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Abstract

The therapeutic potential of stem cell-based therapies may be largely dependent on the ability of stem cells to modulate host cells rather than on their differentiation into host tissues. Within the last decade, there has been considerable interest in the inter-cellular communication mediated by the transfer of cytoplasmic material and organelles between cells. Numerous studies have shown that mitochondria and lysosomes are transported between cells by various mechanisms, such as tunnelling nanotubes, microvesicles and cellular fusion. This review will focus on the known instances of organelle transfer between stem cells and differentiated cells, what effects it has on recipient cells and how organelle transfer is regulated.

Introduction

Previous research has provided convincing evidence that stem cell-based therapies hold great therapeutic potential for many diseases (1). While their mechanisms of action are still under investigation, the therapeutic influence of stem cells may be largely dependent on their modulation of host cells rather than their differentiation in host tissues as they have relatively poor survival and engraftment following transplantation. Therefore, attention has now focused on examining the intercellular interactions underlying stem cell effects and the role of their secretome in cell-to-cell communication.

Intercellular communication is vital for all biological processes, including the maintenance of tissue homeostasis, regulation of normal cellular function and response to external environmental signals that impact cell survival. Cells continuously interact with each other and have the capacity to connect with both

contiguous and distant cells through a wide range of communication networks, such as: paracrine signalling, transport through gap junctions and electrical coupling, tunnelling nanotubes and extracellular vesicle secretion. Within the last decade, there has been considerable interest in the different types of cell-to-cell interaction which include the transfer of cytoplasmic material and organelles between cells which will be the main focus of this review. We will discuss the major mechanisms of stem cell-mediated organelle transfer, the consequences of such transfer to recipient cells and how organelle transfer is regulated. The majority of information on stem cell-mediated organelle transfer has arisen from studies investigating the mechanisms of mesenchymal stem cell (MSC) therapeutic efficacy with only a small proportion of studies describing organelle transfer from other stem cell types. **Table 1** summarises existing studies on intercellular organelle transfer between different types of stem cells and differentiated cells in various disease models.

Mechanisms of Organelle Transfer

Organelle exchange between cells can occur via three potential means: tunnelling nanotubes (TNTs), extracellular vesicles (EVs) and cellular fusion. **Figure 1** diagrammatically illustrates the different modes of organelle transfer between stem and differentiated cells as well as the functional outcomes of such transfer for recipient cells.

Tunnelling Nanotubes

Tunnelling nanotubes (TNTs) are actin-based extensions of the cell cytoplasm that form open-ended channels between communicating cells. TNTs are approximately 50-200 nm in diameter and can span several cell diameters in length (2). Formation

of these tubular structures has mainly been identified in *in vitro* co-culture systems between various cell types, including: stem, immune (monocytes, macrophages and neutrophils), neuronal and cancer cells (3). *In vivo*, TNT-like structures, termed cytonemes, were reported to be involved in signal transduction during the imaginal disc development of *Drosophila* (4,5) and during reproduction of *Plasmodium* in the *Anopheles malaria* midgut (6). TNTs also mediate intercellular communication between immune cells in the lymph nodes (see review by (3,7,8) and between dendritic cells in mouse cornea (9). Furthermore, TNT-like structures were observed in human malignant tumours (10–14) as well as between cardiomyocytes and non-myocytic cells in the heart (15).

TNT biogenesis is not yet fully understood but a number of components are thought to be involved in their formation. It is known that TNTs contain an array of cytoskeletal filaments of which F-actin is the most abundant (2). In addition, microtubules can also exist in TNTs of specific cell types, such as immune cells (16), astrocytes and primary neuronal cells, which form much thicker nanotubules (17). Bukoreshtliev and colleagues reported that nanotubules are formed by extension and retraction of filopodia that physically make contact with and connect contiguous cells (18).

