very little knowledge about membrane GPL composition of MSCs, along with the kind of changes that might take place in membrane composition during expansion. We used lipidomic approach to analyze cellular GPLs and total FA profiles from young and old donors. GPLs were analyzed with ESI-MS/MS techniques and the FA composition of total lipids was measured with gas chromatography. All results are shown as mol % per total lipids in that class or per total FAs.

The GPL class profiles demonstrated that PC and phosphatidylethanolamines (PE) are the most abundant types of phospholipids in MSCs. This result is in line with other reports, apart from the MSCs having a higher proportion of PE than the previously described, otherwise very similar, fibroblasts (Blom et al. 2001)(Publication I, Table 1). When young and old donors were compared, the GPL profiles were highly similar.

During expansion, phosphatidylinositol (PI) totals increased. This phenomenon was more evident when PI totals were compared to phosphatidylserine (PS) totals. The increase of PI was more evident in the BM-MSCs from young donors, since in the BM-MSCs from old donors the PI/PS ratio was already high in the early passage cells (Publication I, Fig. 3).

Expansion of BM-MSCs induced alterations in the mol percentages of numerous phospholipid species especially in the PC and PE classes-. In general, long and unsaturated PL species were increased at the expense of shorter and monounsaturated species. The molar percentage of PC38:4, PC36:4, PC36:1, PE38:4, PE38:1, and PE36:4 increased towards late passages while PC34:1, PC34:2, PE36:1, and PE34:1 decreased. Most noticeable increase was observed in PC38:4 and PE38:4, which happened at the expense of the monounsaturated species PC34:1 and PE36:1 (**Figure 12)**.

The FA profiles measured by GC supported the clearest finding in PC and PE phospholipid species. The increases in PC38:4 and PE38:4 were accompanied by a clear increase in 20:4n-6 in FA profiles. At the same time, the two most prominent n-3 PUFAs, 22:5n-3 and 22:6n-3, decreased, the effect being clearer for 22:6n-3 (**Figure 13**). In addition to measuring the relative quantities of different FAs in the BM-MSCs and FBS, we also measured the total quantity of FAs available for BM-MSCs on the culture dish. These results demonstrated that there is an excessive quantity of essential FAs for cells. (Publication I, Fig. 5B).

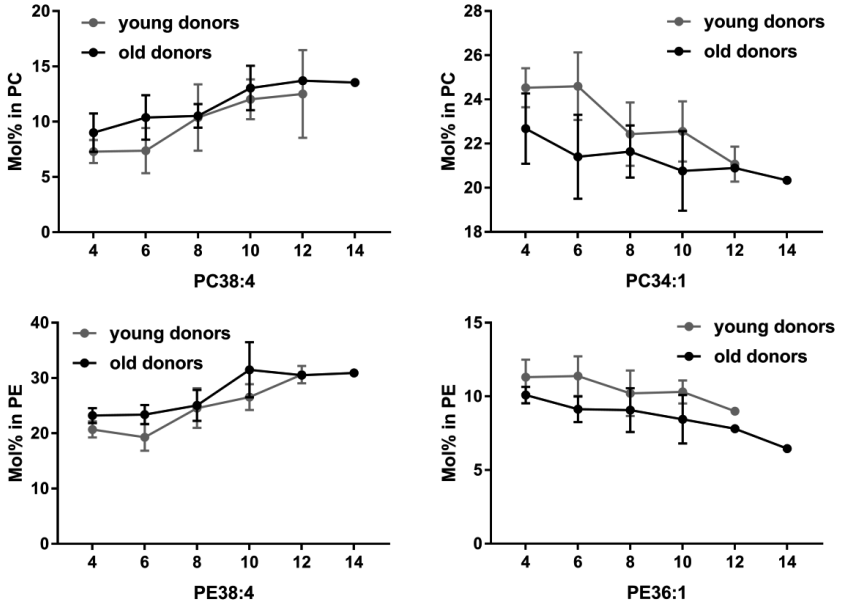

Figure 12 Effect of expansion on selected PC and PE species in hBM-MSCs. Mean values of PC38:4, PC34:1, PE 38:4, and PE 36:1 over different cell passages (x-axis). Error bar represents SD of individual BM donors, n=5

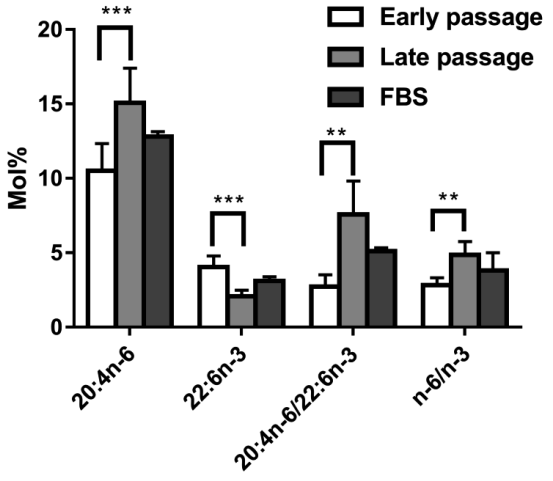

Figure 13 Effect of expansion on selected PUFAs and threis ratios in hBM-MSCs. Results are mean+SD of all donors. (n=10). For FBS, three replicates were analysed.

5.2.2 Gene expression changes related to donor age and cell expansion

In order to elucidate the role of gene exression regulation during expansion, we analysed mRNA expression and miRNA expression in passage 4 and passage 8 cells. Previous findings on proliferation kinetics and lipidomic changes had shown that most dramatic changes occur at passage 8. Therefore, p8 was selected as the late passage time point for microarray experiments. Gene expression analysis demonstrated that mRNA expression was remarkably different already in the early passage BM-MSCs of young and old donors (Publication I, Fig. 6). MiRNA expression, on the other hand, was very robust, and only minor changes between young and old donors were observed *(data not shown)*. Expansion seems to have a different effect on the BM-MSCs from young and old donors. The expression of only 36 mRNAs and 12 miRNAs was changed during the expansion in both young and old donors' BM-MSCs. Thus, the majority of the observed differences were unique to either the young or old donors (Publication I, Fig. 6; Publication II, Fig. 1).

We selected five common miRNAs for qPCR validation. The qPCR results confirmed our microarray results, and we also showed that while miRNA expression mostly increases gradually, it takes a leap from passage p6 to p8 (**Table 5**, Publication II, Table 1 and Fig. 2). BM-MSCs were passaged when confluency was 80%, resulting in different culture times. At p8, the culture time varied between 31 and 71 days. We noticed that miRNA expression levels measured as dct values showed similar variation. We performed a correlation analysis, whichshowed that miRNA expression correlates significantly with the expansion time (at passage 8) (**Table 5**).

Table 5. miRNA expression and correlation analysis between miRNA expression and expansion time. *BMMSC donors 081 and 271 were removed from FC calculations. Correlation of dct values at passage 8 with expansion tile (days) was calculated using spearman's correlation (n=9).*

miRNA	*FC*	*p*	*r*	*p*
miR-1207-5p	*1.3*	0.004	-0.8	0.007
miR-1915-3p	*2.5*	<0.001	-0.8	0.01
miR-3665	2.6	0.002	-0.5	0.07
miR-4281	1.42	0.004	-0.7	0.02
miR-762	2.13	0.006	-0.7	0.02

Detailed analysis of differentially expressed genes revealed that the expression of several genes operating in lipid metabolism were decreased in the old donors' cells already at passage 4. These genes included FA desaturases *FADS1, FADS2, SCD*, lysoPC acyltransferase genes *LPCAT2* and *LPCAT3*, FA elongase 3 (*ELOVL3)*, and leukotriene C4 synthase *(LTC4S)*. In addition, the *PTGS1* gene coding cyclooxygenase 1 enzyme (COX1), which is

responsible for PGE2 synthesis, were more highly expressed in passage 8 cells (old donors) than in passage 4 cells (Publication I, Table 3).

As an indicator for the modulation of immune functions, the genes for the suppressors of cytokine signaling (*SOCS1* and *SOCS3)* were expressed at a lower level in the old donor cells (at passage 4). In addition, the expression of SOCS3 decreased during passaging in the old donors group. Interestingly, SOCS3 is a predicted target for three miRNAs (miR-181-5p, miR-762, and miR-2861) whose expression increased during expansion (Publication I, Table3, Publication II, and Fig. 2).

A more detailed analysis of miRNA changes demonstrated that the expression of miRNA cluster 17/92 was decreased either in old donors' cells (miR-17, miR-18a, miR-19a, and miR-20a) or in young donors' cells (miR-93, miR-25, and miR-92a-3p) (**Figure 14**). This cluster has been studied extensively, with the results indicating downregulation of miR-17, miR-20a and miR-106a in aging (Hackl et al. 2010). MiRNAs are found as clusters in the chromosomes, with miRNAs belonging to the same cluster most likely expressed together. In addition, miRNA in miR17/92 cluster and its paralogues miR106a/363 and miR106b/25) are divided into four families according to their seed sequences (see **Figure 14**). MiRNAs with similar seed sequences are likely to target the same genes and to share similar functions.

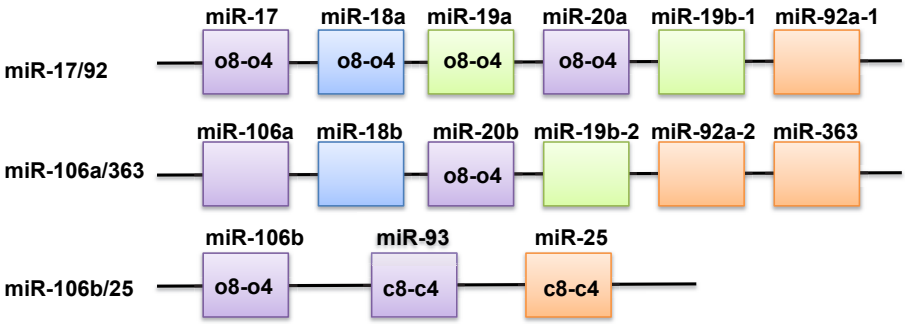

Figure 14 miR17/92 cluster. Differential expression in BM-MSCs of members of the miR-17/92 cluster and its two paralogues miR-106a/363 and miR-106b/25 and their differential expression in BM-MSCs during expansion. o8-o4 old donors group, y8-y4 young donors group, c8-c4 common group. Purple: members of the miR-17 family; blue members of the miR-18 family; green members of the miR-19 family; orange; member of miR-92 family

6 DISCUSSION

Cells are extremely complex, yet organized entities. In a single cell, there are myriad of genes, protein, and lipids, which are all strictly regulated in order to allow the cells to fulfill their tasks and functions. The development of new technologies and bioinformatic tools has led to an era of "omics", a field where the numerous molecules of a cell are examined as an entity. This approach presents certain challenges: How to comprehend the massive data? How the relevant information is filtered from the irrelevant? What do these results mean in a context of cell biology? In this study, one of the challenges was to utilize omics data and to convert it to applicable information about MSCs functionality.

MSCs contribute to the maintenance of the hematopoietic niche in the bone marrow and placenta. They are rare in the body, and therefore extensive *in vitro* passaging is required before use in therapeutic applications. MSCs, like all other primary cells, do not divide infinitely, but their proliferative capacity is limited. The effects of expansion on MSCs' molecular composition and functionality are poorly understood. Donor age is one factor that is associated with a loss of proliferative capacity and cellular senescence of MSCs. Biological markers for cellular senescence indicate that MSCs from old donors show accelerated senescence (Stenderup et al. 2003). In the field of advanced cell therapy, the use of autologous MSCs also for older patients is continuously increasing.

