Emerging therapeutic potential of mesenchymal stem/stromal cells in preeclampsia

Suvakov S[1,2*], Richards C[3*], Nikolic V[4], Simic T[2,5], McGrath K[3], Krasnodembskaya A[6], McClements L[3]

[1]Department of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA

[2]Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[3] School of Life Sciences, Faculty of Science, University of Technology Sydney, NSW, Australia

[4]Department of Pharmacology and Toxicology, Medical Faculty, University of Nis, Nis, Serbia

[5]Serbian Academy of Sciences and Arts, Belgrade, Serbia

[6] The Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Northern Ireland, UK

*Authors contribute equally

Abstract

Purpose of review: Preeclampsia is a dangerous pregnancy condition affecting both the mother and offspring. It is a multifactorial disease with poorly understood pathogenesis, lacking effective treatments. Maternal immune response, inflammation and oxidative stress leading to endothelial dysfunction, are the most prominent pathogenic processes implicated in preeclampsia development. Here, we give a detailed overview of the therapeutic applications and mechanisms of mesenchymal stem/stromal cells (MSCs) as a potential new treatment for preeclampsia.

Recent findings: MSCs have gained growing attention due to low immunogenicity, easy cultivation, and expansion *in vitro*. Accumulating evidence now suggests that

MSCs act primarily through their secretomes facilitating paracrine signalling that leads to potent immunomodulatory, pro-angiogenic and regenerative therapeutic effects.

Summary: MSCs have been studied in different animal models of preeclampsia demonstrating promising result, which support further investigations into the therapeutic effects and mechanisms of MSC-based therapies in preeclampsia, steering these therapies into clinical trials.

Key words: pre-eclampsia; preeclampsia; mesenchymal stem cells; extracellular vesicles; biological therapies

Introduction

Preeclampsia is a severe cardiovascular disorder that affects 2-8% of pregnancies and it is a leading cause of maternal and neonatal morbidity and mortality [1]. Preeclampsia usually occurs during the second half of pregnancy and is characterised by the new onset of hypertension, proteinuria and end organ dysfunction; such as that of the liver and kidneys [2, 3]. Mild to moderate preeclampsia often does not display obvious symptoms and generally can be managed well without complications, unlike severe preeclampsia, which is defined as very high blood pressure (≥160/110) and substantial protein in the urine (≥300 mg of protein), or deterioration in liver, cerebrovascular and clotting function, as well as progressive renal damage [4]. If preeclampsia is left untreated it can result in the manifestation of seizures (eclampsia), HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome and other serious morbidities and death [5]. Early-onset preeclampsia is diagnosed prior to 34 weeks of gestation whereas late-onset preeclampsia is

diagnosed from 34 weeks of gestation onwards [6, 7]. While the development of early-onset preeclampsia appears to be closely associated with abnormal placentation, late-onset preeclampsia is believed to be predominantly caused by irregular growth of the placenta in combination with underlying maternal cardiovascular, metabolic and inflammatory conditions [6, 8]. However, a distinct delineation between these two types of preeclampsia is still not well understood and, therefore, further elucidation of the pathogenesis of the disease in the context of early-onset and late-onset preeclampsia is needed [9]. Cases of postpartum preeclampsia following delivery of the placenta and baby have also been observed [10].

The pathogenesis of preeclampsia

The complex heterogeneity of preeclampsia, ethical implications in obtaining placental samples early in pregnancy and difficulty in developing representative pre-clinical models, have hindered the progression of better understanding the molecular regulation of the pathogenesis of this disease. Despite these obstacles, inappropriate spiral uterine artery (SUA) remodelling due to inadequate trophoblast invasion and function has been identified as one of the main underlying causes. During normal placentation, two subtypes of extravillous trophoblasts (EVTs), referred to as interstitial and endovascular, migrate from the placental villi into the decidual layer of the uterus and begin invading the maternal SUAs. This process is followed by the apoptosis and replacement of maternal endothelial cells and the establishment of high calibre, low resistance vessels that enable increased blood flow to the developing feto-placental interface without damaging the placental villi [11]. Inability of trophoblast cells to migrate, invade and remodel the SUAs leads to poor perfusion of the placenta and

subsequent ischaemic conditions. While a low oxygen gradient has been reported as essential in the proliferation and differentiation of extravillous trophoblasts, persistent hypoxia or low oxygen tension as well as reperfusion injury is believed to lead to an oxidative stress response, the release of antiangiogenic factors and endothelial dysfunction [12].

A number of studies have highlighted the role of decidual immune cells, namely uterine natural killer (uNK) cells and macrophages, in inducing physiological changes in the SUAs prior to invasion of trophoblast cells [13]. Studies suggest that decidual uNK cells and macrophages begin the process of disrupting the vasculature of maternal SUAs, likely by inducing apoptosis of smooth muscle cells and digesting extracellular matrix components by secreting matrix metalloproteinases (MMPs) [14–17]. Data has also suggested that decidual leukocytes promote the migration of invading EVTs to the SUAs by secreting chemokines, which leads to vascular development and the secretion of angiogenic growth factors [18–20]. In fact, failure of this leukocyte regulation may impair appropriate remodelling of SUAs and the establishment of maternal blood flow to the feto-placental unit [21–23].

In addition to regulating the invasion of EVTs, decidual immune cells appear to play a pivotal role in the tolerance of the maternal immune system to the semi-allograft fetus [24–26]. Protection of the feto-placental unit from maternal rejection has been considered a vital process in the establishment of healthy pregnancy, which can be inappropriately developed in cases of nulliparity, new paternal partner and donor oocyte conception, all of which represent risk factors for preeclampsia [27]. Harnessing of uNK cells and macrophages by expressing unique forms of HLA class I molecules and secretion of chemokines by EVTs, are likely to prevent an immune response to feto-placental cells and ensure the initiation of SUA remodelling [20, 28–

30]. In addition, cytokine production by uterine macrophages has been implicated in their immunosuppressive role in pregnancy [25, 31–33]. Recently, extracellular vesicles (EVs) have been demonstrated to play an important role in the exchange between immune cells and trophoblast cells in achieving immunotolerance [34••, 35]. Inappropriate regulation of these immunomodulatory processes may inhibit the release of chemokines and angiogenic factors by uNK cells, impairing implantation and adequate perfusion of the placental bed.

Poor perfusion or placental ischaemia-reperfusion (hypoxia/reoxygenation) injury can cause an imbalance between reactive oxygen species (ROS) and antioxidants resulting in systemic inflammation and endothelial dysfunction [12]. Increased oxidative stress due to impaired perfusion of the placenta or reperfusion episodes following periodic vasoconstriction of inadequately remodelled SUAs may increase the production of ROS, as seen in pregnancies complicated by preeclampsia. Generation of ROS could induce increased apoptosis of the placental syncytiotrophoblast, which form a continuous and multinucleated maternal-fetal syncytium. This subsequently leads to the release of syncytiotrophoblast microvesicles, inflammatory factors such as tumour necrosis factor (TNF-α) and antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) into the maternal circulation [36]. These soluble proteins inhibit the actions of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) as well as transforming growth factor β1 (TGF-β1), which are all important for maintaining endothelial function and vasodilation [37]. The combination of these factors released into the intervillous space appear to trigger the systemic inflammatory response and peripheral endothelial dysfunction of the maternal disease. Underlying vascular conditions may increase a woman’s susceptibility to vascular inflammation

and risk of developing preeclampsia when pregnant [38]. This is often the case in pregnant women with pre-existing conditions such as obesity, hypertension and diabetes, which are well-established risk factors for the development of preeclampsia [6, 39, 40]. Our previous work has demonstrated a significant amount of overlapping pathogenic pathways and biomarkers between preeclampsia and adult hypertension [41•].

