REVIEW

Tunneling nanotubes and mesenchymal stem cells: New insights into the role of melatonin in neuronal recovery

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Abstract

Efficient cell-to-cell communication is essential for tissue development, homeostasis, and the maintenance of cellular functions after injury. Tunneling nanotubes (TNTs) have emerged as a new important method of cell-to-cell communication. TNTs are primarily established between stressed and unstressed cells and can transport a variety of cellular components. Mitochondria are important trafficked entities through TNTs. Transcellular mitochondria transfer permits the incorporation of healthy mitochondria into the endogenous network of recipient cells, changing the bioenergetic profile and other functional properties of the recipient and may allow the recipient cells to recuperate from apoptotic processes and return to a normal operating state. Mesenchymal cells (MSCs) can form TNTs and transfer mitochondria and other constituents to target cells. This occurs under both physiological and pathological conditions, leading to changes in cellular energy metabolism and functions. This review summarizes the newly described capacity of melatonin to improve mitochondrial fusion/fission dynamics and promote TNT formation. This new evidence suggests that melatonin's protective effects could be attributed to its ability to prevent mitochondrial damage in injured cells, reduce senescence, and promote anastasis, a natural cell recovery phenomenon that rescues cells from the brink of death. The modulation of these new routes of intercellular communication by melatonin could play a key role in increasing the therapeutic potential of MSCs.

KEYWORDS
intercellular communication, ischemic injury, mesenchymal stem cells, mitochondria, tunneling nanotubes

1 | INTRODUCTION

Efficient cell-to-cell communication is essential for tissue development, homeostasis, and the maintenance of cellular functions after injury. There are a variety of means by which cells communicate; these include exosomes, secreted microRNAs, or gap junctions.[1–3] Tunneling nanotubes (TNTs), initially observed by Roustom and coworkers in 2004 in cultured rat pheochromocytoma PC12 cells[4] and later found in other cell lines,

including primary rat neurons, astrocytes, and immune cells,[5,6] have emerged as a new important method of cell-to-cell communication. TNTs are thin-extended membrane protrusions that connect cells over long distances allowing the exchange of various-sized cargoes, such as small molecules (e.g., calcium ions), macromolecules (nucleic acids, proteins, etc.), and organelles (vesicles, lysosomes, mitochondria, autophagosomes, etc.) between cells.[7] When TNTs connect multiple cells, this leads to functional cellular networks.[8]

Interest in TNTs has significantly increased despite the difficulties in studying them in in vivo experiments, mainly due to the preservation of their delicate structure, the possible confusion with other cellular protrusions, and their functional heterogeneity. Homotypic and heterotypic TNTs, however, play a vital role in disease progression and therapy. TNTs have been linked to developmental processes and signaling between different cell types, including immune cells, mesenchymal stem/stromal cells (MSCs), astrocytes, and neurons, and have been implicated in pathological conditions such as cancer progression and chemotherapy resistance, neurodegeneration[9] and neuroprotection,[10] as well as anastasis, a natural cell recovery phenomenon that rescues cells from the brink of death.[11] TNTs are therefore identified as novel bridges of intercellular communication in physiological and pathological life processes (Figure 1). Here, we review the processes involved in TNTs cellular communication highlighting their role in MSCs cell survival after stress and their possible modulation by melatonin.

2 | TUNNELING NANOTUBES STRUCTURE AND FORMATION

TNTs are nano-scaled membranous tubes of about 50–200 nm diameter and up to several cell diameters long, freely hovering in the medium. TNTs are different entities from filopodia. Indeed, for example, in cell cultures, TNTs are free-floating while filopodia, which are unable to mediate vesicular transport, are connected to the cell plate. Moreover, the regulatory mechanisms responsible for filopodia and TNTs formation work in direct opposition; when a cell encounters a TNT, it retracts its existing filopodia.[12]

To date, two different mechanisms have been proposed to form TNTs. The first commonly referred to as the "cell dislodgement mechanism," describes two cells closely opposed to each other, fusing transiently and retaining a thin membrane thread while they move apart.[13] The second, known as the actin-driven protrusion mechanism, proposes the formation of a filopodium-like protrusion from one cell to another, followed by membrane fusion of the tip upon physical contact. In both mechanisms, actin plays a key role since drugs inhibiting actin polymerization block TNT formation.[14]