The aim of this study was to investigate the effects of donor age and extensive expansion on the proliferation, quality, and immunosuppressive capacity of hBM-MSCs. We used the omic approach to analyze the molecular changes in hBM-MSCs during extensive expansion.

6.1 Lipid metabolism is connected to MSCs functionality

In this study, we analyzed the membrane GPL and cellular FA composition of hBM-MSCs. In general, we showed that the GPL composition of membranes is highly similar to that previously reported for fibroblasts: only the PE level is higher in MSCs than in fibroblasts, which in other respects are very similar to MSCs. Next, we studied how expansion changes the GPL species composition of hBM-MSCs. We observed that the proportion of 20:4n-6 containing PC and PE species (38:4 and 36:4) increased, whereas n-3 PUFA containing species (40:5, 40:6 and 40:7) and shorter monounsaturated species decreased. These findings were supported by FA analysis. Aging MSCs accumulated 20:4n-6 and lost 22:6n-3 and other n-3 PUFAs. The 20:4n-6 is a precursor for several pro-and anti-inflammatory lipid

mediators, such as prostaglandins and lipoxins (**Figure 15**) (Stables and Gilroy 2011).

The shift in the balance between n-6 and n-3 PUFAs may influence responsiveness to inflammatory signals and capability for immunomodulation (Schoeniger et al. 2011). This hypothesis was strengthened by the observation that both expansion and a high content of 20:4n-6 resulted in decreased capacity for T-cell proliferation. In addition, our gene expression data revealed that the expression of COX-1 gene *PTGS1* was elevated in late passage cells compared to early passage cells. A similar pattern was seen in the *PTGS2* gene, but the difference was not statistically significant.

A simultaneous decrease in the n-3 PUFA content is of immunological importance as well. Traditional eicosanoids have been found to be accompanied by SPMs, including resolvins, protectins, and maresins, which are derivatives of 22:6n-3 and 20:5n-3 FAs. SPMs are needed in the resolution phase of inflammation (Serhan et al. 2015, Stables and Gilroy 2011). SPMs, which are mainly produced by macrophages and neutrophils, prevent inflammation from spreading and halt the formation of chronic inflammation. SPMs are described as modulators of innate immunity but little is known about their effects on the cellular components of adaptive immunity. Both CD4+ and CD8+ T cell responses are reduced by RvD1, RvD2, and Mar1. Particularly the differentiation of Th17 cells was inhibited (Chiurchiu et al. 2016). Even though MSCs have several properties that suggest a potential role also in the resolution phase of inflammation, little evidence of the secretion of SPMs is available. Human periodontal stem cells with MSC-like characteristics have been shown to produce several 20:4n-6, 20:5n-3, and 22:6n-3-derived mediators, but their role in MSC mediated immune modulation remains to be elucidated (Cianci et al. 2016).

It is important to highlight that same cyclooxygenase and lipoxygenase pathways produce different lipid mediators with completely different, even opposing, functions (**Figure 15**). Different GPL/FA compositions in the cell may cause hBM-MSCs to respond very differently to the same external stimuli. The availability of GPL/FA substrate is reflected also in the produced lipid mediators and ultimately in the functionality of MSCs.

In this study, MSCs were cultured in FBS. Due to the constant excess quantity of FAs in the surrounding medium compared to the cells, we can assume that the observed changes in hBM-MSC lipidome were due to an active senescence-associated process. However, it is possible that supplementing the culture medium with even more n-3 PUFAs might support hBM-MSCs growth and maintain their physiological functionality. Interestingly, our group has shown that MSCs are not able to synthesize long chain PUFAs from the short chain precursors (18:2n-6 or 18:3n-3). Thus, 22:6n-3 should be added to culture medium in order to increase 22:6n-3 levels in MSCs (Tigistu-Sahle et al. 2017). However, there may be a limit as too high n-3 PUFA contents in membranes may even impair raft-related

functions and signaling events occurring in lipid rafts (Fan et al. 2004, Turk and Chapkin 2013).

In therapeutic applications, FBS is more often replaced by human platelet lysate or other animal-free supplements. These products show more variation between batches, which may be caused by differences in growth factor concentrations, but the FA composition of these products may also vary. It would be interesting to compare the lipid profile and its changes during passaging in xeno-free cultured MSCs and compare the results with MSCs cultured in FBS.

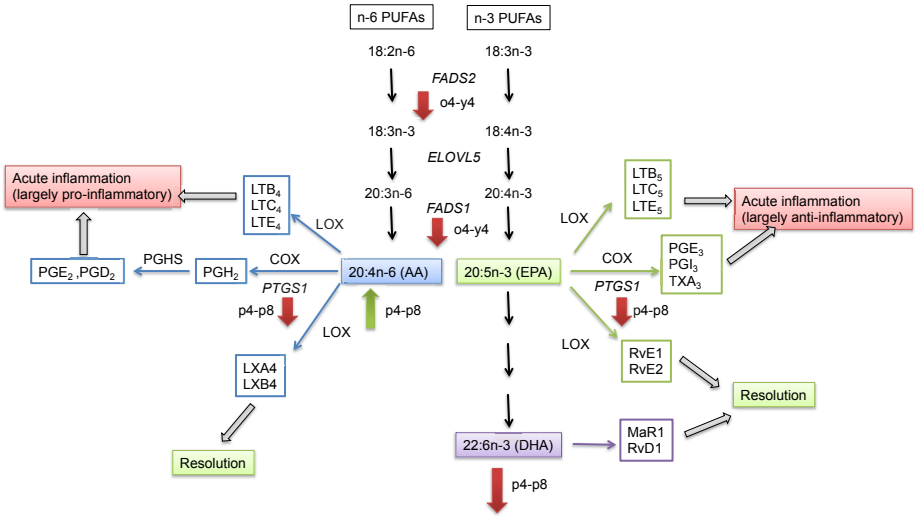

Figure 15 Scheme of n-6 and n3 PUFA metabolism and release of lipid mediators. Green and red arrows indicate either increase or decrease, respectively of FA or mRNA levels observed in this study. p4-p8 refers to change during expansion and o4-y4 refers to difference between young and old donors at passage 4. Abbreviations: AA, arachidonic acid; COX cyclooxygenase; DHA, Docosahexaenoic acid, ELOVL, fatty acid elongase; EPA eicosapentaenoic acid; FADS fatty acid desaturase, LOX lipoxygenase, LT, leukotriene; MaR, maresin; PG, prostaglandin; PUFA, polyunsaturated fatty acid; Rv, resolvin; TX, thromboxane.

6.2 MicroRNA regulation connects cell cycle and immunoregulation

In the second publication in the thesis, we further investigated expansion-induced changes in hBM-MSCs. We noticed that miRNA levels were relatively robust during expansion. In this study, the most highly expressed miRNAs were generally the same that have previously been described and reviewed by Clark *et al.* (2014). This indicates that MSCs have a particular miRNA expression pattern that is not susceptible to changes.

In this study, we had the opportunity to investigate the possible miRNA-mRNA interactions and their role in the proliferation of MSCs. We used a microarray platform to analyze miRNA and mRNA expression in the same cells. We used an ingenuity pathway analysis tool to predict potential targets

for miRNAs and then combined this information with the actual expression levels in the cells. As expected, we saw that in many cases the expression of miRNA and its potential target changed in opposite directions (Publication II, Fig.4). However, in many cases the expression of miRNA and its target mRNA changed in same direction. There are several possible explanations for this. The most likely one is that our experiment did not catch the direct and short-lived causality of the miRNA and its target mRNA. Also, the expression of miRNA and its target may be regulated by the same factor or mechanism. Further, as miRNA regulation is post-trancriptional, it is not self-explanatory that miRNA regulation is seen automatically as reduced expression of its target mRNA. And again, miRNAs regulate tens to thousands of mRNA molecules. Development of prediction tools offers an excellent starting point for narrowing down the potential targets or signaling routes, but further experimental validation is needed to fully understand the role of spesific miRNAs in MSCs functionality.

MiRNAs are involved in many cellular processes, and especially in the development and differentiation of stem cells. In the context of MSCs biology, several miRNAs have been shown to regulate the differentiation, proliferation, and immunosuppression of MSCs. Our analysis revealed that several members of the miR17/92 cluster were down-regulated in old donor cells and in a few members of the young donors group. This miRNA cluster is of a particular interest since miR-17, miR-20a, and miR-106a are down-regulated in aging (Hackl et al. 2010). It is also known that the cell cycle regulator p21, whose expression is increased in senescent cells, is regulated by this cluster (Gomez-Cabello et al. 2013, Ivanovska et al. 2008).

6.3 Extracellular vesicles mediate immunoregulative functions of MSCs

The therapeutic effect of MSCs is currently explained by their capacity to regulate immune cells through paracrine mechanisms. MSCs secrete EVs that are immunosuppressive (Di Trapani et al. 2016) and repair injured tissues in ischemic animal models (Bruno et al. 2009, Collino et al. 2015, He et al. 2012). Although the effects of external cytokine stimulus in the MSC-mediated immunosuppressive mechanisms have been widely studied, the effect on EV production is less well-known. In this study, we show that hCB-MSCs secrete constitutively (in serum deprivated culture media) EVs that remarkably attenuate IRI in a rat model. Our results are thus in line with previous reports demonstrating that BM-MSC-derived EVs are renoprotective in the early phase of AKI. Innate immunity plays a major role in regulating the early phase of AKI. MSCs are known to interact with innate immune cells regulating, for instance, the macrophage polarization toward an anti-inflammatory M2 phenotype. Recently, the role of MSC-derived EVs in macrophage polarization was also demonstrated (Lo Sicco 2017). In

literature, the IFNγ concentration used to stimulate MSCs ranges from a few ng/ml up to 100 ng/ml. In this study, we used the highest concentration, 100 ng/ml, to stimulate CB-MSCs to produce EVs. We noticed that whenMSCs were stimulated with a high quantity of IFNγ, the protective effect of EVs was not seen.

The proteomics analysis of stimulated and non-stimulated CB-MSC EVs showed clear differences between these two groups. In stimulated CB-MSC EVs, the presence of MHC I was observed, which at least partly may explain why they were not protective in the AKI model. Therefore, it is highly possible that innate immune cells recognize these EVs as foreign and consequently they are lysed. Further, we identified larger number of proteins from stimulated EVs compared to non-stimulated EVs. The further analysis of individual proteins gave us some candidates that could explain the differences observed in the functionality of EVs. Strikingly, our proteomic data imply that IFNγ stimulation induces EV production via a secretory route, whereas constitutively produced EVs were produced by either membrane blebbing or by rapid recycling route. This could explain the observed differences in protein content, such as exosomal markers that were found only from stimulated EVs. EVs also contain other molecules in addition to proteins, such as lipids, miRNAs, and mRNAs, whose role in EV-mediated immunosuppression requires further studies.

6.4 Conclusions and future perspectives

In this study, we provide new insight into the importance of the membrane lipid composition and lipid metabolism for the functionality of MSCs. We show for the first time that major changes take place in the composition of GPL species and total FAs during expansion of therapeutically important hBM-MSCs. We demonstrate that the cellular relative content of 20:4n-6 increases while that of n-3 PUFAs decrease. The PUFA alterations reported here presumably affect the production of pro- or anti-inflammatory eicosanoids, as well as SPMs needed for resolution of inflammation. Thus, our results will serve as a basis for further studies aimed at understanding the role of lipid mediators in MSC-mediated immunosuppression. Modification of culture conditions may serve as one way of preventing the harmful effects of long-term cell culture. On the other hand, we show that the immunosuppressive capacity of BM-MSCs shows variation that correlates with the PUFA composition of the cells. Therefore, selected lipid or FA molecules may serve as biomarkers for the good quality and functionality of MSCs.