In addition to maternal disease, preeclampsia can lead to perinatal morbidities such as stillbirth and fetal growth restriction due to reduced placental perfusion [5]. Although preventative low-dose aspirin use has showed promising results, particularly in relation to early-onset preeclampsia, the current treatment of preeclampsia upon diagnosis remains the delivery of the placenta and the baby, often at preterm [42]. Beyond life-threatening complications in pregnancy, preeclampsia is also associated with increased maternal and offspring morbidity in later life. Studies have demonstrated that women and their offspring affected by preeclampsia have a higher risk of developing additional cardiovascular, neurological and metabolic disorders such as diabetes mellitus and heart disease following the pregnancy [43].

Considering that preeclampsia development is usually associated with inappropriate remodelling of maternal SUAs, impaired immune response, oxidative stress and irregular angiogenesis, mesenchymal stem/stromal cells (MSCs) with the potential to ameliorate these aberrant processes are emerging as a promising therapeutic option for preeclampsia [44]. MSCs' low immunogenicity, self-renewal capabilities, and easy cultivation gives them an advantage over other types of cell-based therapies [45]. More specifically, MSCs have been shown to have immunomodulatory, pro-angiogenic, anti-inflammatory and anti-oxidant effects (Figure 1) [46, 47].

MSC-based therapies as a novel therapeutic strategy for preeclampsia

MSCs are the most widely studied stem cells. These fibroblast-like, easy-to-propagate *in vitro* cells were discovered over 50 years ago as they exhibited potential to differentiate into adipose, bone, cartilage and muscle tissue [48]. MSCs can be isolated from tissues such as bone marrow, adipose tissue, umbilical cord and the placenta [49–52]. Interestingly, MSCs residing in the maternal decidua are believed to be involved in regulating the pro-angiogenic, immunomodulatory and anti-inflammatory environment of the maternal-fetal interface during placentation. In fact, abnormalities in decidual MSC cytokine production and micro-RNA have been detected in patients with preeclampsia [53]. Recent study assessing the function of adipose tissue-derived MSCs from women with and without preeclampsia, demonstrated impaired survival, proliferation and migration of MSCs isolated from women with preeclampsia. These cells also showed lower angiogenic potential likely due to senescence, which was improved when a senolytic agent was added to the MSC culture *ex vivo* [54]●●.

MSCs act through both cell contact-dependent regulation of the host cells and by secreting soluble factors. Direct intercellular communication between MSCs and their target cells can occur through tunnelling nanotubule (TNT) formation or cell fusion (reviewed in [55]•). In both cases, direct exchange of cytoplasmic content (including organelles such as mitochondria and lysosomes) can take place, resulting in the restoration of function of the host cells injured by disease microenvironment. More recently, MSC-derived EVs have attracted significant attention as key to intercellular communication. EVs serve as plasma membrane-wrapped vehicles which carry diverse cargo present in the cytoplasm of the producer cells. Vesicle content can include cytosolic and membrane proteins, mRNA and non-coding RNA including

miRNA, as well as organelles such as mitochondria and lysosomes. The nature of the EV cargo of MSCs can be influenced by the extracellular environment. A growing number of studies have demonstrated that treatment with MSC-derived EVs can recapitulate the therapeutic effects of MSCs in pre-clinical models of liver, kidney, heart, skin, lung and other diseases [56–58], however, their role in preeclampsia has only been addressed recently.

MSCs are considered immunoprivileged cells as they can be infused into either autologous or allogeneic hosts owing to their lack of host immune reactivity [59]. MSCs have demonstrated therapeutic effects in animal models of multiple sclerosis, rheumatoid arthritis, myasthenia gravis and diabetes mellitus [60–63]. While MSC-based therapy in other autoimmune experimental models has been extensively studied *in vivo*, there are only a small number of studies performed in preeclampsia models. Notably, when MSCs were injected in an LPS-induced rat model of preeclampsia, it was demonstrated that regulatory mechanisms to recover Th1/Th2 immune response balance were restored and placental inflammation ameliorated [64]. Following MSCs intravenous injection, rats demonstrated lower plasma levels of TNF-a, IL-6, IL-12, and ICAM-1, while IL10 was increased [64]. In separate studies, using Th-1 or endotoxin-induced or angiotensin receptor agonistic autoantibody (AT1-AA) models of preeclampsia, the therapeutic effect of MSCs, which included reduced systolic blood pressure and proteinuria, was attributed to significant attenuation of TNF-α expression in uterine and splenic lymphocytes [65, 66, 67●]. Interestingly, it appears that the mechanism of these MSC-mediated therapeutic effects is through secreted factors rather than cell-to-cell contact, which was also confirmed in another pre-clinical preeclampsia study using MSC-derived exosomes [66**,** 67●, 68]. Indeed, paracrine effects of MSCs have been highlighted as important factors in angiogenesis

and promotion of wound repair [69]. Thus, it was demonstrated that treatment with MSC conditioned media is capable of restoring angiogenic potential of villous explants from women with preeclampsia by decreasing the expression of IL-6 and sFlt-1 [70]. Another study demonstrated that intravenous infusion of MSCs into preeclampsia model of AT1-AA induced pregnant rats can ameliorate SUA remodelling impairment and intrauterine growth retardation by regulating trophoblast invasion; this was also confirmed using MSCs derived from placenta [67•, 71].

Given that MSCs and associated EVs have shown therapeutic effects in murine and rat hypertension models (Table 1) as well as in cardiovascular diseases (Table 2), this type of stem cell-based therapy represents a viable therapeutic option for preeclampsia. A summary of pre-clinical studies investigating the therapeutic potential of MSC-based therapies in preeclampsia is presented in Table 1. As a number of studies highlighted that women with pre-eclampsia are at higher risk of developing cardiovascular disorders such as chronic hypertension, ischaemic heart disease and stroke later in life [72], we reviewed a few of these studies [73], [74], [75], [76], [77], and summarised the findings in Table 2.

Immunomodulatory properties of MSCs

A growing body of evidence suggests that preeclampsia could be considered an autoimmune-like disease affecting the maternal-fetal interface, as described above [78]. Indeed, normotensive pregnancies are found to be a Th2 type immunological state where an immune-tolerant environment is favoured, while preeclampsia has been characterized as a pro-inflammatory state with Th1 predominance [79, 80]. However, the well accepted Th1/Th2 paradigm has changed into the Th1/Th2/Th17-

Treg in light of accumulating evidence that T-regulatory cells (Tregs) contribute to the maintenance of tolerance during pregnancy [81]. Notably, the immunosuppressive function of Treg cells comes from the functional characteristics of dendritic cells (DCs), which constantly induce immunosuppressive functions of Treg cells [82]. A large body of evidence is focused on the ability of MSCs to modulate immune and inflammatory responses, particularly in endometrial tissue, where an adequate immuno-tolerant environment is essential for successful implantation and the normal invasion process of trophoblasts. Paracrine immunomodulation by MSCs targets T-lymphocytes, B-lymphocytes, DCs and natural-killer cells (NKs) [83, 84]. By altering the cytokine profile of DCs, MSCs suspend their pro-inflammatory potential and influence Treg cells generation [85]. Also, MSCs alter Th17 differentiation in two different ways: i) by inducing IL-4 production, needed for Th2 phenotype, and ii) by inhibiting IFN-Ɣ production, needed for Th1. MSCs may shift Th1 towards Th2 response by promoting an immature DC phenotype, preventing the Th1 response, which is favoured by mature DCs [85, 86]. These stem cells are able to directly induce an increase in Treg cell number most likely by suppressing monocyte production of IL-6 and IL-1β in preeclampsia. In the same manner, MSCs may reduce exaggerated inflammation caused by Th17 differentiation, therefore contributing to the immune homeostasis required during pregnancy [85]. Apart from the possibility of altering the cytokine profile, MSCs can act in direct MSC-to-cell contact through PD1-PD1L pathway, which has a central role as a suppressor of immune response during pregnancy [87, 88].