TNT-mediated cell contact results in the transfer of intracellular content between communicating cells. The presence of several motor proteins, like calcium-sensitive dynamin related Rho-GTPases Miro1 and Miro2 (19,20), KLF 5 kinesin motor protein (21) and accessory proteins like TRAK 1 and TRAK 2 (22,23), Myo 19 and Myo 10 (24) permit the efficient shipping of cargo between cells via an actin-myosin-

dependent mechanism (25). Transported cargo includes large organelles, like mitochondria (26–30) and smaller membranous vesicles of the endocytic pathway, such as lysosomes, which are typically transported bi-directionally (31,32). Once the tubule protrudes from the donor cell's plasma membrane, it makes contact and fuses with the recipient's plasma membrane allowing organelle deposition into the cytosol (2). However, it is not clear yet if the fusion process is specific and requires some recognition event or if it occurs spontaneously. Liu et al demonstrated that establishment of TNTs between MSCs and oxidative stress-injured endothelial cells (HUVECs) 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 (29). Additionally, TNTs were shown to be responsible for the transmission of electrical signals between kidney cells (33) as well as calcium signalling between dendritic and monocyte cells (34), reflecting the multifaceted nature of TNTs in normal cellular functioning.

Many environmental cues are known to induce TNT development between cells, such as hydrogen peroxide, serum starvation (35) and cytokines (36). Wang et al demonstrated that TNT formation in astrocytes and neurons involved p53, EGFR, Akt, PI3K and mTOR activation (35). They also showed that it is the stressed cells that always develop TNTs which extend to the unstressed cells but whether or not these findings are applicable to other cell types requires further investigation.

In the pioneering work of Rustom et al, it was shown that TNTs actively transfer vesicles positive for markers of the endosomal/lysosomal system and plasma

membrane components but do not transfer soluble cytoplasmic proteins (2). Koyangi et al were the first to report that TNTs are also capable of transferring mitochondria from cardiomyocytes to human endothelial progenitor cells in co-culture (37). Since then, numerous studies have demonstrated that MSCs utilise TNTs to transfer mitochondria to various cell types. Similarly, TNT-mediated transport of lysosomes from early progenitor cells to injured or stressed endothelial cells results in the reconstitution of the lysosomal pool which attenuates premature cell senescence and tension of blood vessels through increased vaso-relaxation (38) (**Figure 1 A**).

In addition to the transfer of organelles, TNTs are also involved in the transport of cytoplasmic content (26,39-41). With regards to stem cells, transfer of cytoplasmic content from differentiated cells was associated with MSC differentiatiation towards cardiomyocytes (39) or renal tubular cells (26) but not neurons (41). In these studies, it was observed that transport of cytoplasmic contents was predominantly directed from differentiated cells towards MSCs. Figeac and colleagues demonstrated that communication between MSCs and stressed cardiomyocytes via TNTs (through bi-directional cytoplasmic exchange and mitochondrial transfer) was crucial for MSC therapeutic efficacy in a mouse model of myocardial infarction and enhanced the secretion by cardioprotective soluble factors by MSCs (40).

Extracellular Vesicles

Organelle transfer between cells can also occur through their secretion in extracellular vesicles (EVs). This is an umbrella term used to describe a heterogeneous group of biologically active membrane-encompassed vesicles released by cells (42,43). EVs are found in numerous bodily fluids, including: urine, plasma, whole-blood as well as *in vitro* culture medium. They can carry lipids, proteins, enzymes, coding and non-coding RNA molecules. Upon interaction with their target cells, EVs can be internalised and their cargo released inside the recipient cell(s). Therefore, EVs act as envoys for long-distance cross-talk between cells (44–46) and are capable of heavily influencing target cell function (47). To date, documented evidence of organelle transfer in EVs only exists for mitochondria but it is plausible to suggest that ribosomes and lysosome-like structures may also be transported. According to their size and biogenesis, EVs can be categorised into three main subtypes: exosomes, microvesicles and apoptotic bodies (48,49).

- Exosomes are small homogenous membrane-coated vesicles ranging from 30-100 nm in diameter (50,51). They are generated from the late endosomal pathway. During endosome maturation, parts of the endosomal outer membrane bud inside as intraluminal vesicles forming multivesicular bodies (MVBs) (50,51) MVBs subsequently move to and fuse with the plasma membrane of the cell leading to the extrusion of exosomes into the surrounding extracellular environment (52,53) Exosome release is typically regulated by activation of the cell cytoskeleton but not the influx of calcium (54,55). Exosomes are known to contain a multitude of endosomal markers,

such as CD9 and CD63 as well as some heat-shock proteins, including Hsp90, Hsp60 and Hsp70. They also carry a variety of molecular cargo, like proteins (56,57) and genetic components, such as messenger and microRNAs (58). Due to their small size, it is unlikely that exosomes could carry larger organelles, like mitochondria. On the contrary, they may transfer organelle fragments (such as protein complexes of the mitochondrial electron transfer chain) and ribosomes.