In addition to changes in lipid molecules, we investigated gene expression changes in hBM-MSCs. We demonstrated that gene expression of MSCs from young and old donors differed already at passage 4, but the expansion

induced changes were minor. In general, the changes observed in both miRNA and mRNA expression levels were moderate. This may be because MSCs are an extremely heterogenous cell population and a given cell culture probably contains many different types of MSCs. Gene expression of single cells should be studied using single cell analytics to gain a more profound understanding of different populations and their characteristics in this heterogeneous pool of MSCs. This new analytics method will most likely help us to understand the puzzling question of whether all MSCs are equally multipotent or whether we need to admit that MSCs are actually a collection of cells with different abilities

We further investigated the role of miRNA regulation of BM-MSCs. MiRNAs have been shown to have a role in many aspects of MSC biology. In this study, we report comparative analysis of miRNA expression in young and old donors at the early and late passages. We demonstrate the regulative network that connects the regulation of the cell cycle and immune regulation. This observation needs further validation. We also report that MSCs secrete EVs that are immunosuppressive *in vitro* and protect against acute kidney injury *in vivo*. In this study, we used CB-MSCs instead of BM-MSCs since they seemed to be more active in producing EVs, critical study material for many different experimental steps. Research should next be extended to BM-MSCs, since BM is still the most frequently used MSC source in cell therapy. In future experiments, the mRNA and miRNA content of EVs should be investigated. Since EVs are membranous structures it is plausible that they transport also lipid mediators, or enzymes that may catalyze the production of these mediators from the lipids of EVs *in situ*. Whether the lipid composition of EVs resembles the cell membrane of the mother MSCs and what role EVs play in the immunosuppression of MSCs are questions that require further investigations.

ACKNOWLEDGEMENTS

This work was carried out at the Finnish Red Cross Blood Service, Research and Development Department in Helsinki. This project was financially supported by state research funding (EVO grants), SalWe Research Programs for IMO (Tekes-the Finnish Funding Agency for Technology and Innovation grant 648/10), University of Helsinki Research Foundation, and Finnish Cultural Foundation.

I am grateful for the Chief Executive of the Blood Service, Professor Martti Syrjälä, as well as the former and present directors of the Research and Development department, adj.prof Kari Aranko, adj.prof Jaana Mättö, and Professor Jukka Partanen for providing excellent working facilities.

My warmest and sincerest thanks go to my supervisors Dr Saara Laitinen and adj. prof Reijo Käkelä. Saara's enthusiasm and craziness have motivated me and given me the courage to carry on throughout the years. I am grateful that I had the opportunity to take this journey with you. Reijo is thanked for his support and help with the confusing lipids. Thank you for being there for me whenever I have needed guidance.

I thank the reviewers of the thesis, Dr Juan Falcón-Pérez and and Dr Luc Sensebé for their valuable comments and suggestions for improving this thesis. I also thank thesis committee members Professor Vesa Olkkonen and adj. prof Pentti Somerharju for their encouragement and support throughout the project.

Research is all about the people. I am grateful for all the co-authors of the accompanying publications for your contribution to the thesis and for fruitful collaboration. Especially, Professor Petri Lehenkari and adj. prof Matti Korhonen are thanked for providing the study material. Sofia Oja is thanked for characterizing MSCs. Feven Tigistu-Sahle is thanked for her valuable work with the lipidomics analysis. Adj. prof Päivi Saavalainen, Dr Dario Greco, and Dr Amarjit Parmar, are thanked for their expertise in gene expression analysis. Dr Janne Nikkilä, Dr Jarno Tuimala and Dr Sami Kilpinen are thanked for their help with statistics and bioinformatics. Dr Ulla Impola, Lotta Sankkila, Maria Aatonen, adj. prof. Pia Siljander, Ilja Ritamo, adj. prof Leena Valmu, Dr Piet Finckenberg, Dr Jouko Levijoki, Adj. prof Esko Kankuri, and Professor Eero Mervaala are all warmly thanked for their contribution to the vesicle study.

My heartfelt thanks go to the excellent technical staff of R&D. Lotta S deserves thanks for her help with vesicles and friendship and Gitta for

teaching me a lot about cell culture, research, and life itself. I also thank all the former and present people in the R&D as well as other departments for creating a friendly and enthusiastic working place. Minna, Joni and Vera, the future doctors, are all thanked for making the writing process of this thesis less annoying. Ani, thanks for finding time to listen my worries and answer to my questions.

I acknowledge Marja-Leena Hyvönen for the library services and Piia Loponen and Pirjo Nick for the professional secretarial support. I also thank Niina Woolley for the efficient revision of the language of this thesis.

Finally, I my sincere gratitude goes to my family and friends. I am grateful to my parents Anja and Matti and my parents-in-law Marjo and Seppo for endless support. My deepest thanks are reserved for my husband Sami for all the love and support and for our daughter Sanni, for bringing so much joy and giggle in my life.

April 2017, Helsinki

Lotta

REFERENCES

Adams GB, Chabner KT, Alley IR, Olson DP, Szczepiorkowski ZM, Poznansky MC, Kos CH, Pollak MR, Brown EM, Scadden DT (2006) Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature 439:599-603

Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815-1822

Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S (2012) Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 10:544-555

Alfarano C, Roubeix C, Chaaya R, Ceccaldi C, Calise D, Mias C, Cussac D, Bascands JL, Parini A (2012) Intraparenchymal injection of bone marrow mesenchymal stem cells reduces kidney fibrosis after ischemia-reperfusion in cyclosporine-immunosuppressed rats. Cell Transplant 21:2009-2019

Alikhan MA, Summers SA, Gan PY, Chan AJ, Khouri MB, Ooi JD, Ghali JR, Odobasic D, Hickey MJ, Kitching AR, Holdsworth SR (2016) Endogenous Toll-Like Receptor 9 Regulates AKI by Promoting Regulatory T Cell Recruitment. J Am Soc Nephrol 27:706-714

Al-Nbaheen M, Vishnubalaji R, Ali D, Bouslimi A, Al-Jassir F, Megges M, Prigione A, Adjaye J, Kassem M, Aldahmash A (2013) Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Stem Cell Rev 9:32-43

Ameres SL, Zamore PD (2013) Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 14:475-488

Ankrum JA, Ong JF, Karp JM (2014) Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol 32:252-260

Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T (2004) Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 118:149-161

Ascon DB, Lopez-Briones S, Liu M, Ascon M, Savransky V, Colvin RB, Soloski MJ, Rabb H (2006) Phenotypic and Functional Characterization of Kidney-Infiltrating Lymphocytes in Renal Ischemia Reperfusion Injury. The Journal of Immunology 177:3380-3387

Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G (2005) Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 35:1482-1490

Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Perez Lanzon M, Zini N, Naaijkens B, Perut F, Niessen HW, Baldini N, Pegtel DM (2015) Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem Cell Res Ther 6:127-015-0116-z

Baglio SR, Devescovi V, Granchi D, Baldini N (2013) MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene 527:321-331

Barcia RN, Santos JM, Filipe M, Teixeira M, Martins JP, Almeida J, Agua-Doce A, Almeida SC, Varela A, Pohl S, Dittmar KE, Calado S, Simoes SI, Gaspar MM, Cruz ME, Lindenmaier W, Graca L, Cruz H, Cruz PE (2015) What Makes Umbilical Cord Tissue-Derived Mesenchymal Stromal Cells

Superior Immunomodulators When Compared to Bone Marrow Derived Mesenchymal Stromal Cells?. Stem Cells Int 2015:583984

Barlow S, Brooke G, Chatterjee K, Price G, Pelekanos R, Rossetti T, Doody M, Venter D, Pain S, Gilshenan K, Atkinson K (2008) Comparison of human placenta- and bone marrow-derived multipotent mesenchymal stem cells. Stem Cells Dev 17:1095-1107

Bartel DP (2009) MicroRNAs: Target Recognition and Regulatory Functions. Cell 136:215-233

Beiral HJ, Rodrigues-Ferreira C, Fernandes AM, Gonsalez SR, Mortari NC, Takiya CM, Sorenson MM, Figueiredo-Freitas C, Galina A, Vieyra A (2014) The impact of stem cells on electron fluxes, proton translocation, and ATP synthesis in kidney mitochondria after ischemia/reperfusion. Cell Transplant 23:207-220

Bernardo ME, Fibbe WE (2013) Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell 13:392-402

Bieback K, Hecker A, Kocaomer A, Lannert H, Schallmoser K, Strunk D, Kluter H (2009) Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. Stem Cells 27:2331-2341

Blom TS, Koivusalo M, Kuismanen E, Kostiainen R, Somerharju P, Ikonen E (2001) Mass Spectrometric Analysis Reveals an Increase in Plasma Membrane Polyunsaturated Phospholipid Species upon Cellular Cholesterol Loading. Biochemistry (N Y) 40:14635-14644

Bobrie A, Colombo M, Raposo G, Thery C (2011) Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 12:1659-1668

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185-193

Bonventre JV, Yang L (2011) Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest 121:4210-4221

Bork S, Horn P, Castoldi M, Hellwig I, Ho AD, Wagner W (2011) Adipogenic differentiation of human mesenchymal stromal cells is down-regulated by microRNA-369-5p and up-regulated by microRNA-371. J Cell Physiol 226:2226-2234

Bork S, Pfister S, Witt H, Horn P, Korn B, Ho AD, Wagner W (2010) DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell 9:54-63

Brügger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD (1997) Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. Proc Natl Acad Sci U S A 94:2339-2344

Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C, Camussi G (2009) Mesenchymal Stem Cell-Derived Microvesicles Protect Against Acute Tubular Injury. J Am Soc Nephrol 20:1053-1067

Bruns I, Lucas D, Pinho S, Ahmed J, Lambert MP, Kunisaki Y, Scheiermann C, Schiff L, Poncz M, Bergman A, Frenette PS (2014) Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. Nat Med 20:1315-1320

Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425:841-846

Campos AM, Maciel E, Moreira AS, Sousa B, Melo T, Domingues P, Curado L, Antunes B, Domingues MR, Santos F (2016) Lipidomics of Mesenchymal Stromal Cells: Understanding the Adaptation of Phospholipid Profile in Response to Pro-Inflammatory Cytokines. J Cell Physiol 231:1024-1032

Candini O, Spano C, Murgia A, Grisendi G, Veronesi E, Piccinno MS, Ferracin M, Negrini M, Giacobbi F, Bambi F, Horwitz EM, Conte P, Paolucci P, Dominici M (2015) Mesenchymal Progenitors Aging Highlights a miR-196 Switch Targeting HOXB7 as Master Regulator of Proliferation and Osteogenesis. Stem Cells 33:939-950

Caplan AI (2009) Why are MSCs therapeutic? New data: new insight. J Pathol 217:318-324

Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K (2015) Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes 64:587-592

Castrechini NM, Murthi P, Gude NM, Erwich JJHM, Gronthos S, Zannettino A, Brennecke SP, Kalionis B (2010) Mesenchymal stem cells in human placental chorionic villi reside in a vascular Niche. Placenta 31:203-212

Castro-Manrreza ME, Mayani H, Monroy-Garcia A, Flores-Figueroa E, Chavez-Rueda K, Legorreta-Haquet V, Santiago-Osorio E, Montesinos JJ (2014) Human mesenchymal stromal cells from adult and neonatal sources: a comparative in vitro analysis of their immunosuppressive properties against T cells. Stem Cells Dev 23:1217-1232