Apart from having anti-inflammatory properties, MSCs are described to also be able to produce a pro-inflammatory environment, depending on a stimuli. Waterman et al. demonstrated that MSCs' polarization depends on specific toll-like receptors (TLRs) expression affecting ability to migrate, invade, and secrete immune modulating

factors [89]. Thus, TLR3 stimulation of MSCs will give immunosuppressive effects, while TLR4 activation will provide a pro-inflammatory signature.

Angiogenic properties of MSCs

MSCs have been shown to promote endogenous angiogenesis in a variety of *in vitro* assays and *in vivo* models of diseases such as acute lung injury, stroke, breast cancer, wound healing and other types of ischaemic injury [90–99]. Further, MSCs have been investigated for their ability to stimulate angiogenesis in *in vitro* trophoblast cultures and animal models of preeclampsia. As described above, when Nuzzo *et al* treated pre-eclamptic villous explants with placenta-derived MSC conditioned media (MSC-CM), a neutralization of pro-inflammatory and anti-angiogenic mRNA expression was observed [70]. While the exact mechanism of MSC-mediated regulation of angiogenesis is still unclear, it is now believed that their effects are predominantly induced by paracrine factors rather than their capacity to differentiate into endothelial cells [46, 100, 101]. More specifically, EVs secreted by MSCs are able to transfer biologically active membrane and cytosolic components to target cells, as described above. Exosomes, a subtype of EVs, have recently been recognised for their role in intercellular communication by transporting proteins, lipids and genetic material including non-coding RNAs such as miRNAs in order to regulate the biological functions of target cells [102].

MSC-CM and isolated EVs have been used in various *in vitro* experiments to determine the role of MSC-based paracrine factors in promoting angiogenesis. Komaki et al (2017) tested MSC-CM and isolated exosomes from placenta-derived MSCs to evaluate their regulation of human umbilical vein endothelial cell (HUVEC)

angiogenesis. The MSC-CM contained angiogenic factors that enhanced HUVEC tube formation, however, when exosomes were removed from the media, the angiogenic effect was significantly reduced. Further, the isolated exosomes were successfully incorporated into the HUVEC cells, following which angiogenic marker expression was increased; with the pro-angiogenic effects of these exosomes confirmed in a murine auricle ischaemic injury model [103]. Several other studies including our own work, demonstrated similar pro-angiogenic capabilities of MSCs on endothelial and trophoblast migration, invasion and tube formation [104–107].

In addition, a growing number of animal models of ischaemic injury and preeclampsia have begun examining the mechanism of MSC promotion of angiogenesis [64, 67, 108]. Xiong et al (2018) using L-name induced rat model of preeclampsia investigated the effects and mechanism of varying concentrations of human umbilical cord MSC-derived exosomes. Following treatment, the rat models treated with exosomes demonstrated a substantial decrease in blood pressure, cell apoptosis and expression of anti-angiogenic sFlt-1. Further beneficial effects of MSV-derived exosomes included an increase in the number of fetuses per pregnancy, restored morphology, micro-vascular density and VEGF expression, in placenta, in a dose-dependent manner. However, the exosomal cargo responsible for these effects was not investigated [68••]. A number of pro-angiogenic factors regulated by MSC or associated EVs, have also been implicated in the pathogenesis of preeclampsia, however, further research is required to elucidate this association between preeclampsia and MSC-mediated mechanism of repair [109–111].

Anti-inflammatory effects of MSCs

Compelling evidence has demonstrated that during the inflammatory process, MSCs modulate the balance between effector and regulatory immune functions in favour of the latter. Most notably, in almost every pre-clinical model of inflammatory disease, including models of preeclampsia, MSC administration leads to robust amelioration of inflammatory response reflected in the reduction of inflammatory cell influx, improvement of epithelial and endothelial barrier integrity associated with the decreased expression of endothelial adhesion molecules and significantly lower levels of pro-inflammatory cytokines, both locally and systemically [64, 67•, 112]. The therapeutic effects of MSCs result in deactivation (or reprogramming) of both innate and adaptive inflammatory immune cells, such as monocytes, macrophages, DCs, CD4+, CD8+, NK, and B cells, while up-regulating regulatory subsets of cells such as alternatively activated monocytes and macrophages, and regulatory T cells to facilitate resolution of inflammation and restore function.

As introduced earlier, an important mechanism of MSC modulation of the host cells is mediated through their capacity to secrete multiple paracrine factors. Soluble mediators act on multiple cell targets, changing their phenotype and function. A constantly growing number of soluble mediators have been implicated in MSC-induced anti-inflammatory effects, including indoleamine 2,3-dioxygenase (IDO), nitric oxide, TNF-a stimulated Gene/Protein 6 (TSG6), TGF-β, Prostaglandin-E_2 (PGE_2) and LipoxinA4 [113–117]. These findings paved the way to improving effectiveness of MSC-based therapies through gene modification, to overexpress several different soluble mediators, such as TGFβ or IL-10 [118, 119]. Interestingly, Gonzales-King et al. demonstrated that exosomes derived from HIF-1α overexpressing MSCs were enriched in the Notch-1 ligand Jagged-1, and subsequently were able to trigger transcriptional changes in Notch target genes in endothelial cells and induce

angiogenesis in both an *in vitro* model of capillary-like tube formation and matrigel plug assay *in vivo* [120]. MSC-derived EVs are capable of improving endothelial barrier integrity through transferring of Ang-1 mRNA, which results in expression and secretion of Ang-1 protein by human lung microvascular endothelial cells [121]. We have demonstrated that MSC-derived EVs containing functional mitochondria metabolically reprogram macrophages from glycolysis governed M1 pro-inflammatory phenotype towards oxidative-phosphorylation-dependent M2 anti-inflammatory phenotype [122]. A growing area of research is focussed on investigating the functional role of EV miRNA cargo. Thus, Pan et al, demonstrated that mouse bone marrow derived MSCs were able to ameliorate hypoxia-reperfusion induced injury in HUVECs *in vitro* by exosomal transfer of miRNA-126 which subsequently activated PI3K/Akt/eNOS pathway [123].

Given that EVs have several advantages over whole cell therapy such as lower risk of tumorigenic effect, lower susceptibility to damage by hostile injury microenvironment (e.g. hypoxia and high concentrations of cytokines), ability to retain efficacy after freezing and therefore avoiding the need to have expensive GMP cell manufacturing facilities on site (which could be critical for smaller hospitals), EVs are increasingly considered as an attractive alternative to the whole cell based therapy.

Interestingly, new evidence suggests that after administration *in vivo,* MSCs undergo apoptosis, possibly targeted by NK cells [124]. These apoptotic MSCs induce anti-inflammatory effects through modulation of phagocytic cells involved in their clearance (reviewed in [125–127]). Galleu et al. were the first to report that graft-versus-host-disease (GvHD) patients could be stratified into two categories based on their cytotoxic activity towards MSCs. Those who had high cytotoxic activity against MSCs responded to MSC infusion, whereas those with low cytotoxic activity did not.

After infusion, a recipient phagocytes engulfed apoptotic MSCs and produces indoleamine 2,3-dioxygenase (IDO), which was necessary for effective immunosuppression observed following MSC administration [124]. The follow-up study by the same group identified PGE-2 as a key soluble factor upstream of IDO-induced monocytes after engulfment of apoptotic MSCs, which is responsible for IDO upregulation and could be used as a biomarker of MSC efficacy in the patients receiving MSC therapy [128]. These findings are in line with the studies from the Hoogduijn's group suggesting that infused MSCs are rapidly phagocytosed by monocytes [129, 130]. Phagocytosis of MSCs induces phenotypical and functional changes in monocytes polarising the cells towards non-classical Ly6C^{low} phenotype. These monocytes were able to induce Foxp3+ regulatory T-cell formation in mixed lymphocyte reactions. Therefore, these findings highlight that the therapeutic effects of MSCs are dependent on interactions between MSCs and monocytes/macrophages and emphasize the important contribution of innate immune modulation to MSC therapeutic efficacy.