Interaction with target cells can be achieved through receptor-ligand signalling (59). It has been suggested that integrins may play a vital role in the homing of exosomes to endothelial cells, which express the VCAM-1 integrin receptor, or cardiomyocytes which express ICAM-1 following myocardial ischaemia-reperfusion injury (60). Additionally, tetraspanin proteins could assist in the uptake of exosomes as these are primarily involved in invasion and fusion events within cells (61). Other likely means of exosomal internalisation by cells include: endocytosis, phagocytosis and membrane fusion (59).

- Microvesicles are heterogeneous structures that are formed by the protrusion, external budding and fission of the cell's plasma membrane which subsequently liberates spherical structures, containing cargo, into the extracellular space (62). They are the largest of the three vesicle types, ranging from 50-1000 nm in diameter (51) but can also reach sizes up to 10 μM in the case of cancer cells were they are commonly referred to as oncosomes (62). One of the best known mechanisms of microvesicle

biogenesis is the recruitment of TSG101 protein to the cell surface by arrestin domain-containing protein 1 (ARRDC1). Unlike MVB-derived exosomes, ARRDC1-mediated microvesicles (ARMMs) do not express late endosomal markers. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at the plasma membrane is mediated by the recruitment of TSG101 protein (63). Microvesicle biogenesis is influenced by both intracellular calcium concentrations and cytoskeleton activation. Like exosomes, they transport lipids, proteins, mRNAs and microRNAs and also communicate with their cellular target through receptor-ligand interactions, phagocytosis, membrane contact and endocytic pathways (59). Cargo sorting and microvesicle shedding are regulated by small GTPases, including members of the ARF, Rab and Rho families (reviewed in (62)).

Islam et al were the first group to report the phenomenon of EV-mediated mitochondrial transfer from MSCs to lung alveolar epithelial cells (28), followed by the elegant study by Phinney and colleagues which demonstrated that MSC-derived EVs contained functional mitochondria which were subsequently internalised by macrophages and as a result, enhanced their levels of oxidative phosphorylation. Simultaneously, shedding of exosomes from MSCs regulated toll-like receptor signalling and cytokine production through the transfer of regulatory microRNAs, particularly miR-451 (64). According to their study, mitochondria-containing EVs were positive for microtubule-associated protein 1 light chain 3 (LC3) and autophagy-related protein 12 which are characteristic of autophagosomes. These EVs also expressed the endosomal sorting complex required for transport (ESCRT) TSG101 and ARRDC1, suggesting that their biogenesis was mediated through the

microvesicle formation pathway (64) (**Figure 1B**). Besides mitochondria, EVs can also transfer ribosomes. It was previously demonstrated by Court and colleagues that shwann cells use EVs to transfer polyribosomes to axons (65). Although no reports have yet identified the presence of ribosomes in EVs originating from stem cells, this could be a potential mechanism for modifying gene expression and protein production within recipient cells.

- Cells undergoing apoptosis also release heterogeneous populations of EVs. These can be small (50–1,000 nm), exosomal-like vesicles which carry a variety of potentially biologically active components, including small molecules, proteins and nucleic acids. Larger vesicles (1 to several microns in diameter), referred to as "apoptotic bodies," can carry organelles, such as mitochondria, nuclear fragments and endoplasmic reticulum. Some of these vesicles are released through blebbing of cellular membranes whereas some may originate from the endosomal pathway (47). Dieudé et al discovered that endothelial cells undergoing apoptosis release small exosomal-like vesicles containing active proteosomal complexes which are released following caspase-3 activation. The presence of proteosomal complexes was found to be a major trigger for the production of anti-perlecan antibodies and acceleration of aortic graft rejection in mice (66).

Recently, other mechanisms of EV formation during apoptosis have been reported. Apoptotic T cells were found to form fine protusions, termed 'apoptopodia' that appear to be involved in the release of EVs greater than 1 μm (67). EV production