Chabannes D, Hill M, Merieau E, Rossignol J, Brion R, Soulillou JP, Anegon I, Cuturi MC (2007) A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. Blood 110:3691-3694

Chang MK, Raggatt L, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, Maylin ER, Ripoll VM, Hume DA, Pettit AR (2008) Osteal Tissue Macrophages Are Intercalated throughout Human and Mouse Bone Lining Tissues and Regulate Osteoblast Function In Vitro and In Vivo. The Journal of Immunology 181:1232-1244

Chen K, Wang D, Du WT, Han ZB, Ren H, Chi Y, Yang SG, Zhu D, Bayard F, Han ZC (2010) Human umbilical cord mesenchymal stem cells hUC-MSCs exert immunosuppressive activities through a PGE2-dependent mechanism. Clin Immunol 135:448-458

Chen L, HolmstrØm K, Qiu W, Ditzel N, Shi K, Hokland L, Kassem M (2014) MicroRNA-34a Inhibits Osteoblast Differentiation and In Vivo Bone Formation of Human Stromal Stem Cells. Stem Cells 32:902-912

Chen W, Huang Y, Han J, Yu L, Li Y, Lu Z, Li H, Liu Z, Shi C, Duan F, Xiao Y (2016) Immunomodulatory effects of mesenchymal stromal cells-derived exosome. Immunol Res 64:831-840

Chen X, Wang C, Yin J, Xu J, Wei J, Zhang Y (2015) Efficacy of Mesenchymal Stem Cell Therapy for Steroid-Refractory Acute Graft-Versus-Host Disease following Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. PLoS One 10:e0136991

Chhabra A, Lechner A, Ueno M, Acharya A, Van Handel B, Wang Y, Iruela-Arispe M , Tallquist M, Mikkola HA (2012) Trophoblasts Regulate the Placental Hematopoietic Niche through PDGF-B Signaling. Developmental Cell 22:651-659

Chinnadurai R, Copland IB, Patel SR, Galipeau J (2014) IDO-Independent Suppression of T Cell Effector Function by IFN-γ–Licensed Human Mesenchymal Stromal Cells. J Immunol 192:1491-1501

Chiurchiu V, Leuti A, Dalli J, Jacobsson A, Battistini L, Maccarrone M, Serhan CN (2016) Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. Sci Transl Med 8:353ra111

Cianci E, Recchiuti A, Trubiani O, Diomede F, Marchisio M, Miscia S, Colas RA, Dalli J, Serhan CN, Romano M (2016) Human Periodontal Stem Cells Release Specialized Proresolving Mediators and Carry Immunomodulatory and Prohealing Properties Regulated by Lipoxins. Stem Cells Translational Medicine 5:20-32

Clark EA, Kalomoiris S, Nolta JA, Fierro FA (2014) Concise review: MicroRNA function in multipotent mesenchymal stromal cells. Stem Cells 32:1074-1082

Cle DV, Santana-Lemos B, Tellechea MF, Prata KL, Orellana MD, Covas DT, Calado RT (2015) Intravenous infusion of allogeneic mesenchymal stromal cells in refractory or relapsed aplastic anemia. Cytotherapy 17:1696-1705

Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. Cell 130:223-233

Collino F, Bruno S, Incarnato D, Dettori D, Neri F, Provero P, Pomatto M, Oliviero S, Tetta C, Quesenberry PJ, Camussi G (2015) AKI Recovery Induced by Mesenchymal Stromal Cell-Derived Extracellular Vesicles Carrying MicroRNAs. J Am Soc Nephrol 26:2349-2360

Consentius C, Reinke P, Volk HD (2015) Immunogenicity of allogeneic mesenchymal stromal cells: what has been seen in vitro and in vivo?. Regen Med 10:305-315

Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A (2006) Human mesenchymal stem cells modulate B-cell functions. Blood 107:367-372

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

Davies LC, Heldring N, Kadri N, Le Blanc K (2017) Mesenchymal Stromal Cell Secretion of Programmed Death-1 Ligands Regulates T Cell Mediated Immunosuppression. Stem Cells 35:766-776

De Luca L, Trino S, Laurenzana I, Simeon V, Calice G, Raimondo S, Podesta M, Santodirocco M, Di Mauro L, La Rocca F, Caivano A, Morano A, Frassoni F, Cilloni D, Del Vecchio L, Musto P (2016) MiRNAs and piRNAs from bone marrow mesenchymal stem cell extracellular vesicles induce cell survival and inhibit cell differentiation of cord blood hematopoietic stem cells: a new insight in transplantation. Oncotarget 7:6676-6692

Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen JF, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC (2007) Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 204:1257-1265

Del Fattore A, Luciano R, Pascucci L, Goffredo BM, Giorda E, Scapaticci M, Fierabracci A, Muraca M (2015) Immunoregulatory Effects of Mesenchymal Stem Cell-Derived Extracellular Vesicles on T Lymphocytes. Cell Transplant 24:2615-2627

Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838-3843

Di Trapani M, Bassi G, Midolo M, Gatti A, Kamga PT, Cassaro A, Carusone R, Adamo A, Krampera M (2016) Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions. Sci Rep 6:24120

Di Trapani M, Bassi G, Ricciardi M, Fontana E, Bifari F, Pacelli L, Giacomello L, Pozzobon M, Feron F, De Coppi P, Anversa P, Fumagalli G, Decimo I, Menard C, Tarte K, Krampera M (2013) Comparative study of immune regulatory properties of stem cells derived from different tissues. Stem Cells Dev 22:2990-3002

Ding L, Morrison SJ (2013) Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature 495:231-235

Ding L, Saunders TL, Enikolopov G, Morrison SJ (2012) Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 481:457-462

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

Du WJ, Chi Y, Yang ZX, Li ZJ, Cui JJ, Song BQ, Li X, Yang SG, Han ZB, Han ZC (2016) Heterogeneity of proangiogenic features in mesenchymal stem cells derived from bone marrow, adipose tissue, umbilical cord, and placenta. Stem Cell Res Ther 7:163

Duffy MM, Pindjakova J, Hanley SA, McCarthy C, Weidhofer GA, Sweeney EM, English K, Shaw G, Murphy JM, Barry FP, Mahon BP, Belton O, Ceredig R, Griffin MD (2011) Mesenchymal stem cell inhibition of T-helper 17 cell- differentiation is triggered by cell-cell contact and mediated by prostaglandin E2 via the EP4 receptor. Eur J Immunol 41:2840-2851

Ebert PJ, Jiang S, Xie J, Li QJ, Davis MM (2009) An endogenous positively selecting peptide enhances mature T cell responses and becomes an autoantigen in the absence of microRNA miR-181a. Nat Immunol 10:1162-1169

Eguchi T, Watanabe K, Hara ES, Ono M, Kuboki T, Calderwood SK (2013) OstemiR: a novel panel of microRNA biomarkers in osteoblastic and osteocytic differentiation from mesencymal stem cells. PLoS One 8:e58796

English K (2013) Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol 91:19-26

English K, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP (2009) Cell contact, prostaglandin E2 and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25Highforkhead box P3+ regulatory T cells. Clinical & Experimental Immunology 156:149-160

Erpicum P, Detry O, Weekers L, Bonvoisin C, Lechanteur C, Briquet A, Beguin Y, Krzesinski JM, Jouret F (2014) Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion. Nephrol Dial Transplant 29:1487-1493

Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M (2011) MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci U S A 108:6139-6144

Espagnolle N, Balguerie A, Arnaud E, Sensebe L, Varin A (2017) CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells. Stem Cell Reports ((Epub ahead of print)

Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS (2004) Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. J Immunol 173:6151-6160

Fazzina R, Iudicone P, Fioravanti D, Bonanno G, Totta P, Zizzari IG, Pierelli L (2016) Potency testing of mesenchymal stromal cell growth expanded in human platelet lysate from different human tissues. Stem Cell Res Ther 7:122-016-0383-3

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497-509

Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, Phillips M, Herrmann RP (2014) A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. Clin Gastroenterol Hepatol 12:64-71

Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP (1968) Heterotopic of bone marrow.Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6:230-247

Frohlich H, Speer N, Poustka A, Beissbarth T (2007) GOSim--an R-package for computation of information theoretic GO similarities between terms and gene products. BMC Bioinformatics 8:166

Fuchs B, Schiller J, Cross MA (2007) Apoptosis-associated changes in the glycerophospholipid composition of hematopoietic progenitor cells monitored by 31P NMR spectroscopy and MALDI-TOF mass spectrometry. Chem Phys Lipids 150:229-238

Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871-1875

Furuichi K, Shintani H, Sakai Y, Ochiya T, Matsushima K, Kaneko S, Wada T (2012) Effects of adipose-derived mesenchymal cells on ischemia–reperfusion injury in kidney. Clinical and Experimental Nephrology 16:679-689

Galipeau J, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, Dominici M, Fibbe WE, Gee AP, Gimble JM, Hematti P, Koh MB, LeBlanc K, Martin I, McNiece IK, Mendicino M, Oh S, Ortiz L, Phinney DG, Planat V, Shi Y, Stroncek DF, Viswanathan S, Weiss DJ, Sensebe L (2016) International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. Cytotherapy 18:151-159

Gandolfo MT, Jang HR, Bagnasco SM, Ko GJ, Agreda P, Satpute SR, Crow MT, King LS, Rabb H (2009) Foxp3+ regulatory T cells participate in repair of ischemic acute kidney injury. Kidney Int 76:717-729

Gao J, Yang T, Han J, Yan K, Qiu X, Zhou Y, Fan Q, Ma B (2011) MicroRNA expression during osteogenic differentiation of human multipotent mesenchymal stromal cells from bone marrow. J Cell Biochem 112:1844-1856

Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK (2005) The placenta is a niche for hematopoietic stem cells. Dev Cell 8:365-375

Ghannam S, Pène J, Torcy-Moquet G, Jorgensen C, Yssel H (2010) Mesenchymal Stem Cells Inhibit Human Th17 Cell Differentiation and Function and Induce a T Regulatory Cell Phenotype. The Journal of Immunology 185:302-312

Gieseke F, Kruchen A, Tzaribachev N, Bentzien F, Dominici M, Muller I (2013) Proinflammatory stimuli induce galectin-9 in human mesenchymal stromal cells to suppress T-cell proliferation. Eur J Immunol 43:2741-2749

Goff LA, Boucher S, Ricupero CL, Fenstermacher S, Swerdel M, Chase LG, Adams CC, Chesnut J, Lakshmipathy U, Hart RP (2008) Differentiating human multipotent mesenchymal stromal cells regulate microRNAs: prediction of microRNA regulation by PDGF during osteogenesis. Exp Hematol 36:1354-1369

Gojo S, Gojo N, Takeda Y, Mori T, Abe H, Kyo S, Hata J, Umezawa A (2003) In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 288:51-59

Gomez-Cabello D, Adrados I, Gamarra D, Kobayashi H, Takatsu Y, Takatsu K, Gil J, Palmero I (2013) DGCR8-mediated disruption of miRNA biogenesis induces cellular senescence in primary fibroblasts. Aging Cell 12:923-931

Gooch A, Doty J, Flores J, Swenson L, Toegel FE, Reiss GR, Lange C, Zander AR, Hu Z, Poole S, Zhang P, Westenfelder CH (2008) Initial report on a phase I clinical trial: Prevention and treatment of post-operative Acute Kidney Injury with allogeneic Mesenchymal Stem Cells in patients who require on-pump cardiac surgery. Cell Ther Transplant. Cellular Therapy and Transplantation 1:31-35