Anti-oxidant effects of MSCs

Oxidative stress is a key mechanism involved in early inflammation, and reactive oxygen and nitrogen species have been implicated in the pathogenesis of preeclampsia [12, 41•, 131]. A number of studies have shown that MSCs are able to secrete relatively high levels of heme oxygenase-1 (HO-1) and that HO-1 overexpression in MSCs enhances their therapeutic potential in pre-clinical models of lung and liver injury [132, 133]. Heme oxygenases degrades heme to biliverdin, iron, and carbon monoxide, which has beneficial vasodilatory effect. Expression of HO-1

modulates oxidative stress and confers protection from apoptosis [134]. In the models of acute kidney injury, molecules associated with the release of free radicals, such as the inducible nitric oxide synthases (iNOS), endothelial nitric oxide synthases (eNOS) and 8-hydroxy-2-deoxyguanosine (8-OHdG) are decreased after MSC administration [135, 136].

In the model of ischemia-reperfusion, AKI mice after MSC administration were found to have higher expression levels of NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione reductase (GSH-Rx) and glutathione peroxidase (GSH-Px) when compared with control groups. Moreover, the global oxidative index had decreased after MSC treatment [137]. Zhuo et al. reported that MSC infusion also significantly improved the activity of superoxide dismutase (SOD), a potent molecule responsible for reducing oxidative stress, and increased GSH-Px expression, an antioxidant enzyme, in renal tissues [138].

Another mechanism implicated in the anti-oxidant effect of MSCs is their capacity to transfer functional mitochondria to the target cells in affected tissues and thus alleviate oxidative stress induced by mitochondrial dysfunction. As mentioned earlier, mitochondria can be transferred via TNTs as well as secreted in EVs. Mitochondrial transfer is associated with a decrease in mitochondrial ROS, restoration of mitochondrial membrane potential ($\Delta\Psi m$) and restoration of oxidative phosphorylation levels in recipient cells leading to improved functional activity (e.g., surfactant secretion, phagocytosis, wound healing and viability; Reviewed in [55•, 139, 140]). Liu et al. demonstrated that the establishment of TNTs between MSCs and oxidative stress-injured endothelial cells (HUVECs) resulted in the rescue of aerobic respiration and protection of endothelial cells from apoptosis. TNT formation required recognition of the surface-exposed phosphatidylserines (PSs) on the injured HUVECs

by MSCs. Shielding of PSs with Annexin V resulted in the failure of TNT-mediated cell contact between the two cell types [141]. In the follow up study, the same group showed that mitochondrial transfer from MSCs promoted cerebral microvasculature recovery in the rat model of ischemic stroke. The same group demonstrated that the host cells of injured cerebral microvasculature accepted the mitochondrial transfer from the transplanted MSCs. Mitochondrial transfer was associated with significantly improved mitochondrial activity of injured microvasculature, enhanced angiogenesis, reduced infarct volume, and improved overall functional recovery [92]. We have recently demonstrated that mitochondrial transfer from MSC to pulmonary epithelial cells restores epithelial cell mitochondrial membrane potential significantly reduced by inflammatory environment [142•]. Mitochondrial dysfunction has been implicated in preeclampsia and targeting mitochondrial-mediated oxidative stress has been shown to alleviate endothelial dysfunction in preeclampsia [143, 144].

Translating MSCs therapies into clinical trials for preeclampsia treatment

MSCs have been in focus for several years for a number of therapeutic applications. Their potent anti-inflammatory, pro-angiogenic and immunomodulatory potential, easy isolation, capacity for self-renewal and the lack of immunogenicity, represent a promising tool for future therapeutic applications. Although a number of molecular pathways have been identified, the exact mechanisms by which MSCs exert their therapeutic function in preeclampsia, or any other disease, are still not completely clear. It seems that direct cell-to-cell contact is not crucial, as findings suggest that no fluorescent-labelled MSCs were present in any of the organs which restored their function after MSC treatment [67•]. Considering the very complex pathogenesis of

preeclampsia, it is likely that each of the secreted molecules play only a partial role in restoring the normal function of an end-organ. An interesting approach to identify the exact molecule(s) would be to inhibit or knockout molecules individually or simultaneously and investigate the therapeutic potential.

While a number of studies utilising *in vivo* models of preeclampsia have demonstrated promising results, there are a number of concerns, which need to be addressed in order to steer MSCs into clinical trials for the treatment of preeclampsia. Most of these studies reported data up to 10 days following administration of MSC-based injections. No results in relation to prolonged exposure to MSC were reported and not much is known about the effect of MSCs on fetal health, apart from an apparent increase in birth weight. Since only a limited number of *in vivo* studies have investigated the use of MSCs as a treatment of preeclampsia, further pre-clinical and clinical studies are necessary to evaluate the therapeutic potential as well as the safety profile of MSCs in preeclampsia. Based on the current knowledge of MSC properties, a concern still remains regarding their role in tumour development [145]. Since MSCs have regenerative and pro-angiogenic roles, their capacity to promote malignancies needs to be fully addressed prior to any treatment. Also, there are only a limited number of pre-clinical models of preeclampsia, which are all induced and poorly representative of human preeclampsia [146].

Considering that MSCs can be harvested from different sources of tissues, there is likely a difference in stages of differentiations, as well as in proteomic and genomic profiles, which results in different functional efficacies of these cells. Therefore, this heterogeneity could affect their therapeutic potential and immunogenicity. Another important aspect of MSC-based therapies is their mechanism of action, which is poorly understood. Overall, before MSCs can be used

in the clinical trial settings, standardised procedures need to be developed in relation to their isolation, propagation and administration, as well as patient's suitability, which will maximise their therapeutic potential and minimise possible side effects.

Conclusion

Although preeclampsia remains the leading cause of maternal and fetal morbidity and mortality, the only cure remains the delivery of the placenta and the baby. While this can reduce short-term pregnancy complications, long-term increased incidence of diabetes and cardiovascular diseases still remains. MSCs and associated EVs have demonstrated therapeutic potential in a variety of *in vitro* and *in vivo* models of various diseases and MSCs have begun investigation in the clinical trial context. In preeclampsia pre-clinical models, MSC-based therapies have demonstrated improvement in symptoms of preeclampsia and immuno-modulatory, pro-angiogenic, anti-inflammatory and anti-oxidant effects. However, the complex pathogenesis of preeclampsia and the lack of mechanistic insight into MSC-mediated repair requires further elucidation before MSCs or MSC-EVs can be introduced in the clinical context.