from late stage apoptotic monocytes but not neuronal cells, squamous epithelial cells and cervical epithelial cells has been observed to involve fragmentation of membrane protrusions resembling 'beads on a string' (68). While the significance of these observations remains to be fully elucidated, EVs produced from fragmentation of beaded apoptopodia were found to be enriched in mitochondria and acidic organelles but devoid of nuclear components, including histones and DNA (68) that are well-known constituents of apoptotic bodies. Intriguingly, Galleu et al showed that the immunosuppressive properties of MSCs in graft-verses-host disease was dependent on the capacity of cytotoxic host cells to induce perforin-dependent apoptosis in MSCs and on subsequent engulfment of apoptotic MSCs by host phagocytes. Furthermore, the authors demonstrated that the presence of activated cytotoxic cells in human patients is predictive of MSC therapeutic efficacy and postulated that the capacity of recipient cells to induce apoptosis in MSCs is necessary for MSC therapeutic effects (69). Although the immunosupressive effect of apoptotic MSCs was largely explained by enhanced Indoleamine-pyrrole 2,3-dioxygenase (IDO) secretion by phagocytes, organelle transfer from MSCs to host cells was not investigated in this study. However, it is definitely possible and it's impact for the observed immunosuppressive effect will require further investigation.

Cell fusion

Cell fusion is a form of intercellular communication where the plasma membrane of two independent cells merge together whilst retaining nuclear morphology. In doing so, cytosolic constituents and organelles are shared between these cells, particularly if there is permanent fusion. On the other hand, partial cell fusion involves direct but

transient exchange of subcellular organelles, like mitochondria, and protein complexes between cells. These events are extremely rare and should only occur under certain conditions which is why stringent regulation of fusion protein and receptor expression is vital (70). The physical relevance of these processes between cells remains elusive but some studies have reported that both embryonic and adult stem cells can fuse with cardiomyocytes (27), neurons (71) and hepatocytes (72), resulting in the generation of hybrid multinucleated cells with simultaneous expression of markers specific for progenitor and differentiated cells (73). Even though the factors that initiate cellular fusion events are still under investigation, well-known insults, such as inflammation and injury, considerably drive this process in recipient cells and tissues (49). Acquistapace et al observed that both human adipose tissue- and bone marrow-derived MSCs reprogram mouse cardiomyocytes towards a progenitor-like state upon partial cell fusion. These hybrid cells expressed early proliferation and commitment markers, including myocyte enhancer factor 2C and GATA-4, indicating a potential regenerative mechanism whereby cardiomyocytes regain their proliferative and survival properties to repair damaged heart tissue (27). The authors further noted that restoration of cardiomyocyte function and survival was facilitated by the transfer and persistence of stem cell mitochondria within distressed heart cells (**Figure 1 C**).

<u>Mitochondrial transfer</u>

Accumulating evidence now suggests that MSCs transport mitochondria to numerous cell types, including: endothelial, epithelial, cardiac, renal, corneal and immune cells, particularly under conditions of stress or injury **(Figure 1)**. Islam et al

reported that bone marrow-derived MSCs effectively transfer mitochondria to type 2 alveolar epithelial cells (ATII) in a lipopolysaccharide (LPS)-induced mouse model of acute lung injury. Transfer resulted in enhanced ATP production, restoration of surfactant secretion by ATII cells, amelioration of acute lung injury and improved survival, highlighting a key role of exogenous mitochondria in the improvement of cell bioenergetics (28). Interestingly, although mitochondrial transfer occurred through the secretion of EVs, it was demonstrated that the formation of connexin 43-containing gap junctions between MSCs and ATII cells was required for successful mitochondrial transfer. Subsequently, TNT-mediated transfer of mitochondria from MSCs to bronchial epithelial cells was shown to be protective in chronic obstructibe pulmonary disease (COPD) and asthma mouse models (19,74) Rho-like GTPases located on the outer mitochondrial membrane, such as Miro-1, are intricately involved in the regulation of TNT-mediated mitochondrial transfer from MSCs to lung epithelial cells (19). Moreover, over-expression of Miro-1 in MSCs enhances mitochondrial transfer and substantially attenuates rotenone-induced lung injury and hyper-responsiveness in the airways of asthmatic mice (19). However, the exact role of connexin-43 in MSC intercellular communication remains controversial. Recent studies have shown that blocking connexin-43 gap junctions does not affect the ability of MSCs to establish contact with macrophages or bronchial epithelial cells via TNTs as well as the rate of mitochondrial transport (30) (75).