Gotherstrom C, Westgren M, Shaw SW, Astrom E, Biswas A, Byers PH, Mattar CN, Graham GE, Taslimi J, Ewald U, Fisk NM, Yeoh AE, Lin JL, Cheng PJ, Choolani M, Le Blanc K, Chan JK (2014) Pre- and postnatal transplantation of fetal mesenchymal stem cells in osteogenesis imperfecta: a two-center experience. Stem Cells Transl Med 3:255-264

Gouveia de Andrade AV, Bertolino G, Riewaldt J, Bieback K, Karbanova J, Odendahl M, Bornhauser M, Schmitz M, Corbeil D, Tonn T (2015) Extracellular vesicles secreted by bone marrow- and adipose tissue-derived mesenchymal stromal cells fail to suppress lymphocyte proliferation. Stem Cells Dev 24:1374-1376

Greenbaum A, Hsu YM, Day RB, Schuettpelz LG, Christopher MJ, Borgerding JN, Nagasawa T, Link DC (2013) CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature 495:227-230

Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ (2006) miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 34:D140-4

Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM (2001) Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 189:54-63

Hackl M, Brunner S, Fortschegger K, Schreiner C, Micutkova L, Muck C, Laschober GT, Lepperdinger G, Sampson N, Berger P, Herndler-Brandstetter D, Wieser M, Kuhnel H, Strasser A, Rinnerthaler M, Breitenbach M, Mildner M, Eckhart L, Tschachler E, Trost A, Bauer JW, Papak C, Trajanoski Z, Scheideler M, Grillari-Voglauer R, Grubeck-Loebenstein B, Jansen-Durr P, Grillari J (2010) miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. Aging Cell 9:291-296

Haimi P, Uphoff A, Hermansson M, Somerharju P (2006) Software tools for analysis of mass spectrometric lipidome data. Anal Chem 78:8324-8331

Ham O, Song BW, Lee SY, Choi E, Cha MJ, Lee CY, Park JH, Kim IK, Chang W, Lim S, Lee CH, Kim S, Jang Y, Hwang KC (2012) The role of microRNA-23b in the differentiation of MSC into chondrocyte by targeting protein kinase A signaling. Biomaterials 33:4500-4507

Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB,Jr, Reisman MA, Schaer GL, Sherman W (2009) A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 54:2277-2286

Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. Experimental Cell Research 37:614-636

He J, Wang Y, Sun S, Yu M, Wang C, Pei X, Zhu B, Wu J, Zhao W (2012) Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model. Nephrology 17:493-500

Heo JS, Choi Y, Kim HS, Kim HO (2016) Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. Int J Mol Med 37:115-125

Hermankova B, Zajicova A, Javorkova E, Chudickova M, Trosan P, Hajkova M, Krulova M, Holan V (2016) Suppression of IL-10 production by activated B cells via a cell contact-dependent cyclooxygenase-2 pathway upregulated in IFN-gamma-treated mesenchymal stem cells. Immunobiology 221:129-136

Hermansson M, Uphoff A, Käkelä R, Somerharju P (2005) Automated quantitative analysis of complex lipidomes by liquid chromatography/mass spectrometry. Anal Chem 77:2166-2175

Herrera MB, Bussolati B, Bruno S, Morando L, Mauriello-Romanazzi G, Sanavio F, Stamenkovic I, Biancone L, Camussi G (2007) Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. Kidney Int 72:430-441

Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G (2004) Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med 14:1035-1041

Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD (2011) Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 134:1790-1807

Hoogduijn MJ (2015) Are mesenchymal stromal cells immune cells?. Arthritis Res Ther 17:88-015-0596-3

Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, Kopp HG, Shido K, Petit I, Yanger K, James D, Witte L, Zhu Z, Wu Y, Pytowski B, Rosenwaks Z, Mittal V, Sato TN, Rafii S (2009) Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. Cell Stem Cell 4:263-274

Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44-57

Huang F, Chen M, Chen W, Gu J, Yuan J, Xue Y, Dang J, Su W, Wang J, Zadeh HH, He X, Rong L, Olsen N, Zheng SG (2017) Human Gingiva-Derived Mesenchymal Stem Cells Inhibit Xeno-Graft-versus-Host Disease via CD39-CD73-Adenosine and IDO Signals. Front Immunol 8:68

Huang J, Zhao L, Xing L, Chen D (2010) MicroRNA-204 Regulates Runx2 Protein Expression and Mesenchymal Progenitor Cell Differentiation. Stem Cells 28:357-364

Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, Li H, Han Q, Zhao RC (2012) Upregulation of miR-22 promotes osteogenic differentiation and inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. Stem Cells Dev 21:2531-2540

Imberti B, Morigi M, Tomasoni S, Rota C, Corna D, Longaretti L, Rottoli D, Valsecchi F, Benigni A, Wang J, Abbate M, Zoja C, Remuzzi G (2007) Insulin-like growth factor-1 sustains stem cell mediated renal repair. J Am Soc Nephrol 18:2921-2928

Ivanova-Todorova E, Bochev I, Mourdjeva M, Dimitrov R, Bukarev D, Kyurkchiev S, Tivchev P, Altunkova I, Kyurkchiev DS (2009) Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunol Lett 126:37-42

Ivanovska I, Ball AS, Diaz RL, Magnus JF, Kibukawa M, Schelter JM, Kobayashi SV, Lim L, Burchard J, Jackson AL, Linsley PS, Cleary MA (2008) MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol Cell Biol 28:2167-2174

Ivey KN, Muth A, Arnold J, King FW, Yeh R, Fish JE, Hsiao EC, Schwartz RJ, Conklin BR, Bernstein HS, Srivastava D (2008) MicroRNA Regulation of Cell Lineages in Mouse and Human Embryonic Stem Cells. Cell Stem Cell 2:219-229

Jackson MV, Morrison TJ, Doherty DF, McAuley DF, Matthay MA, Kissenpfennig A, O'Kane C,M., Krasnodembskaya AD (2016) Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. Stem Cells 34:2210-2223

Jang HR, Park JH, Kwon GY, Lee JE, Huh W, Jin HJ, Choi SJ, Oh W, Oh HY, Kim YG (2014) Effect of preemptive treatment with human umbilical cord blood-derived mesenchymal stem cells on the development of renal ischemia-reperfusion injury in mice. Am J Physiol Renal Physiol 307:F1149-61

Jensen AR, Manning MM, Khaneki S, Drucker NA, Markel TA (2016) Harvest tissue source does not alter the protective power of stromal cell therapy after intestinal ischemia and reperfusion injury. J Surg Res 204:361-370

Jeon YJ, Kim J, Cho JH, Chung HM, Chae JI (2016) Comparative Analysis of Human Mesenchymal Stem Cells Derived From Bone Marrow, Placenta, and Adipose Tissue as Sources of Cell Therapy. J Cell Biochem 117:1112-1125

Jiang D, Muschhammer J, Qi Y, Kugler A, de Vries JC, Saffarzadeh M, Sindrilaru A, Beken SV, Wlaschek M, Kluth MA, Ganss C, Frank NY, Frank MH, Preissner KT, Scharffetter-Kochanek K (2016) Suppression of Neutrophil-Mediated Tissue Damage-A Novel Skill of Mesenchymal Stem Cells. Stem Cells 34:2393-2406

Jiang X, Zhang Y, Liu B, Zhang S, Wu Y, Yu X, Mao N (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105:4120-4126

Jin HJ, Bae YK, Kim M, Kwon SJ, Jeon HB, Choi SJ, Kim SW, Yang YS, Oh W, Chang JW (2013) Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. Int J Mol Sci 14:17986-18001

Juhl M, Tratwal J, Follin B, Sondergaard RH, Kirchhoff M, Ekblond A, Kastrup J, Haack-Sorensen M (2016) Comparison of clinical grade human platelet lysates for cultivation of mesenchymal stromal cells from bone marrow and adipose tissue. Scand J Clin Lab Invest 76:93-104

Käkelä R, Somerharju P, Tyynela J (2003) Analysis of phospholipid molecular species in brains from patients with infantile and juvenile neuronal-ceroid lipofuscinosis using liquid chromatography-electrospray ionization mass spectrometry. J Neurochem 84:1051-1065

Kang CM, Kim H, Song JS, Choi BJ, Kim SO, Jung HS, Moon SJ, Choi HJ (2016) Genetic Comparison of Stemness of Human Umbilical Cord and Dental Pulp. Stem Cells Int 2016:3453890

Kang T, Lu W, Xu W, Anderson L, Bacanamwo M, Thompson W, Chen YE, Liu D (2013) MicroRNA-27 (miR-27) Targets Prohibitin and Impairs Adipocyte Differentiation and Mitochondrial Function in Human Adipose-derived Stem Cells. Journal of Biological Chemistry 288:34394-34402

Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM (2010) miR-29 Modulates Wnt Signaling in Human Osteoblasts through a Positive Feedback Loop. Journal of Biological Chemistry 285:25221-25231

Karbiener M, Fischer C, Nowitsch S, Opriessnig P, Papak C, Ailhaud G, Dani C, Amri EZ, Scheideler M (2009) microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. Biochem Biophys Res Commun 390:247-251

Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, Bulte JW, Petrou P, Ben-Hur T, Abramsky O, Slavin S (2010) Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 67:1187-1194

Katayama Y, Battista M, Kao W, Hidalgo A, Peired AJ, Thomas SA, Frenette PS (2006) Signals from the Sympathetic Nervous System Regulate Hematopoietic Stem Cell Egress from Bone Marrow. Cell 124:407-421

Katz JB, Muller AJ, Prendergast GC (2008) Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. Immunol Rev 222:206-221

Kerkelä E, Laitinen A, Rabina J, Valkonen S, Takatalo M, Larjo A, Veijola J, Lampinen M, Siljander P, Lehenkari P, Alfthan K, Laitinen S (2016) Adenosinergic Immunosuppression by Human Mesenchymal Stromal Cells Requires Co-Operation with T cells. Stem Cells 34:781-790

Kern S, Eichler H, Stoeve J, KlÃ¼ter H, Bieback K (2006) Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue. Stem Cells 24:1294-1301

Ketterl N, Brachtl G, Schuh C, Bieback K, Schallmoser K, Reinisch A, Strunk D (2015) A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance. Stem Cell Res Ther 6:236-015-0233-8

Kim YJ, Hwang SJ, Bae YC, Jung JS (2009a) MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 27:3093-3102

Kim YJ, Bae SW, Yu SS, Bae YC, Jung JS (2009b) miR-196a Regulates Proliferation and Osteogenic Differentiation in Mesenchymal Stem Cells Derived From Human Adipose Tissue. Journal of Bone and Mineral Research 24:816-825

Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, Harley CB, Aviv A (2010) Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. Nat Protocols 5:1596-1607

Kinsey GR, Huang L, Vergis AL, Li L, Okusa MD (2010) Regulatory T cells contribute to the protective effect of ischemic preconditioning in the kidney. Kidney Int 77:771-780

Koivusalo M, Haimi P, Heikinheimo L, Kostiainen R, Somerharju P (2001) Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response. J Lipid Res 42:663-672

Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Sztainberg Y, Tesio M, Samstein RM, Goichberg P, Spiegel A, Elson A, Lapidot T (2006) Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med 12:657-664

Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F (2006) Role for Interferon-? in the Immunomodulatory Activity of Human Bone Marrow Mesenchymal Stem Cells. Stem Cells 24:386-398

Kroesen BJ, Teteloshvili N, Smigielska-Czepiel K, Brouwer E, Boots AM, van den Berg A, Kluiver J (2015) Immuno-miRs: critical regulators of T-cell development, function and ageing. Immunology 144:1-10

Laitinen A, Oja S, Kilpinen L, Kaartinen T, Moller J, Laitinen S, Korhonen M, Nystedt J (2016a) A robust and reproducible animal serum-free culture method for clinical-grade bone marrow-derived mesenchymal stromal cells. Cytotechnology 68:891-906