References:

Recent papers of particular importance have been highlighted as:

• Of importance

•• Of major importance

1. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller A-B, Daniels J, Gülmezoglu AM, Temmerman M, Alkema L (2014) Global causes of maternal death: a WHO systematic analysis. Lancet Glob Heal 2:e323-33

2. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R (2010) Pre-eclampsia. Lancet 376:631–644

3. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, Zeeman GG, Brown MA (2014) The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens An Int J Women's Cardiovasc Heal 4:97–104

4. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ (2016) Pre-eclampsia. Lancet 387:999–1011

5. Duley L (2009) The Global Impact of Pre-eclampsia and Eclampsia. Semin Perinatol 33:130–137

6. Staff AC (2019) The two-stage placental model of preeclampsia: An update. J Reprod Immunol 134–135:1–10

7. Xiong X, Demianczuk NN, Saunders LD, Wang F-L, Fraser WD (2002) Impact of Preeclampsia and Gestational Hypertension on Birth Weight by Gestational Age. Am J Epidemiol 155:203–209

8. Redman CW, Sargent IL, Staff AC (2014) IFPA Senior Award Lecture: Making sense of pre-eclampsia – Two placental causes of preeclampsia? Placenta 35:S20–S25

9. Staff AC, Redman CWG (2018) The Differences Between Early- and Late-Onset Pre-eclampsia. Springer, Singapore, pp 157–172

10. Redman CWG (1991) Pre-eclampsia and the placenta. Placenta 12:301–308

11. Burton GJ, Woods AW, Jauniaux E, Kingdom JCP (2009) Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta 30:473–482

12. Wu F, Tian F-J, Lin Y, Xu W-M (2016) Oxidative Stress: Placenta Function and Dysfunction. Am J Reprod Immunol 76:258–271

13. Faas MM, Vos P De (2018) Innate immune cells in the placental bed in healthy pregnancy and preeclampsia. Placenta 69:125–133

14. Smith SD, Dunk CE, Aplin JD, Harris LK, Jones RL (2009) Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. Am J Pathol 174:1959–1971

15. Naruse K, Lash GE, Innes BA, Otun HA, Searle RF, Robson SC, Bulmer JN (2008) Localization of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitors for MMPs (TIMPs) in uterine natural killer cells in early human pregnancy. Hum Reprod 24:553–561

16. Lyall F, Robson SC, Bulmer JN (2013) Spiral Artery Remodeling and Trophoblast Invasion in Preeclampsia and Fetal Growth Restriction. Hypertension 62:1046–1054

17. Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, Aplin JD, Baker PN, Robson SC, Bulmer JN (2012) Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. https://doi.org/101096/fj12-210310. https://doi.org/10.1096/FJ.12-210310

18. Hanna J, Goldman-Wohl D, Hamani Y, et al (2006) Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 12:1065–1074

19. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, Robson SC, Bulmer JN (2006) Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. J Leukoc Biol 80:572–580

20. Lash GE, Naruse K, Robson A, Innes BA, Searle RF, Robson SC, Bulmer JN (2011) Interaction between uterine natural killer cells and extravillous trophoblast cells: effect on cytokine and angiogenic growth factor production. Hum Reprod 26:2289–2295

21. Fraser R, Whitley GS, Johnstone AP, Host AJ, Sebire NJ, Thilaganathan B, Cartwright JE (2012) Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. J Pathol 228:322–332

22. Renaud SJ, Postovit L-M, Macdonald-Goodfellow SK, McDonald GT, Caldwell JD, Graham CH (2005) Activated Macrophages Inhibit Human Cytotrophoblast Invasiveness In Vitro1. Biol Reprod 73:237–243

23. Fraser R, Whitley GSJ, Thilaganathan B, Cartwright JE (2015) Decidual natural killer cells regulate vessel stability: Implications for impaired spiral artery remodelling. J Reprod Immunol 110:54–60

24. Warning JC, McCracken SA, Morris JM (2011) A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. REPRODUCTION 141:

25. Heikkinen J, Möttönen M, Komi J, Alanen A, Lassila O (2003) Phenotypic characterization of human decidual macrophages. Clin Exp Immunol 131:498–505

26. Trundley A, Moffett A (2004) Human uterine leukocytes and pregnancy. Tissue Antigens 63:1–12

27. Levron Y, Dviri M, Segol I, Yerushalmi GM, Hourvitz A, Orvieto R, Mazaki-Tovi S, Yinon Y (2014) The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations. Am J Obstet Gynecol 211:383.e1-383.e5

28. Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, Chazara O, Bauer J, Hiby SE, Colucci F, Moffett A (2013) Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. J Clin Invest 123:4264–4272

29. Choudhury RH, Dunk CE, Lye SJ, Aplin JD, Harris LK, Jones RL (2017) Extravillous Trophoblast and Endothelial Cell Crosstalk Mediates Leukocyte Infiltration to the Early Remodeling Decidual Spiral Arteriole Wall. J Immunol 198:4115 LP-4128

30. Fu B, Li X, Sun R, Tong X, Ling B, Tian Z, Wei H (2013) Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. Proc Natl Acad Sci U S A 110:E231-40

31. Lidström C, Matthiesen L, Berg G, Sharma S, Ernerudh J, Ekerfelt C (2003) Cytokine Secretion Patterns of NK Cells and Macrophages in Early Human Pregnancy Decidua and Blood: Implications for Suppressor Macrophages in Decidua. Am J Reprod Immunol 50:444–452

32. Engert S, Rieger L, Kapp M, Becker JC, Dietl J, Kämmerer U (2007) Profiling Chemokines, Cytokines and Growth Factors in Human Early Pregnancy Decidua By Protein Array. Am J Reprod Immunol 58:129–137

33. Gustafsson C, Mjösberg J, Matussek A, Geffers R, Matthiesen L, Berg G, Sharma S, Buer J, Ernerudh J (2008) Gene Expression Profiling of Human Decidual Macrophages: Evidence for Immunosuppressive Phenotype. PLoS One 3:1–9

34. Nair S, Salomon C (2018) Extracellular vesicles and their immunomodulatory functions in pregnancy. Semin Immunopathol 40:425–437

•• *This review evaluates and summarises important communication pathways between immune and trophoblast cells via placental extracellular vesicles in pregnancy. It also outlines the gaps in the knowledge in relation to characterisation and isolation of different sub-types of vesicles and poorly understood molecular mechanisms of these interactions.*

35. Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, Rice GE, Salomon C (2015) Placental exosomes in normal and complicated pregnancy. Am J Obstet Gynecol 213:S173–S181

36. Cim N, Kurdoglu M, Ege S, Yoruk I, Yaman G, Yildizhan R (2017) An analysis on the roles of angiogenesis-related factors including serum vitamin D, soluble endoglin (sEng), soluble fms-like tyrosine kinase 1 (sFlt1), and vascular endothelial growth factor (VEGF) in the diagnosis and severity of late-onset preeclampsia. J Matern Neonatal Med 30:1602–1607

37. Walshe TE, Dole VS, Maharaj ASR, Patten IS, Wagner DD, D'Amore PA (2009) Inhibition of VEGF or TGF-β Signaling Activates Endothelium and Increases Leukocyte Rolling. Arterioscler Thromb Vasc Biol 29:1185–1192

38. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ (2010) Hypertension in Pregnancy and Later Cardiovascular Risk. Circulation 122:579–584

39. Avagliano L, Bulfamante G Pietro, Morabito A, Marconi AM (2011) Abnormal spiral artery remodelling in the decidual segment during pregnancy: from histology to clinical correlation. J Clin Pathol 64:1064–1068

40. National Institute for Health and Clinical Excellence (NICE). (2011) Hypertension in pregnancy. RCOG Press

41. Lopez-Campos G, Bonner E, McClements L (2019) An Integrative Biomedical Informatics Approach to Elucidate the Similarities Between Pre-Eclampsia and Hypertension. Stud Health Technol Inform 264:988–992

• *This paper identifies for the first time the overlapping mechanisms between preeclampsia and hypertension. While an association between these diseases was previously understood, several overlapping pathogenic pathways were uncovered through bioinformatics analysis that contribute to understanding the complex pathogenesis of preeclampsia.*

42. Rolnik DL, Wright D, Poon LC, et al (2017) Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. N Engl J Med 377:613–622

43. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ (2009) Hypertensive Pregnancy Disorders and Subsequent Cardiovascular Morbidity and Type 2 Diabetes Mellitus in the Mother. Hypertension 53:944–951

44. McNally R, Alqudah A, Obradovic D, McClements L (2017) Elucidating the Pathogenesis of Pre-eclampsia Using In Vitro Models of Spiral Uterine Artery Remodelling. Curr Hypertens Rep. 19:93-