Phinney and authors demonstrated the outsourcing of partially depolarised mitochondria released from MSCs within microvesicles to recipient macrophages which were engulfed by phagocytosis. Transfer of mitochondria greatly improved ATP turnover in macrophages *in vitro* but also served as a survival mechanism for MSCs under oxidative stress conditions (64). We have also demonstrated

mitochondrial transfer from MSCs to macrophages via TNTs which resulted in the enhancement of macrophage bioenergetics and improvement of their phagocytic activity both in *in vitro* and *in vivo* models of *Escherichia-coli*-induced lung injury (30). Interestingly in this study, we found that when TNTs were blocked by Cytochalasin B, mitochondrial transfer to macrophages still occurred via EV secretion, suggesting that both mechanisms act simultaneously. In a subsequent study, we have found that mitochondrial transfer in MSC EVs augmented levels of oxidative phosphorylation in macrophages, resulting in their metabolic reprogramming from a pro-inflammatory towards an anti-inflammatory phenotype with enhanced phagocytic activity (76).

Numerous studies have reported mitochondrial transfer directed from MSCs to cardiomyocytes (27,36,39,77) via partial cell fusion or TNTs. Interestingly, Koyanagi et al (37) observed mitochondrial transfer from cardiomyocytes to endothelial progenitor cells (EPCs), resulting in stem cells expression of cardiomycyte-specific proteins. Also, in the study of Mahrouf-Yourgof and colleagues, it was found that in the situation of oxidative stress, damaged mitochondria are transferred from cardiomyocytes or endothelial cells to MSCs, although the mode of such transfer was not investigated in detail (84), this transfer acted as a trigger of mitochondrial donation from MSC to stressed cells and promoted MSC anti-apoptotic properties..

Liu et al demonstrated TNT-mediated mitochondrial transfer from MSCs to endothelial HUVEC cells injured by oxidative stress (29).
Jiang and authors reported that MSC-mediated mitochondrial transfer rescued corneal epithelial cells from Rotenone (Rot)-induced oxidative stress which was

further detected in their *in vivo* rabbit model of alkali eye injury. Notably, in this study, MSCs with impaired mitochondrial function were not able to improve corneal epithelial cell wound healing *in vivo* (78).

Unidirectional TNT-mediated transfer of mitochondria between MSCs and neural cells and astrocytes was detected *in vitro* and confirmed in rat brains *in vivo* (41)*.* In this study, rat brains were injected with mitoGFP-expressing MSCs. Interestingly, GFP-positive particles were found in the cellular bodies of neurons in rat brain slices.

Consequences of mitochondrial transfer to recipient cells

At present, the majority of studies suggest that transfer of functional mitochondria results in the improvement of mitochondrial respiration, ATP production and/or mitigation of mitochondrial ROS levels in recipient cells leading to improved functional activity (e.g. surfactant secretion, phagocytosis and wound healing) and viability (**Table 1**). In addition, there is evidence alluding to the involvement of mitochondrial transfer in shaping the nuclear transcriptional landscape and contributing to cellular reprogramming. Acquistapace et al revealed that MSC mitochondria were responsible for reprogramming cardiomyocytes towards earlier progenitors (27). Zhao and collaborators showed that platelets were modulated in diabetic patients who received a novel type of stem cell educator therapy. This technique utilised autologous patient lymphocytes which were briefly exposed to adherent stem cells from cord blood in the therapy device (79). Treatment with a single dose of this therapy caused permanent reversal of T cell autoimmunity,

regeneration of pancreatic islet β cells and improved metabolic control in patients with diabetes. The authors discovered that after interaction with cord blood stem cells, platelets exhibited immune-tolerance markers, such as autoimmune regulator (AIRE) which could alter immune cell function and proliferation. Human cord- and peripheral blood-derived platelets were also found to express embryonic stem cell markers (OCT4, SOX2, KLF4, and C-MYC), specific human islet B-cell transcription factor MAFA and the pancreatic progenitor-associated marker SOX9. Remarkably, all of these markers were associated with the platelets' mitochondria. Co-culture of cord blood-derived platelets with human islet cells resulted in platelet mitochondrial uptake and improved islet cell viability, proliferation and increases C-peptide release *in vitro.* Furthermore, migration of platelets into pancreatic islets was demonstrated immunohistochemically on biopsies from diabetic subjects. The authors hypothesized that migration of platelets to the pancreas and transfer of mitochondria to pancreatic islet cells were responsible for β-cell neogenesis and long-term clinical improvements in the diabetic patients observed in this trial (79). This is the first report directly demonstrating a key role of mitochondria in tissue regeneration, possibly through the transfer of stemness factors.