Laitinen A, Lampinen M, Liedtke S, Kilpinen L, Kerkelä E, Sarkanen J, Heinonen T, Kogler G, Laitinen S (2016b) The effects of culture conditions on the functionality of efficiently obtained mesenchymal stromal cells from human cord blood. Cytotherapy 18:423-437

Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ, Canadian Critical Care Trials Group (2012) Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One 7:e47559

Le Blanc K, Mougiakakos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12:383-396

Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O, Developmental Committee of the European Group for Blood and Marrow Transplantation (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371:1579-1586

Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31:890-896

Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY, STARTING collaborators (2010) A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells 28:1099-1106

Lee JM, Jung J, Lee H, Jeong SJ, Cho KJ, Hwang S, Kim GJ (2012) Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. Int Immunopharmacol 13:219-224

Lempiäinen J, Finckenberg P, Levijoki J, Mervaala E (2012) AMPK activator AICAR ameliorates ischaemia reperfusion injury in the rat kidney. Br J Pharmacol 166:1905-1915

Lepelletier Y, Lecourt S, Renand A, Arnulf B, Vanneaux V, Fermand JP, Menasche P, Domet T, Marolleau JP, Hermine O, Larghero J (2010) Galectin-1 and semaphorin-3A are two soluble factors conferring T-cell immunosuppression to bone marrow mesenchymal stem cell. Stem Cells Dev 19:1075-1079

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15-20

Li H, Li T, Wang S, Wei J, Fan J, Li J, Han Q, Liao L, Shao C, Zhao RC (2013a) miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 10:313-324

Li J, Dong J, Zhang ZH, Zhang DC, You XY, Zhong Y, Chen MS, Liu SM (2013b) miR-10a restores human mesenchymal stem cell differentiation by repressing KLF4. J Cell Physiol 228:2324-2336

Li X, Bai J, Ji X, Li R, Xuan Y, Wang Y (2014) Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. Int J Mol Med 34:695-704

Li W, Ren G, Huang Y, Su J, Han Y, Li J, Chen X, Cao K, Chen Q, Shou P, Zhang L, Yuan ZR, Roberts AI, Shi S, Le AD, Shi Y (2012) Mesenchymal stem cells: a double-edged sword in regulating immune responses. Cell Death Differ 19:1505-1513

Liotta F, Angeli R, Cosmi L, Fili L, Manuelli C, Frosali F, Mazzinghi B, Maggi L, Pasini A, Lisi V, Santarlasci V, Consoloni L, Angelotti ML, Romagnani P, Parronchi P, Krampera M, Maggi E, Romagnani S, Annunziato F (2008) Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing Notch signaling. Stem Cells 26:279-289

Liu H, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X (2012a) The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS One 7:e34608

Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, Yan C (2014) Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. Microvasc Res 92:10-18

Liu K, Chen Y, Zeng Y, Xu L, Liu D, Chen H, Zhang X, Han W, Wang Y, Zhao T, Wang J, Wang J, Han Q, Zhao C, Huang X (2011a) Coinfusion of mesenchymal stromal cells facilitates platelet recovery without increasing leukemia recurrence in haploidentical hematopoietic stem cell transplantation: a randomized, controlled clinical study. Stem Cells Dev 20:1679-1685

Liu L, Wang Y, Fan H, Zhao X, Liu D, Hu Y, Kidd AR, Bao J, Hou Y (2012b) MicroRNA-181a Regulates Local Immune Balance by Inhibiting Proliferation and Immunosuppressive Properties of Mesenchymal Stem Cells. Stem Cells 30:1756-1770

Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B, Luo H, Tang L, Kong X, Chen X, Liu N, Ding Y, Jin Y (2011b) MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 29:1804-1816

Llufriu S, Sepulveda M, Blanco Y, Marin P, Moreno B, Berenguer J, Gabilondo I, Martinez-Heras E, Sola-Valls N, Arnaiz JA, Andreu EJ, Fernandez B, Bullich S, Sanchez-Dalmau B, Graus F,

Villoslada P, Saiz A (2014) Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. PLoS One 9:e113936

Lo Sicco C, Reverberi D, Balbi C, Ulivi V, Principi E, Pascucci L, Becherini P, Bosco MC, Varesio L, Franzin C, Pozzobon M, Cancedda R, Tasso R (2017) Mesenchymal Stem Cell-Derived Extracellular Vesicles as Mediators of Anti-Inflammatory Effects: Endorsement of Macrophage Polarization. Stem Cells Transl Med (Epub ahead of print)

Lu TP, Lee CY, Tsai MH, Chiu YC, Hsiao CK, Lai LC, Chuang EY (2012) miRSystem: an integrated system for characterizing enriched functions and pathways of microRNA targets. PLoS One 7:e42390

Luan X, Li G, Wang G, Wang F, Lin Y (2013) Human placenta-derived mesenchymal stem cells suppress T cell proliferation and support the culture expansion of cord blood CD34(+) cells: a comparison with human bone marrow-derived mesenchymal stem cells. Tissue Cell 45:32-38

Luzi E, Marini F, Sala SC, Tognarini I, Galli G, Brandi ML (2008) Osteogenic Differentiation of Human Adipose Tissue-Derived Stem Cells Is Modulated by the miR-26a Targeting of the SMAD1 Transcription Factor. Journal of Bone and Mineral Research 23:287-295

Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M (2013) Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229:176-185

Maragkakis M, Alexiou P, Papadopoulos GL, Reczko M, Dalamagas T, Giannopoulos G, Goumas G, Koukis E, Kourtis K, Simossis VA, Sethupathy P, Vergoulis T, Koziris N, Sellis T, Tsanakas P, Hatzigeorgiou AG (2009) Accurate microRNA target prediction correlates with protein repression levels. BMC Bioinformatics 10:295-2105-10-295

Matic I, Matthews BG, Wang X, Dyment NA, Worthley DL, Rowe DW, Grcevic D, Kalajzic I (2016) Quiescent Bone Lining Cells Are a Major Source of Osteoblasts During Adulthood. Stem Cells 34:2930-2942

Mattar P, Bieback K (2015) Comparing the Immunomodulatory Properties of Bone Marrow, Adipose Tissue, and Birth-Associated Tissue Mesenchymal Stromal Cells. Front Immunol 6:560

Matula Z, Nemeth A, Lorincz P, Szepesi A, Brozik A, Buzas EI, Low P, Nemet K, Uher F, Urban VS (2016) The Role of Extracellular Vesicle and Tunneling Nanotube-Mediated Intercellular Cross-Talk Between Mesenchymal Stem Cells and Human Peripheral T Cells. Stem Cells Dev 25:1818-1832

Maumus M, Peyrafitte JA, D'Angelo R, Fournier-Wirth C, Bouloumie A, Casteilla L, Sengenes C, Bourin P (2011) Native human adipose stromal cells: localization, morphology and phenotype. Int J Obes (Lond) 35:1141-1153

Mei Y, Bian C, Li J, Du Z, Zhou H, Yang Z, Zhao RC (2013) miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. J Cell Biochem 114:1374-1384

Meirelles LdS, Chagastelles PC, Nardi NB (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204-2213

Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D (2004) Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase–mediated tryptophan degradation. Blood 103:4619-4621

Melief SM, Zwaginga JJ, Fibbe WE, Roelofs H (2013) Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their bone marrow-derived counterparts. Stem Cells Transl Med 2:455-463

Mellor AL, Munn DH (2004) Ido expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 4:762-774

Menard C, Pacelli L, Bassi G, Dulong J, Bifari F, Bezier I, Zanoncello J, Ricciardi M, Latour M, Bourin P, Schrezenmeier H, Sensebe L, Tarte K, Krampera M (2013) Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: standardization of immune quality controls. Stem Cells Dev 22:1789-1801

Mendez-Ferrer S, Lucas D, Battista M, Frenette PS (2008) Haematopoietic stem cell release is regulated by circadian oscillations. Nature 452:442-447

Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS (2010) Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466:829-834

Mendonca MV, Larocca TF, de Freitas Souza BS, Villarreal CF, Silva LF, Matos AC, Novaes MA, Bahia CM, de Oliveira Melo Martinez AC, Kaneto CM, Furtado SB, Sampaio GP, Soares MB, dos Santos RR (2014) Safety and neurological assessments after autologous transplantation of bone marrow mesenchymal stem cells in subjects with chronic spinal cord injury. Stem Cell Res Ther 5:126

Mennan C, Brown S, McCarthy H, Mavrogonatou E, Kletsas D, Garcia J, Balain B, Richardson J, Roberts S (2016) Mesenchymal stromal cells derived from whole human umbilical cord exhibit similar properties to those derived from Wharton's jelly and bone marrow. FEBS Open Bio 6:1054-1066

Mikkola HK, Orkin SH (2006) The journey of developing hematopoietic stem cells. Development 133:3733-3744

Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM (2006) Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 24:376-385

Montespan F, Deschaseaux F, Sensebe L, Carosella ED, Rouas-Freiss N (2014) Osteodifferentiated mesenchymal stem cells from bone marrow and adipose tissue express HLA-G and display immunomodulatory properties in HLA-mismatched settings: implications in bone repair therapy. J Immunol Res 2014:230346

Morigi M, Introna M, Imberti B, Corna D, Abbate M, Rota C, Rottoli D, Benigni A, Perico N, Zoja C, Rambaldi A, Remuzzi A, Remuzzi G (2008) Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. Stem Cells 26:2075-2082

Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M, Remuzzi G (2004) Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. J Am Soc Nephrol 15:1794-1804

Morrison SJ, Scadden DT (2014) The bone marrow niche for haematopoietic stem cells. Nature 505:327-334

Mougiakakos D, Jitschin R, Johansson CC, Okita R, Kiessling R, Le Blanc K (2011) The impact of inflammatory licensing on heme oxygenase-1-mediated induction of regulatory T cells by human mesenchymal stem cells. Blood 117:4826-4835

Najar M, Raicevic G, Fayyad-Kazan H, De Bruyn C, Bron D, Toungouz M, Lagneaux L (2013) Impact of different mesenchymal stromal cell types on T-cell activation, proliferation and migration. Int Immunopharmacol 15:693-702

Najar M, Raicevic G, Fayyad-Kazan H, De Bruyn C, Bron D, Toungouz M, Lagneaux L (2012) Immune-related antigens, surface molecules and regulatory factors in human-derived mesenchymal stromal cells: the expression and impact of inflammatory priming. Stem Cell Rev 8:1188-1198

Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ (2009) Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. Nature 460:259-263

Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, Mahoney JE, Park SY, Lu J, Protopopov A, Silberstein LE (2013) Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nat Cell Biol 15:533-543

Obermajer N, Popp FC, Soeder Y, Haarer J, Geissler EK, Schlitt HJ, Dahlke MH (2014) Conversion of Th17 into IL-17A(neg) regulatory T cells: a novel mechanism in prolonged allograft survival promoted by mesenchymal stem cell-supported minimized immunosuppressive therapy. J Immunol 193:4988-4999

Oh KW, Moon C, Kim HY, Oh SI, Park J, Lee JH, Chang IY, Kim KS, Kim SH (2015) Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem Cells Transl Med 4:590-597

Omatsu Y, Sugiyama T, Kohara H, Kondoh G, Fujii N, Kohno K, Nagasawa T (2010) The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity 33:387-399

Ong S, Lee WH, Kodo K, Wu JC (2015) MicroRNA-mediated regulation of differentiation and trans-differentiation in stem cells. Adv Drug Deliv Rev 88:3-15