45. Hass R, Kasper C, Böhm S, Jacobs R (2011) Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 9:12

46. da Silva Meirelles L, Fontes AM, Covas DT, Caplan AI (2009) Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 20:419–427

47. Grimes S, Bombay K, Lanes A, Walker M, Corsi DJ (2019) Potential biological therapies for severe preeclampsia: a systematic review and meta-analysis. BMC Pregnancy Childbirth 19:163

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

49. Soleimani M, Nadri S (2009) A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. Nat Protoc 4:102–106

50. Araña M, Mazo M, Aranda P, Pelacho B, Prosper F (2013) Adipose Tissue-Derived Mesenchymal Stem Cells: Isolation, Expansion, and Characterization. In: Kao RL (ed) Cell. Cardiomyoplasty Methods Protoc. Humana Press, Totowa, NJ, pp 47–61

51. Huang P, Lin LM, Wu XY, Tang QL, Feng XY, Lin GY, Lin X, Wang HW, Huang TH, Ma L (2010) Differentiation of human umbilical cord Wharton's jelly-derived mesenchymal stem cells into germ-like cells in vitro. J Cell Biochem 109:747–754

52. Pelekanos RA, Sardesai VS, Futrega K, Lott WB, Kuhn M, Doran MR (2016) Isolation and Expansion of Mesenchymal Stem/Stromal Cells Derived from Human Placenta Tissue. J Vis Exp. https://doi.org/10.3791/54204

53. Zhao G, Zhou X, Chen S, Miao H, Fan H, Wang Z, Hu Y, Hou Y (2014) Differential expression of microRNAs in decidua-derived mesenchymal stem cells from patients with pre-eclampsia. J Biomed Sci 21:81

54. Suvakov S, Cubro H, White WM, et al (2019) Targeting senescence improves angiogenic potential of adipose-derived mesenchymal stem cells in patients with preeclampsia. Biol Sex Differ 10:49

●●*This study demonstrated that mesenchymal stem cells (MSCs) derived from adipose tissue from women with diagnosed preeclampsia undergoing Caesarian section display impaired angiogenic function. Impaired function of MSCs is attributed to senescence suggesting a possible new insight into pathophysiological mechanism of preeclampsia and novel therapeutic approach using MSCs.*

55. Murray LMA, Krasnodembskaya AD (2019) Concise Review: Intercellular Communication Via Organelle Transfer in the Biology and Therapeutic Applications of Stem Cells. Stem Cells 37:14–25

• *This review compiles evidence of the mechanisms that mesenchymal stem cells use to modulate host cells, including transfer of cytoplasmic material and organelles. This information is critical for understanding the intercellular communication modes of MSCs in the context of their therapeutic application.*

56. Lai RC, Arslan F, Lee MM, et al (2010) Exosome secreted by {MSC} reduces myocardial ischemia/reperfusion injury. Stem Cell Res 4:214–222

57. Lou G, Chen Z, Zheng M, Liu Y (2017) Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med 49:e346--e346

58. Wu P, Zhang B, Shi H, Qian H, Xu W (2018) {MSC}-exosome: A novel cell-free therapy for cutaneous regeneration. Cytotherapy 20:291–301

59. Prockop DJ (2009) Repair of Tissues by Adult Stem/Progenitor Cells (MSCs): Controversies, Myths, and Changing Paradigms. Mol Ther 17:939–946

60. Liu S, Wang J, Han R, et al (2019) Therapeutic effect of transplanted umbilical cord mesenchymal stem cells in a cynomolgus monkey model of multiple sclerosis. Am J Transl Res 11:2516–2531

61. Abdelmawgoud H, Saleh A (2018) Anti-inflammatory and antioxidant effects of mesenchymal and hematopoietic stem cells in a rheumatoid arthritis rat model. Adv Clin Exp Med 27:873–880

62. Yu J, Zheng C, Ren X, Li J, Liu M, Zhang L, Liang L, Du W, Chao Han Z (2010) Intravenous Administration of Bone Marrow Mesenchymal Stem Cells Benefits Experimental Autoimmune Myasthenia Gravis Mice Through an Immunomodulatory Action. Scand J Immunol 72:242–249

63. Mohammadi Ayenehdeh J, Niknam B, Rasouli S, Hashemi SM, Rahavi H, Rezaei N, Soleimani M, Liaeiha A, Niknam MH, Tajik N (2017) Immunomodulatory and protective effects of adipose tissue-derived mesenchymal stem cells in an allograft

islet composite transplantation for experimental autoimmune type 1 diabetes. Immunol Lett 188:21–31

64. Wang L-L, Yu Y, Guan H-B, Qiao C (2016) Effect of Human Umbilical Cord Mesenchymal Stem Cell Transplantation in a Rat Model of Preeclampsia. Reprod Sci 23:1058–1070

65. Fu L, Liu Y, Zhang D, Xie J, Guan H, Shang T (2015) Beneficial effect of human umbilical cord-derived mesenchymal stem cells on an endotoxin-induced rat model of preeclampsia. Exp Ther Med 10:1851–1856

66. Liu L, Zhao G, Fan H, Zhao X, Li P, Wang Z, Hu Y, Hou Y (2014) Mesenchymal stem cells ameliorate Th1-induced pre-eclampsia-like symptoms in mice via the suppression of TNF-α expression. PLoS One 9:e88036

67. Zhang D, Fu L, Wang L, Lin L, Yu L, Zhang L, Shang T (2017) Therapeutic benefit of mesenchymal stem cells in pregnant rats with angiotensin receptor agonistic autoantibody-induced hypertension: Implications for immunomodulation and cytoprotection. Hypertens Pregnancy 36:247–258

• *This paper uses a rat model to demonstrate the potential of mesenchymal stem cells to ameliorate the effects of hypertension in pregnancy via immunomodulation and paracrine action.*

68. Xiong Z-H, Wei J, Lu M-Q, Jin M-Y, Geng H-L (2018) Protective effect of human umbilical cord mesenchymal stem cell exosomes on preserving the morphology and angiogenesis of placenta in rats with preeclampsia. Biomed Pharmacother 105:1240–1247

•• *This paper demonstrates, for the first time, pre-clinical data of a dose-dependent therapeutic response to mesenchymal stem cell-derived exosomes in rats with induced preeclampsia.*

69. Sun J, Zhang Y, Song X, Zhu J, Zhu Q (2019) The Healing Effects of Conditioned Medium Derived from Mesenchymal Stem Cells on Radiation-Induced Skin Wounds in Rats. Cell Transplant 28:105–115

70. Nuzzo A, Giuffrida D, Piccoli E, Zenerino C, Barrile R, Todros T, Rolfo A (2014) Anti-inflammatory and pro-angiogenic effects of placental mesenchymal stromal cells conditioned media on preeclamptic placental tissue. Placenta 35:A87

71. Choi JH, Jung J, Na K-H, Cho KJ, Yoon TK, Kim GJ (2014) Effect of Mesenchymal Stem Cells and Extracts Derived from the Placenta on Trophoblast Invasion and Immune Responses. Stem Cells Dev 23:132–145

72. Lui NA, Jeyaram G, Henry A (2019) Postpartum Interventions to Reduce Long-Term Cardiovascular Disease Risk in Women After Hypertensive Disorders of Pregnancy: A Systematic Review. Front Cardiovasc Med 6:160

73. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY (2010) A Long-Term Follow-Up Study of Intravenous Autologous Mesenchymal Stem Cell Transplantation in Patients With Ischemic Stroke. Stem Cells 28:1099–1106

74. Steinberg GK, Kondziolka D, Wechsler LR, et al (2016) Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke. Stroke 47:1817–1824

75. Hare JM, Traverse JH, Henry TD, et al (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

76. Mathiasen AB, Qayyum AA, Jørgensen E, Helqvist S, Kofoed KF, Haack-Sørensen M, Ekblond A, Kastrup J Bone marrow-derived mesenchymal stromal cell treatment in patients with ischaemic heart failure: final 4-year follow-up of the MSC-HF trial. Eur J Heart Fail. https://doi.org/10.1002/ejhf.1700