However, mitochondrial transfer does not always have beneficial effects. Stem cells have the capacity to deliver functional mitochondria to opportunistic cancer cells which could be detrimental as this may optimise or restore tumor dysfunctional metabolic machinery (80,81,97-100). Tumours devoid of mitochondrial DNA can obtain new DNA from surrounding stromal cells via TNT formation so that respiratory function is re-established for further tumour initiation and even dissemination. This has been observed in acute myeloid leukaemia where MSC mitochondrial transfer confers resistance to chemotherapy treatment (80). By contrast, some reports have

noted that increased mitochondrial transfer from MSCs to malignant cells actually perturbs their proliferative and invasive potential so the role of mitochondria in cancer remains ambiguous (81).

- Regulation of mitochondrial transport

Transport of mitochondria is typically initiated by a complete absence or loss of functional mitochondria from recipient cells, either as a result of mitochondrial DNA damage/depletion due to stress or the artificial inhibition of mitochondria *in vitro*. Interestingly, cells bearing pathogenic DNA mutations that interfere with mitochondrial function do not act as mitochondrial donors (82,83). Mahrouf-Yorgov et al demonstrated that MSCs sense cell stress via engulfment and subsequent degradation of mitochondria from damaged somatic cells (cardiomyocytes and endothelial cells) and this process leads to enhancement of MSC mitochondrial biogenesis via the activation of cytoprotective protein HO-1. This ultimately allows MSCs to donate more mitochondria (84). Similarly, mitochondrial transfer from MSCs to astrocytes was more efficient when recipient cells were exposed to ischemic damage associated with elevated ROS levels (85).

Ahmad at al were the first to demonstrate that over-expression of the Miro-1 protein (mitochondrial Rho-GTPase1 that regulates intercellular mitochondrial movement) in MSCs, enhances efficiency of transfer to bronchial epithelial cells and results in better therapeutic effect in mouse models of Rot-induced airway injury and allergic airway inflammation (19). Furthermore, it was demonstrated that Miro-1 overexpression improves MSC therapeutic efficacy in models of cardiomyopathy (36) and ischemic stroke in rats (85).

Zhang et al found that TNT formation by bone marrow-derived MSCs and induced pluripotent stem cell (iPSC)-derived MSCs is regulated by TNF-a via the TNF-α/NF-κB/TNFαIP2 pathway. This mechanism was critical for a superior therapeutic effect of iPSC-derived MSCs in an *in vivo* model of anthracycline-induced cardiomyopathy which permitted more effective mitochondrial transfer to cardiomyocytes (36).

Mitochondrial transfer from MSCs not only results in protection and/or rejuvenation of injured recipient cells but it is also an important part of MSC survival as it allows them to eliminate partially depolarised and dysfunctional mitochondria. Phinney and colleagues demonstrated that mitochondrial release into EVs is mediated through unaccomplished mitophagy, where instead of fusion with lysosomes and degradation in autolysosome, LC3 positive vesicles with mitochondria were incorporated into the outward budding blebs of the plasma membrane and extruded into the extracellular space. The authors hypothesised that this intricate process could be a part of the MSC cell-survival mechanism to counteract mitochondrial dysfunction induced by oxidative stress (64).

What still remains a mystery is the degree of damage required to incite stem cell-mediated mitochondrial transfer. It is possible that injured cells can resolve their own endogenous organelle network before relying on exogenous sources for recovery but the mechanisms governing this are elusive. Our unpublished observations suggest that mitochondrial transfer from MSCs to recipient cells occurs at a substantial level without any injurious stimuli and is not selective in regard to recipient cell type. However, in the presence of stimulation (inflammatory or oxidative stress), the uptake of MSC mitochondria is significantly enhanced. Another important question that pertains is the fate of exogenous mitochondria once delivered to recipient cells.