Oskowitz AZ, Lu J, Penfornis P, Ylostalo J, McBride J, Flemington EK, Prockop DJ, Pochampally R (2008) Human multipotent stromal cells from bone marrow and microRNA: regulation of differentiation and leukemia inhibitory factor expression. Proc Natl Acad Sci U S A 105:18372-18377

Pachon-Pena G, Yu G, Tucker A, Wu X, Vendrell J, Bunnell BA, Gimble JM (2011) Stromal stem cells from adipose tissue and bone marrow of age-matched female donors display distinct immunophenotypic profiles. J Cell Physiol 226:843-851

Pandey AC, Semon JA, Kaushal D, O'Sullivan RP, Glowacki J, Gimble JM, Bunnell BA (2011) MicroRNA profiling reveals age-dependent differential expression of nuclear factor kappaB and mitogen-activated protein kinase in adipose and bone marrow-derived human mesenchymal stem cells. Stem Cell Res Ther 2:49

Park H, Haynes CA, Nairn AV, Kulik M, Dalton S, Moremen K, Merrill AH,Jr (2010) Transcript profiling and lipidomic analysis of ceramide subspecies in mouse embryonic stem cells and embryoid bodies. J Lipid Res 51:480-489

Pers YM, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, Sensebe L, Casteilla L, Fleury S, Bourin P, Noel D, Canovas F, Cyteval C, Lisignoli G, Schrauth J, Haddad D, Domergue S, Noeth U, Jorgensen C, ADIPOA Consortium (2016) Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial. Stem Cells Transl Med 5:847-856

Petrou P, Gothelf Y, Argov Z, Gotkine M, Levy YS, Kassis I, Vaknin-Dembinsky A, Ben-Hur T, Offen D, Abramsky O, Melamed E, Karussis D (2016) Safety and Clinical Effects of Mesenchymal Stem Cells Secreting Neurotrophic Factor Transplantation in Patients With Amyotrophic Lateral Sclerosis: Results of Phase 1/2 and 2a Clinical Trials. JAMA Neurol 73:337-344Pinho S, Lacombe J, Hanoun M, Mizoguchi T, Bruns I, Kunisaki Y, Frenette PS (2013) PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J Exp Med 210:1351-1367

Pinzon N, Li B, Martinez L, Sergeeva A, Presumey J, Apparailly F, Seitz H (2017) microRNA target prediction programs predict many false positives. Genome Res 27:234-245

Pires AO, Mendes-Pinheiro B, Teixeira FG, Anjo SI, Ribeiro-Samy S, Gomes ED, Serra SC, Silva NA, Manadas B, Sousa N, Salgado AJ (2016) Unveiling the Differences of Secretome of Human Bone Marrow Mesenchymal Stem Cells, Adipose Tissue-Derived Stem Cells, and Human Umbilical Cord Perivascular Cells: A Proteomic Analysis. Stem Cells Dev 25:1073-1083

Plotnikov EY, Khryapenkova TG, Vasileva AK, Marey MV, Galkina SI, Isaev NK, Sheval EV, Polyakov VY, Sukhikh GT, Zorov DB (2008) Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. J Cell Mol Med 12

Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB (2010) Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. PLoS One 5:e9016

Puissant B, Barreau C, Bourin P, Clavel C, Corre J, Bousquet C, Taureau C, Cousin B, Abbal M, Laharrague P, Penicaud L, Casteilla L, Blancher A (2005) Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. Br J Haematol 129:118-129

Qi S, Wu D (2013) Bone marrow-derived mesenchymal stem cells protect against cisplatin-induced acute kidney injury in rats by inhibiting cell apoptosis. Int J Mol Med 32:1262-1272

Qian H, Yang H, Xu W, Yan Y, Chen Q, Zhu W, Cao H, Yin Q, Zhou H, Mao F, Chen Y (2008) Bone marrow mesenchymal stem cells ameliorate rat acute renal failure by differentiation into renal tubular epithelial-like cells. Int J Mol Med 22:325-332

Ragni E, Banfi F, Barilani M, Cherubini A, Parazzi V, Larghi P, Dolo V, Bollati V, Lazzari L (2017) Extracellular Vesicle-Shuttled mRNA in Mesenchymal Stem Cell Communication. Stem Cells (Epub ahead of print)

Ragni E, Montemurro T, Montelatici E, Lavazza C, Vigano M, Rebulla P, Giordano R, Lazzari L (2013) Differential microRNA signature of human mesenchymal stem cells from different sources reveals an "environmental-niche memory" for bone marrow stem cells. Exp Cell Res 319:1562-1574

Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, Toungouz M, Lagneaux L (2011a) The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. Cell Immunol 270:207-216

Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, Toungouz M, Lagneaux L (2011b) The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. Cell Immunol 270:207-216

Raposo G, Stoorvogel W (2013) Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 200:373-383

Rashedi I, Gomez-Aristizabal A, Wang XH, Viswanathan S, Keating A (2017) TLR3 or TLR4 Activation Enhances Mesenchymal Stromal Cell-Mediated Treg Induction via Notch Signaling. Stem Cells 35:265-275

Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN (2011) MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. The FASEB Journal 25:544-560

Reinders ME, Dreyer GJ, Bank JR, Roelofs H, Heidt S, Roelen DL, Zandvliet ML, Huurman VA, Fibbe WE, van Kooten C, Claas FH, Rabelink TJ, de Fijter JW (2015) Safety of allogeneic bone marrow derived mesenchymal stromal cell therapy in renal transplant recipients: the neptune study. J Transl Med 13:344-015-0700-0

Reinisch A, Etchart N, Thomas D, Hofmann NA, Fruehwirth M, Sinha S, Chan CK, Senarath-Yapa K, Seo EY, Wearda T, Hartwig UF, Beham-Schmid C, Trajanoski S, Lin Q, Wagner W, Dullin C, Alves F, Andreeff M, Weissman IL, Longaker MT, Schallmoser K, Majeti R, Strunk D (2015) Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood 125:249-260

Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, Shi Y (2010) Inflammatory Cytokine-Induced Intercellular Adhesion Molecule-1 and Vascular Cell

Adhesion Molecule-1 in Mesenchymal Stem Cells Are Critical for Immunosuppression. The Journal of Immunology 184:2321-2328

Ribeiro A, Laranjeira P, Mendes S, Velada I, Leite C, Andrade P, Santos F, Henriques A, Graos M, Cardoso CM, Martinho A, Pais M, da Silva CL, Cabral J, Trindade H, Paiva A (2013) Mesenchymal stem cells from umbilical cord matrix, adipose tissue and bone marrow exhibit different capability to suppress peripheral blood B, natural killer and T cells. Stem Cell Res Ther 4:125

Robin C, Bollerot K, Mendes S, Haak E, Crisan M, Cerisoli F, Lauw I, Kaimakis P, Jorna R, Vermeulen M, Kayser M, van der Linden R, Imanirad P, Verstegen M, Nawaz-Yousaf H, Papazian N, Steegers E, Cupedo T, Dzierzak E (2009) Human placenta is a potent hematopoietic niche containing hematopoietic stem and progenitor cells throughout development. Cell Stem Cell 5:385-395

Roemeling-van Rhijn M, Khairoun M, Korevaar SS, Lievers E, Leuning DG, Ijzermans JN, Betjes MG, Genever PG, van Kooten C, de Fijter HJ, Rabelink TJ, Baan CC, Weimar W, Roelofs H, Hoogduijn MJ, Reinders ME (2013a) Human Bone Marrow- and Adipose Tissue-derived Mesenchymal Stromal Cells are Immunosuppressive In vitro and in a Humanized Allograft Rejection Model. J Stem Cell Res Ther Suppl 6:20780

Roemeling-van Rhijn M, Reinders ME, Franquesa M, Engela AU, Korevaar SS, Roelofs H, Genever PG, Ijzermans JN, Betjes MG, Baan CC, Weimar W, Hoogduijn MJ (2013b) Human Allogeneic Bone Marrow and Adipose Tissue Derived Mesenchymal Stromal Cells Induce CD8+ Cytotoxic T Cell Reactivity. J Stem Cell Res Ther 3:004

Rosado MM, Bernardo ME, Scarsella M, Conforti A, Giorda E, Biagini S, Cascioli S, Rossi F, Guzzo I, Vivarelli M, Dello Strologo L, Emma F, Locatelli F, Carsetti R (2015) Inhibition of B-cell proliferation and antibody production by mesenchymal stromal cells is mediated by T cells. Stem Cells Dev 24:93-103

Roson-Burgo B, Sanchez-Guijo F, Del Canizo C, De Las Rivas J (2014) Transcriptomic portrait of human Mesenchymal Stromal/Stem Cells isolated from bone marrow and placenta. BMC Genomics 15:910-2164-15-910

Ryan JM, Barry F, Murphy JM, Mahon BP (2007) Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 149:353-363

Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131:324-336

Saldanha-Araujo F, Ferreira FI, Palma PV, Araujo AG, Queiroz RH, Covas DT, Zago MA, Panepucci RA (2011) Mesenchymal stromal cells up-regulate CD39 and increase adenosine production to suppress activated T-lymphocytes. Stem Cell Res 7:66-74

Satti HS, Waheed A, Ahmed P, Ahmed K, Akram Z, Aziz T, Satti TM, Shahbaz N, Khan MA, Malik SA (2016) Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study. Cytotherapy 18:518-522

Schellenberg A, Lin Q, Schuler H, Koch CM, Joussen S, Denecke B, Walenda G, Pallua N, Suschek CV, Zenke M, Wagner W (2011) Replicative senescence of mesenchymal stem cells causes DNA-methylation changes which correlate with repressive histone marks. Aging (Albany NY) 3:873-888

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Meth 9:671-675

Schoeniger A, Adolph S, Fuhrmann H, Schumann J (2011) The Impact of Membrane Lipid Composition on Macrophage Activation in the Immune Defense against Rhodococcus equi and Pseudomonas aeruginosa. Int J Mol Sci 12:7510-7528

Schoolmeesters A, Eklund T, Leake D, Vermeulen A, Smith Q, Force Aldred S, Fedorov Y (2009) Functional profiling reveals critical role for miRNA in differentiation of human mesenchymal stem cells. PLoS One 4:e5605

Schrier RW, Wang W, Poole B, Mitra A (2004) Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. J Clin Invest 114:5-14

Schuler PJ, Saze Z, Hong CS, Muller L, Gillespie DG, Cheng D, Harasymczuk M, Mandapathil M, Lang S, Jackson EK, Whiteside TL (2014) Human CD4+ CD39+ regulatory T cells produce adenosine upon co-expression of surface CD73 or contact with CD73+ exosomes or CD73+ cells. Clin Exp Immunol 177:531-543

Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N (2008) Widespread changes in protein synthesis induced by microRNAs. Nature 455:58-63

Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F (2008) Human Leukocyte Antigen-G5 Secretion by Human Mesenchymal Stem Cells Is Required to Suppress T Lymphocyte and Natural Killer Function and to Induce CD4+CD25highFOXP3+ Regulatory T Cells. Stem Cells 26:212-222

Seo-Mayer PW, Thulin G, Zhang L, Alves DS, Ardito T, Kashgarian M, Caplan MJ (2011) Preactivation of AMPK by metformin may ameliorate the epithelial cell damage caused by renal ischemia. Am J Physiol Renal Physiol 301:F1346-57

Sepulveda JC, Tome M, Fernandez ME, Delgado M, Campisi J, Bernad A, Gonzalez MA (2014) Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model. Stem Cells 32:1865-1877

Serhan CN, Chiang N, Dalli J (2015) The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol 27:200-215

Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M (2006) In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc 1:2856-2860