77. Premer C, Wanschel A, Porras V, Balkan W, Legendre-Hyldig T, Saltzman RG, Dong C, Schulman IH, Hare JM (2019) Mesenchymal Stem Cell Secretion of SDF-1α Modulates Endothelial Function in Dilated Cardiomyopathy. Front Physiol 10:1182

78. Goldman-Wohl DS, Yagel S (2007) Examination of distinct fetal and maternal molecular pathways suggests a mechanism for the development of preeclampsia. J Reprod Immunol 76:54–60

79. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG (1993) Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol (Baltimore, Md 1950) 151:4562–4573

80. de Groot CJM, van der Mast BJ, Visser W, De Kuiper P, Weimar W, Van Besouw NM (2010) Preeclampsia is associated with increased cytotoxic T-cell capacity to paternal antigens. Am J Obstet Gynecol 203:496.e1-496.e6

81. Saito S (2010) Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia. Immunol Cell Biol 88:615–617

82. Manavalan JS, Rossi PC, Vlad G, Piazza F, Yarilina A, Cortesini R, Mancini D, Suciu-Foca N (2003) High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. Transpl Immunol 11:245–258

83. Siegel G, Schäfer R, Dazzi F (2009) The immunosuppressive properties of mesenchymal stem cells. Transplantation 87:S45-49

84. Yi T, Song SU (2012) Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. Arch Pharm Res 35:213–221

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

86. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE (2006) Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol (Baltimore, Md 1950) 177:2080–2087

87. 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 35:1482–90

88. Taglauer ES, Trikhacheva AS, Slusser JG, Petroff MG (2008) Expression and Function of PDCD1 at the Human Maternal-Fetal Interface1. Biol Reprod 79:562–569

89. 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

90. Fang X, Abbott J, Cheng L, Colby JK, Lee JW, Levy BD, Matthay MA (2015) Human Mesenchymal Stem (Stromal) Cells Promote the Resolution of Acute Lung Injury in Part through Lipoxin A4. J Immunol 195:875–881

91. Lee JW, Fang X, Krasnodembskaya A, Howard JP, Matthay MA (2011) Concise review: Mesenchymal stem cells for acute lung injury: role of paracrine soluble factors. Stem Cells 29:913–919

92. Liu K, Guo L, Zhou Z, Pan M, Yan C (2019) Mesenchymal stem cells transfer mitochondria into cerebral microvasculature and promote recovery from ischemic stroke. Microvasc Res 123:74–80

93. Huang W, Lv B, Zeng H, et al (2015) Paracrine Factors Secreted by MSCs Promote Astrocyte Survival Associated With GFAP Downregulation After Ischemic Stroke via p38 MAPK and JNK. J Cell Physiol 230:2461–2475

94. Ma Y, Hao X, Zhang S, Zhang J (2012) The in vitro and in vivo effects of human umbilical cord mesenchymal stem cells on the growth of breast cancer cells. Breast Cancer Res Treat 133:473–485

95. Chen L, Xu Y, Zhao J, Zhang Z, Yang R, Xie J, Liu X, Qi S (2014) Conditioned medium from hypoxic bone marrow-derived mesenchymal stem cells enhances wound healing in mice. PLoS One 9:e96161

96. Zhang B, Wang M, Gong A, et al (2015) HucMSC-Exosome Mediated-Wnt4 Signaling Is Required for Cutaneous Wound Healing. Stem Cells 33:2158–2168

97. Park HW, Moon H-E, Kim H-SR, et al (2015) Human umbilical cord blood-derived mesenchymal stem cells improve functional recovery through thrombospondin1, pantraxin3, and vascular endothelial growth factor in the ischemic rat brain. J Neurosci Res 93:1814–1825

98. Koch JM, D'Souza SS, Schwahn DJ, Dixon I, Hacker TA (2016) Mesenchymoangioblast-derived mesenchymal stromal cells inhibit cell damage, tissue damage and improve peripheral blood flow following hindlimb ischemic injury in mice. Cytotherapy 18:219–228

99. Al-Rifai R, Nguyen P, Bouland N, Terryn C, Kanagaratnam L, Poitevin G, François C, Boisson-Vidal C, Sevestre M-A, Tournois C (2019) In vivo efficacy of endothelial growth medium stimulated mesenchymal stem cells derived from patients with critical limb ischemia. J Transl Med 17:261

100. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ (2009) Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5:54–63

101. Katsha AM, Ohkouchi S, Xin H, Kanehira M, Sun R, Nukiwa T, Saijo Y (2011) Paracrine factors of multipotent stromal cells ameliorate lung injury in an elastase-induced emphysema model. Mol Ther 19:196–203

102. Yu B, Zhang X, Li X (2014) Exosomes derived from mesenchymal stem cells. Int J Mol Sci 15:4142–4157

103. Komaki M, Numata Y, Morioka C, et al (2017) Exosomes of human placenta-derived mesenchymal stem cells stimulate angiogenesis. Stem Cell Res Ther 8:219

104. Liang X, Zhang L, Wang S, Han Q, Zhao RC (2016) Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. J Cell Sci 129:2182 LP-2189

105. Xiong X, Sun Y, Wang X (2019) HIF1A/miR-20a-5p/TGFβ1 axis modulates adipose-derived stem cells in a paracrine manner to affect the angiogenesis of human dermal microvascular endothelial cells. J Cell Physiol 1–11

106. Li X, Song Y, Liu F, et al (2017) Long Non-Coding RNA MALAT1 Promotes Proliferation, Angiogenesis, and Immunosuppressive Properties of Mesenchymal Stem Cells by Inducing VEGF and IDO. J Cell Biochem 118:2780–2791

107. Todd N, Mcnally R, Alqudah A, Krasnodembskaya A, Mcclements L (2018) MESENCHYMAL STEM CELLS INFLUENCE TROPHOBLAST AND ENDOTHELIAL CELL FUNCTIONALITY IMPORTANT FOR PREVENTION OF PRE-ECLAMPSIA VIA A NOVEL ANTI-ANGIOGENIC PROTEIN, FKBPL. J Hypertens. https://doi.org/10.1097/01.hjh.0000539414.44731.25

108. Bian S, Zhang L, Duan L, Wang X, Min Y, Yu H (2014) Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model. J Mol Med 92:387–397

109. Hwang JH, Lee MJ, Seok OS, Paek YC, Cho GJ, Seol HJ, Lee JK, Oh MJ (2010) Cytokine expression in placenta-derived mesenchymal stem cells in patients with pre-eclampsia and normal pregnancies. Cytokine 49:95–101

110. Chen S, Zhao G, Miao H, Tang R, Song Y, Hu Y, Wang Z, Hou Y (2015) MicroRNA-494 inhibits the growth and angiogenesis-regulating potential of mesenchymal stem cells. FEBS Lett 589:710–717

111. Li P, Guo W, Du L, Zhao J, Wang Y, Liu L, Hu Y, Hou Y (2013) microRNA-29b contributes to pre-eclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. Clin Sci 124:27–40

112. Hoogduijn MJ, Lombardo E (2019) Concise Review: Mesenchymal Stromal Cells Anno 2019: Dawn of the Therapeutic Era? Stem Cells Transl Med. https://doi.org/10.1002/sctm.19-0073

113. Meisel R (2004) Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103:4619–4621

114. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, Muroi K, Ozawa K (2007) Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 109:228–234

115. Sala E, Genua M, Petti L, et al (2015) Mesenchymal Stem Cells Reduce Colitis in Mice via Release of {TSG}6, Independently of Their Localization to the Intestine. Gastroenterology 149:163--176.e20