It is well established that stem cells utilise glycolysis for their energy needs and switch to oxidative phosphorylation only upon differentiation. Their mitochondria are largely in a dormant state as reflected by their shape (round, undefined cristae), cellular localisation (cytosolic) and number (scarce) as opposed to the mitochondria in cells with higher energetic demands (elongated in shape with well defined cristae and prenuclear localisation) (86). Apparently upon transfer to the recipient cell, these dormant stem cell-derived mitochondria undergo functional and morphological remodelling similar to that observed during differentiation. Here, an increase in mtDNA copy number, enhanced oxygen consumption rate and increased levels of intracellular ATP are observed (87,88) Specific signalling pathways involved in potential stem cell mitochondria remodelling remain to be investigated yet. How do they interconnect with endogenous mitochondria and how does this process alter the phenotype of recipient cells? Both fusion and fission events, involved in mitochondrial quality control, have been suggested to play a major role. Through fusion, mitochondria are able to share their whole contents and is heavily involved in the exchange and repair of mitochondrial DNA, protein complementation and metabolite balance. By contrast, mitochondrial fission allows the removal of aberrant mitochondria from cells through mitophagy and the appropriate segregation of mitochondrial DNA (89). Data from Phinney et al suggest that MSC mitochondria fuse with endogenous macrophage mitochondria and that human mitochondrial DNA (human COXI transcripts) can be found in mice 28 days after MSC administration. On the contrary, MSC nuclear DNA was not detected after 3 days post administration, suggesting that mitochondrial transfer is sustainable and might at least partially explain the long-term therapeutic effects seen after MSC administration (64).

Lysosomal Transfer

Lysosomes are highly dynamic membrane-bound structures that are about 50-500 nm in diameter and are responsible for the internalisation of extracellular material from either endocytosis or phagocytosis as well as intracellular constituents from autophagy (90). Because these organelles contain over 60 potent hydrolytic enzymes together with a highly acidic microenvironment, they also participate in the subsequent degradation of cargo (90,91) which includes: polysaccharides, proteins and complex lipids into their respective constituents (91,92) The mechanism of lysosome locomotion can be both a diffusive and active process, involving ATP and motor proteins, like dyneins and kinesins, which permit the movement of lysosomes along microtubules (93,94) Whether or not the size of lysosomes affects their transport is currently under investigation but one study has noted that their diffusion is inversely proportional to their diameter size (95). Yasuda et al demonstrated that endothelial progenitor cells transfer lysosomes to stressed HUVECs through TNTs. Lysosomal transfer results in the reconstitution of both lysosomal pH and the lysosomal pool during stress, thus enhancing the viability of endothelial cells and mitigating the risk of premature apoptosis or senescence in vasculopathy (38). Similar findings have been described in *in vivo* models of diabetic-induced mice whereby lysosomal transfer of EPCs leads to TNT-dependent attenuation of senescent endothelial cells and therefore, rectifying vaso-relaxation and overall function of endothelial tissue (38). Two types of TNTs, both thick (>0. 7 μm) and thin (<0.7 μm), have been observed between EPCs and HUVECs, indicating that other components may be transferred in addition to organelles, like plasma membrane

proteins and lipids (38). This then raises the important question as to whether or not transfer of individual types or combinations of organelles are implicated in the therapeutic effects of stem cells in various diseases.

Naphade et al showed that hematopetic stem cell (HSC) transplantation is able to correct cystinosis, a multisystemic lysosomal storage disease, caused by a defective lysosomal membrane cystine transporter, cystinosin (CTNS gene). Upon differentiation of HSCs to macrophages, lysosomes carrying the cystinosin transporter are transferred in a bidirectional manner via TNTs between HSC-derived macrophages and cystinosin-deficient fibroblasts. Nanotubular formation was further observed between engrafted HSCs and diseased proximal tubular kidney cells of mice with cystinosis (96).

Conclusions

Stem cells have the capacity to establish very complex and extensive transport networks that allow effective communication with stressed or damaged somatic cells, regardless of lineage. As a result, they serve as important mediators which aim to repair, regenerate and restore cell/tissue function. Despite their huge therapeutic potential in a wide variety of disease states, many questions still remain regarding the mechanisms of such cellular communication, the factors driving this communication and the pathways regulating it. There is convincing evidence to suggest the role of mitochondrial and lysosomal transfer in the therapeutic effects of stem cell-based therapies but the contribution of other organelle transport is ambiguous. Likewise, it is unclear if stem cells are able to replenish their own organelle pool following donation. Further research is imperative to define new ways

of tracking and visualising this process. Additionally, development of more *in vivo*

models may shed some light into the organelles and transport systems that could be

artificially manipulated to optimise the therapeutic potential of stem cells.

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Figure 1 Legend

Different modes of organelle transfer between stem cells and various differentiated cells. Mitochondria are transferred through tunnelling nanotubules (A), extracellular vesicles (B) and cellular fusion (C). Mitochondrial transfer results in improvement in mitochondrial respiration,   restoration of cell function and/ or transcriptional reprogramming. Lysosomes are transferred via tunnelling nanotubules (A).

Table 1 Mechanisms of organelle transfer between different types of stem cells and differentiated cells