Shi M, Zhang Z, Xu R, Lin H, Fu J, Zou Z, Zhang A, Shi J, Chen L, Lv S, He W, Geng H, Jin L, Liu Z, Wang FS (2012) Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl Med 1:725-731

Si X, Liu X, Li J, Wu X (2015) Transforming growth factor-beta1 promotes homing of bone marrow mesenchymal stem cells in renal ischemia-reperfusion injury. Int J Clin Exp Pathol 8:12368-12378

Sioud M, Mobergslien A, Boudabous A, Floisand Y (2011) Mesenchymal stem cell-mediated T cell suppression occurs through secreted galectins. Int J Oncol 38:385-390

Smyth GK (2005) Limma: linear models for microarray data. In: Gentleman R, Carey V, Duboit S, Irizarry R, Huber W (eds) Bioinformatics and Computational Biology Solutions using R and Bioconductor. Springer, New Yourk, pp 397-420

Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M (2006) Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 24:74-85

Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F (2008) Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Ther 15:730-738

Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L (2009) MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. Blood 113:6576-6583

Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L (2008) Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111:1327-1333

Spees JL, Lee RH, Gregory CA (2016) Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Research & Therapy 7:1-13

Stables MJ, Gilroy DW (2011) Old and new generation lipid mediators in acute inflammation and resolution. Prog Lipid Res 50:35-51

Stenderup K, Justesen J, Clausen C, Kassem M (2003) Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells,. Bone 33:919-926

Stubbendorff M, Deuse T, Hua X, Phan TT, Bieback K, Atkinson K, Eiermann TH, Velden J, Schroder C, Reichenspurner H, Robbins RC, Volk HD, Schrepfer S (2013) Immunological properties of extraembryonic human mesenchymal stromal cells derived from gestational tissue. Stem Cells Dev 22:2619-2629

Sugiyama T, Kohara H, Noda M, Nagasawa T (2006) Maintenance of the Hematopoietic Stem Cell Pool by CXCL12-CXCR4 Chemokine Signaling in Bone Marrow Stromal Cell Niches. Immunity 25:977-988

Sullards MC, Merrill AH, Jr. (2001) Analysis of Sphingosine 1-Phosphate, Ceramides, and Other Bioactive Sphingolipids by High-Performance Liquid Chromatography-Tandem Mass Spectrometry. Sci STKE 2001:pl1

Sung SY, Liao CH, Wu HP, Hsiao WC, Wu IH, Jinpu, Yu, Lin SH, Hsieh CL (2013) Loss of let-7 microRNA upregulates IL-6 in bone marrow-derived mesenchymal stem cells triggering a reactive stromal response to prostate cancer. PLoS One 8:e71637

Talwadekar MD, Kale VP, Limaye LS (2015) Placenta-derived mesenchymal stem cells possess better immunoregulatory properties compared to their cord-derived counterparts-a paired sample study. Sci Rep 5:15784

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Tigistu-Sahle F, Lampinen M, Kilpinen L, Holopainen M, Lehenkari P, Laitinen S, Käkelä R (2017) Metabolism and phospholipid assembly of polyunsaturated fatty acids in human bone marrow mesenchymal stromal cells. J Lipid Res 58:92-110

Tögel F, Westenfelder C (2012) Kidney protection and regeneration following acute injury: progress through stem cell therapy. Am J Kidney Dis 60:1012-1022

Tögel F, Zhang P, Hu Z, Westenfelder C (2009) VEGF is a mediator of the renoprotective effects of multipotent marrow stromal cells in acute kidney injury. J Cell Mol Med 13:2109-2114

Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C (2005a) Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. Am J Physiol Renal Physiol 289:F31-42

Tögel F, Isaac J, Hu Z, Weiss K, Westenfelder C (2005b) Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. Kidney Int 67:1772-1784

Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti E, Capelli C, Introna M, Remuzzi G, Benigni A (2013) Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. Stem Cells Dev 22:772-780

Traggiai E, Volpi S, Schena F, Gattorno M, Ferlito F, Moretta L, Martini A (2008) Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. Stem Cells 26:562-569

Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, Johnstone BH, March KL (2008) A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 102:77-85

Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 75:389-397

Turinetto V, Vitale E, Giachino C (2016) Senescence in Human Mesenchymal Stem Cells: Functional Changes and Implications in Stem Cell-Based Therapy. Int J Mol Sci 17:10.3390/ijms17071164

Turk HF, Chapkin RS (2013) Membrane lipid raft organization is uniquely modified by n-3 polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 88:43-47

Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654-659

Valencia J, Blanco B, Yanez R, Vazquez M, Herrero Sanchez C, Fernandez-Garcia M, Rodriguez Serrano C, Pescador D, Blanco JF, Hernando-Rodriguez M, Sanchez-Guijo F, Lamana ML, Segovia JC, Vicente A, Del Canizo C, Zapata AG (2016) Comparative analysis of the immunomodulatory capacities of human bone marrow- and adipose tissue-derived mesenchymal stromal cells from the same donor. Cytotherapy 18:1297-1311

Van Zant G, Liang Y (2003) The role of stem cells in aging. Exp Hematol 31:659-672

Wagner W, Bork S, Horn P, Krunic D, Walenda T, Diehlmann A, Benes V, Blake J, Huber F, Eckstein V, Boukamp P, Ho AD (2009) Aging and Replicative Senescence Have Related Effects on Human Stem and Progenitor Cells. PLoS ONE 4:e5846

Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, Benes V, Blake J, Pfister S, Eckstein V, Ho AD (2008) Replicative Senescence of Mesenchymal Stem Cells: A Continuous and Organized Process. PLoS ONE 3:e2213

Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD (2005) Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Experimental Hematology 33:1402-1416

Wang D, Li J, Zhang Y, Zhang M, Chen J, Li X, Hu X, Jiang S, Shi S, Sun L (2014) Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. Arthritis Res Ther 16:R79

Wang Q, Cai J, Cai XH, Chen L (2013) miR-346 regulates osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting the Wnt/beta-catenin pathway. PLoS One 8:e72266

Wang Y, Fan H, Zhao G, Liu D, Du L, Wang Z, Hu Y, Hou Y (2012) miR-16 inhibits the proliferation and angiogenesis-regulating potential of mesenchymal stem cells in severe pre-eclampsia. FEBS Journal 279:4510-4524

Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010) A New Mesenchymal Stem Cell (MSC) Paradigm: Polarization into a Pro-Inflammatory MSC1 or an Immunosuppressive MSC2 Phenotype. PLoS ONE 5:e10088

Wegmeyer H, Broske AM, Leddin M, Kuentzer K, Nisslbeck AK, Hupfeld J, Wiechmann K, Kuhlen J, von Schwerin C, Stein C, Knothe S, Funk J, Huss R, Neubauer M (2013) Mesenchymal stromal cell characteristics vary depending on their origin. Stem Cells Dev 22:2606-2618

Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR, Trumpp A (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135:1118-1129

Winkler IG, Barbier V, Nowlan B, Jacobsen RN, Forristal CE, Patton JT, Magnani JL, Levesque JP (2012) Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med 18:1651-1657

Winkler IG, Barbier V, Wadley R, Zannettino ACW, Williams S, Lévesque J (2010a) Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood 116:375-385

Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, Poulton IJ, van Rooijen N, Alexander KA, Raggatt LJ, Lévesque J (2010b) Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood 116:4815-4828

Wise AF, Williams TM, Kiewiet MB, Payne NL, Siatskas C, Samuel CS, Ricardo SD (2014) Human mesenchymal stem cells alter macrophage phenotype and promote regeneration via homing to the kidney following ischemia-reperfusion injury. Am J Physiol Renal Physiol 306:F1222-35

Wu LW, Wang YL, Christensen JM, Khalifian S, Schneeberger S, Raimondi G, Cooney DS, Lee WP, Brandacher G (2014) Donor age negatively affects the immunoregulatory properties of both adipose and bone marrow derived mesenchymal stem cells. Transpl Immunol 30:122-127

Xing L, Cui R, Peng L, Ma J, Chen X, Xie RJ, Li B (2014) Mesenchymal stem cells, not conditioned medium, contribute to kidney repair after ischemia-reperfusion injury. Stem Cell Res Ther 5:101

Xishan Z, Baoxin H, Xinna Z, Jun R (2013) Comparison of the effects of human adipose and bone marrow mesenchymal stem cells on T lymphocytes. Cell Biol Int 37:11-18

Xu C, Ren G, Cao G, Chen Q, Shou P, Zheng C, Du L, Han X, Jiang M, Yang Q, Lin L, Wang G, Yu P, Zhang X, Cao W, Brewer G, Wang Y, Shi Y (2013) miR-155 Regulates Immune Modulatory Properties of Mesenchymal Stem Cells by Targeting TAK1-binding Protein 2. Journal of Biological Chemistry 288:11074-11079

Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S, Cai Y, Cheng S, Wang X, Liu Y, Tang L, Ding Y, Jin Y (2013a) Tumor necrosis factor alpha suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. J Bone Miner Res 28:559-573

Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S, Cai Y, Cheng S, Wang X, Liu Y, Tang L, Ding Y, Jin Y (2013b) Tumor necrosis factor ? suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency?induced osteoporosis. Journal of Bone and Mineral Research 28:559-573

Yoo JK, Kim CH, Jung HY, Lee DR, Kim JK (2014) Discovery and characterization of miRNA during cellular senescence in bone marrow-derived human mesenchymal stem cells. Exp Gerontol 58:139-145

Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, Lee JE, Kim YJ, Yang SK, Jung HL, Sung KW, Kim CW, Koo HH (2009) Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. Cell Immunol 259:150-156

Zaragosi LE, Wdziekonski B, Brigand KL, Villageois P, Mari B, Waldmann R, Dani C, Barbry P (2011) Small RNA sequencing reveals miR-642a-3p as a novel adipocyte-specific microRNA and miR-30 as a key regulator of human adipogenesis. Genome Biol 12:R64-2011-12-7-r64

Zarjou A, Kim J, Traylor AM, Sanders PW, Balla J, Agarwal A, Curtis LM (2011) Paracrine effects of mesenchymal stem cells in cisplatin-induced renal injury require heme oxygenase-1. Am J Physiol Renal Physiol 300:F254-62

Zeng Y, Qu X, Li H, Huang S, Wang S, Xu Q, Lin R, Han Q, Li J, Zhao RC (2012) MicroRNA-100 regulates osteogenic differentiation of human adipose-derived mesenchymal stem cells by targeting BMPR2. FEBS Lett 586:2375-2381

Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L (2003) Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425:836-841

Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, Li G, Wang H, Lu G, Hu X, Jiang S, Li JN, Lin MC, Zhang YO, Kung HF (2011) MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol 8:829-838

Zhang W, Zhong W, Wang L (2014) A signal-amplification circuit between miR-218 and Wnt/β-catenin signal promotes human adipose tissue-derived stem cells osteogenic differentiation. Bone 58:59-66

Zhang Z, Lin H, Shi M, Xu R, Fu J, Lv J, Chen L, Lv S, Li Y, Yu S, Geng H, Jin L, Lau GK, Wang FS (2012) Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. J Gastroenterol Hepatol 27 Suppl 2:112-120

Zhao JJ, Liu JL, Liu L, Jia HY (2014a) Protection of mesenchymal stem cells on acute kidney injury. Mol Med Rep 9:91-96

Zhao M, Perry JM, Marshall H, Venkatraman A, Qian P, He XC, Ahamed J, Li L (2014b) Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nat Med 20:1321-1326

Zhou S (2015) Paracrine effects of haematopoietic cells on human mesenchymal stem cells. Scientific Reports 5:10573

Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, LeBoff MS, Glowacki J (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7:335-343