116. de Araújo Farias V, Carrillo-Gálvez AB, Martin F, Anderson P (2018) TGF-B and mesenchymal stromal cells in regenerative medicine, autoimmunity and cancer. Cytokine Growth Factor Rev 43:25–37

117. Németh K, Leelahavanichkul A, Yuen PST, et al (2008) Bone marrow stromal cells attenuate sepsis via prostaglandin E2{\textendash}dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 15:42–49

118. van der Kraan PM (2013) Transforming Growth Factor-Beta Induced Chrondrogenic Differentiation of Bone Marrow-Derived Mesenchymal Stem Cells: Role of Smad Signaling Pathways. In: Stem Cells Cancer Stem Cells, Vol. 10. Springer Netherlands, pp 85–91

119. Jerkic M, Masterson C, Ormesher L, Gagnon S, Goyal S, Rabani R, Otulakowski G, Zhang H, Kavanagh BP, Laffey JG (2019) Overexpression of {IL}-10 Enhances the Efficacy of Human Umbilical-Cord-Derived Mesenchymal Stromal Cells in E. coli Pneumosepsis. J Clin Med 8:847

120. Gonzalez-King H, Garcia NA, Ontoria-Oviedo I, Ciria M, Montero JA, Sepúlveda P (2017) Hypoxia Inducible Factor-1a Potentiates Jagged 1-Mediated Angiogenesis by Mesenchymal Stem Cell-Derived Exosomes. Stem Cells 35:1747–1759

121. Hu S, Park J, Liu A, Lee J, Zhang X, Hao Q, Lee J-W (2018) Mesenchymal Stem Cell Microvesicles Restore Protein Permeability Across Primary Cultures of Injured Human Lung Microvascular Endothelial Cells. Stem Cells Transl Med 7:615–624

122. Morrison TJ, Jackson M V., Cunningham EK, Kissenpfennig A, McAuley DF, O'Kane CM, Krasnodembskaya AD (2017) Mesenchymal Stromal Cells Modulate Macrophages in Clinically Relevant Lung Injury Models by Extracellular Vesicle Mitochondrial Transfer. Am J Respir Crit Care Med 196:1275–1286

123. Pan Q, Wang Y, Lan Q, Wu W, Li Z, Ma X, Yu L (2019) Exosomes Derived from Mesenchymal Stem Cells Ameliorate Hypoxia/Reoxygenation-Injured {ECs} via Transferring {MicroRNA}-126. Stem Cells Int 2019:1–13

124. Galleu A, Riffo-Vasquez Y, Trento C, et al (2017) Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med 9:eaam7828

125. Weiss DJ, English K, Krasnodembskaya A, Isaza-Correa JM, Hawthorne IJ, Mahon BP (2019) The Necrobiology of Mesenchymal Stromal Cells Affects Therapeutic Efficacy. Front Immunol. https://doi.org/10.3389/fimmu.2019.01228

126. Weiss ARR, Dahlke MH (2019) Immunomodulation by Mesenchymal Stem Cells ({MSCs}): Mechanisms of Action of Living, Apoptotic, and Dead {MSCs}. Front Immunol. https://doi.org/10.3389/fimmu.2019.01191

127. Galipeau J, Sensébé L (2018) Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. Cell Stem Cell 22:824–833

128. Cheung TS, Galleu A, von Bonin M, Bornhäuser M, Dazzi F (2019) Apoptotic mesenchymal stromal cells induce prostaglandin E2 in monocytes: implications for the monitoring of mesenchymal stromal cells activity. HemaSphere 3:402–403

129. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, Baan CC, Dahlke MH, Hoogduijn MJ (2012) Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. https://doi.org/10.3389/fimmu.2012.00297

130. de Witte SFH, Luk F, Parraga JMS, et al (2018) Immunomodulation By Therapeutic Mesenchymal Stromal Cells ({MSC}) Is Triggered Through Phagocytosis of {MSC} By Monocytic Cells. Stem Cells 36:602–615

131. Chekir C, Nakatsuka M, Noguchi S, Konishi H, Kamada Y, Sasaki A, Hao L, Hiramatsu Y (2006) Accumulation of Advanced Glycation End Products in Women with Preeclampsia: Possible Involvement of Placental Oxidative and Nitrative Stress. Placenta 27:225–233

132. Chen X, Zhang Y, Wang W, Liu Z, Meng J, Han Z (2018) Mesenchymal Stem Cells Modified with Heme Oxygenase-1 Have Enhanced Paracrine Function and Attenuate Lipopolysaccharide-Induced Inflammatory and Oxidative Damage in Pulmonary Microvascular Endothelial Cells. Cell Physiol Biochem 49:101–122

133. Zhang Z, Zhu W, Ren H, Zhao X, Wang S, Ma H, Shi X (2017) Mesenchymal stem cells increase expression of heme oxygenase-1 leading to anti-inflammatory activity in treatment of acute liver failure. Stem Cell Res Ther. https://doi.org/10.1186/s13287-017-0524-3

134. Camara NOS, Soares MP (2005) Heme oxygenase-1 ({HO}-1), a protective gene that prevents chronic graft dysfunction. Free Radic Biol Med 38:426–435

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

136. Liu H, McTaggart SJ, Johnson DW, Gobe GC (2012) Anti-oxidant pathways are stimulated by mesenchymal stromal cells in renal repair after ischemic injury. Cytotherapy 14:162–172

137. Chen Y-T, Sun C-K, Lin Y-C, et al (2011) Adipose-Derived Mesenchymal Stem Cell Protects Kidneys against Ischemia-Reperfusion Injury through Suppressing Oxidative Stress and Inflammatory Reaction. J Transl Med 9:51

138. Zhuo W, Liao L, Xu T, Wu W, Yang S, Tan J (2011) Mesenchymal Stem Cells Ameliorate Ischemia-Reperfusion-Induced Renal Dysfunction by Improving the Antioxidant/Oxidant Balance in the Ischemic Kidney. Urol Int 86:191–196

139. Torralba D, Baixauli F, Sánchez-Madrid F (2016) Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. Front Cell Dev Biol. https://doi.org/10.3389/fcell.2016.00107

140. Paliwal S, Chaudhuri R, Agrawal A, Mohanty S (2018) Regenerative abilities of mesenchymal stem cells through mitochondrial transfer. J Biomed Sci. https://doi.org/10.1186/s12929-018-0429-1

141. 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{\textendash}reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. Microvasc Res 92:10–18

142. Fergie N, Todd N, McClements L, McAuley D, O'Kane C, Krasnodembskaya A (2019) Hypercapnic acidosis induces mitochondrial dysfunction and impairs the ability of mesenchymal stem cells to promote distal lung epithelial repair. FASEB J 33:5585-5598

• *This paper highlights the altered behaviour of mesenchymal stem cells (MSCs)- specifically, lacking the ability to transfer mitochondria- in hypercapnic/high CO2 patients; suggesting that different environmental conditions can affect the therapeutic potential of MSCs.*

143. Vaka VR, McMaster KM, Cunningham MW, Ibrahim T, Hazlewood R, Usry N, Cornelius DC, Amaral LM, LaMarca B (2018) Role of Mitochondrial Dysfunction and Reactive Oxygen Species in Mediating Hypertension in the Reduced Uterine Perfusion Pressure Rat Model of Preeclampsia. Hypertension 72:703–711

144. McCarthy C, Kenny LC (2016) Therapeutically targeting mitochondrial redox signalling alleviates endothelial dysfunction in preeclampsia. Sci Rep 6:32683

145. Vakhshiteh F, Atyabi F, Ostad SN (2019) Mesenchymal stem cell exosomes: a two-edged sword in cancer therapy. Int J Nanomedicine 14:2847–2859

146. Sunderland N, Hennessy A, Makris A (2011) Animal Models of Pre-eclampsia. Am J Reprod Immunol 65:533–541