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Suvakov, S., Richards, C., Nikolic, V., Simic, T. P., McGrath, K., Krasnodembskaya, A., & McClements, L. (2020). Emerging Therapeutic Potential of Mesenchymal Stem/Stromal Cells in Preeclampsia. *Current Hypertension Reports*, 22, [37]. <https://doi.org/10.1007/s11906-020-1034-8>

Published in:
Current Hypertension Reports

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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Emerging therapeutic potential of mesenchymal stem/stromal cells in preeclampsia

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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 ($\geq 160/110$) 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 β 1 (TGF- β 1), 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].

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 [54•]). 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 effect of MSCs in pre-clinical models of liver, kidney, heart, skin, lung and other diseases, however, their role in preeclampsia has only been addressed recently [55–57].

MSCs are considered immunoprivileged cells as they can be infused into either autologous or allogeneic hosts owing to their lack of host immune reactivity [58]. MSCs have demonstrated therapeutic effects in animal models of multiple sclerosis, rheumatoid arthritis, myasthenia gravis and diabetes mellitus [59–62]. 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 [63]. Following MSCs intravenous injection, rats demonstrated lower plasma levels of TNF- α , IL-6, IL-12, and ICAM-1, while IL10 was increased [63]. In a separate study, using a Th-1 induced model 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 [64, 65]. Interestingly, it appears that the mechanism of these MSC-mediated therapeutic effects is through secreted factors rather than cell-to-cell contact [66•]. Indeed, paracrine effects of MSCs have been highlighted as important factors in angiogenesis and promotion of wound repair [67, 68•]. 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 [69]. 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 [66•, 70].

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 [71, 72]. 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 [73], we reviewed a few of these studies [74], [75], [76], [77], [78], [79] 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 [80]. 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 [81, 82]. 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 [83]. Notably, the immunosuppressive function of Treg cells comes from the functional characteristics of dendritic cells (DCs), which constantly induce immunosuppressive functions of Treg cells [84]. 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) [85, 86]. By altering the cytokine profile of DCs, MSCs suspend their pro-inflammatory potential and influence Treg cells generation [87]. Also, MSCs alter Th17 differentiation in two different ways: i) by inducing IL-4 production, needed for Th2 phenotype, and ii) by inhibiting IFN- γ 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 [87, 88]. 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-1 β in preeclampsia. In the same manner, MSCs may reduce exaggerated inflammation caused by Th17 differentiation, therefore contributing to the immune homeostasis required during pregnancy [87]. 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 [89, 90].

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 [91]. 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 [92–101]. 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 [69]. 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, 102, 103]. 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 [104].

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 [105]. Several other studies including our own work, demonstrated similar pro-angiogenic capabilities of MSCs on endothelial and trophoblast migration, invasion and tube formation [106–109].

In addition, a growing number of animal models of ischaemic injury and preeclampsia have begun examining the mechanism of MSC promotion of angiogenesis [63, 65, 110]. 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 MSC-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 [111–113].

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 [63, 68•, 113]. 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- α stimulated Gene/Protein 6 (TSG6), TGF- β , Prostaglandin-E₂ (PGE₂) and LipoxinA4 [92, 115–119]. These findings paved the way to improving effectiveness of MSC-based therapies through gene modification, to overexpress several different soluble mediators, such as TGF β or IL-10 [120, 121]. Interestingly, Gonzales-King et al. demonstrated that exosomes derived from HIF-1 α 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* [122]. 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 [123]. 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 [124]. 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 [125].

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 GMP cell manufacturing facility 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 [126]). These apoptotic MSCs induce anti-inflammatory effects through modulation of phagocytic cells involved in their clearance (reviewed in [127–129]). 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 [126]. 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 [130]. These findings are in line with the studies from the Hoogduijn's group suggesting that infused MSCs are rapidly phagocytosed by monocytes [131, 132]. 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. Taken together 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•, 132]. 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 [134, 135]. 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 [136]. 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 [137, 138].

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 [139]. 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 [140].

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 [54•, 140, 141]). 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 [143]. 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 [94]. We have recently demonstrated that mitochondrial transfer from MSC to pulmonary epithelial cells restores epithelial cell mitochondrial membrane potential significantly reduced by inflammatory environment [143•]. Mitochondrial dysfunction has been implicated in preeclampsia and targeting mitochondrial-mediated oxidative stress has been shown to alleviate endothelial dysfunction in preeclampsia [145, 146].

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 [66•]. 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 [147]. 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 [148].

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.

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