Onfelt and coworkers[13] described two classes of TNTs between human monocyte-derived macrophages distinguished by their cytoskeletal composition and functional properties. Thin TNTs (diameter < 0.7 nm) contain only F-actin, whereas thicker TNTs (diameter > 0.7 nm) contain both F-actin and microtubules (microtubule-containing TNTs; MT-TNTs). Thick TNTs appear linked
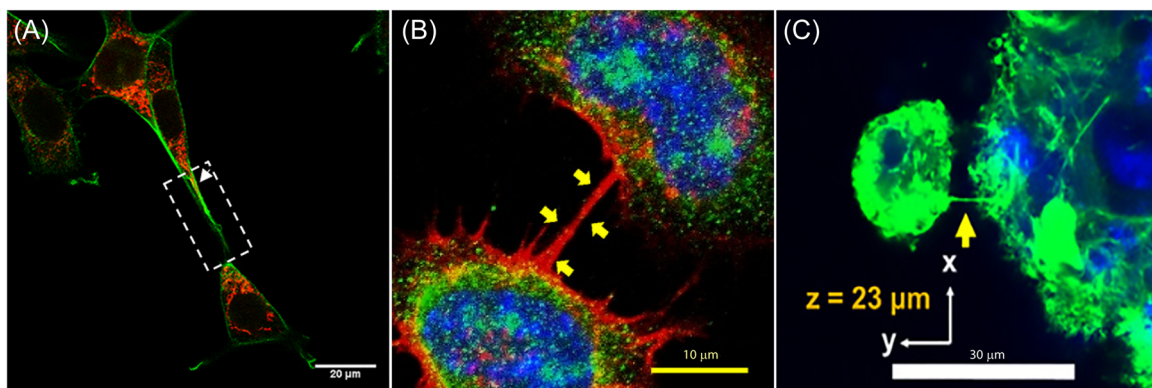

FIGURE 1 Tunneling nanotubes (TNTs) in different cell types. (A) Confocal image of a TNT (white arrow) between HT22 cells exposed to oxygen-glucose deprivation and then treated with melatonin. The overlay shows the co-localization (yellow) of phalloidin (green) and MitoTracker Deep Red (red) indicating the presence of mitochondria within TNT.[100] (B) Confocal image of TNT (yellow arrows) between CAD cells stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue), phalloidin (red), and Ca^{2+}/calmodulin-dependent protein kinase II (βCaMKII, green). The overlay shows the βCaMKII-positive puncta inside a TNT (yellow). Adapted with permission from Vargas et al.[25] C, Confocal image of TNT (yellow arrow) between mesenchymal stem cells (MSCs) and an MSC spheroid stained with Hoechst 33342 (blue) and phalloidin (green). Adapted with permission from Zhang et al. (Copyright [2018] American Chemical Society).[124]

to bidirectional transfers of cellular components, including organelles, whereas thin TNTs containing only F-actin exhibit unidirectional transfers.[15] Thus, TNTs seem heterogeneous in their membrane structure and cell type-specific. Many do not contain microtubules since tubulin blockage does not affect their formation in all cell types.

Stressed and unstressed cells establish different types of TNTs. Wang and Gerdes, for example, showed that UV-treated PC12 cells assembled a type of TNT characterized by continuous microtubule formations (MT-TNTs).[16] These TNTs were different from those formed between untreated cells. They also showed that MT-TNTs could transfer mitochondria and participate in the rescue effects.[16] TNT connections were also observed among neurons and astrocytes. However, only cells containing injured mitochondria at an early apoptotic stage formed MT-TNTs,[16] suggesting that connections are dependent on the vitality status of the cells.

In HBEC-3 cells, it has been shown that cell density affects the ability to form TNTs. In this model, blockage of tubulin did not influence TNTs formation.[17] The authors identified two types of TNTs named TNT1 and TNT2. TNT1 formed a network within groups of cells or linked isolated cells and were probably formed through a directed filopodia-like protrusion, while TNT2 only connected closely adjacent cells. It should be noted that the bulk of the information on TNTs formation between cells originated from experimental work in cell culture since studying TNTs in vivo is significantly more difficult. However, TNT-like structures exist during the embryonic development of chicks[18] and fish.[19] TNT-like structures connecting bone marrow-derived MHC class II cells have been reported in the corneal stroma of GFP chimeric mice[20] and between osteoclasts in the bone destruction sites of arthritic rats.[21] Recently, Chen and colleagues provided in vivo evidence of intercellular transportation of proteins via TNTs in brain cells. They reported TNT development between apical dendrites and nearby astrocytic somas/processes in the prefrontal cortex of adult mice, which allowed intercellular communication, including prion transport.[22]

3 | HOW AND WHERE DO NANOTUBES FORM?

The formation of TNTs is mainly a feature of cells under stress. Hydrogen peroxide or serum deprivation, for example, induces oxidative stress and increases TNTs formation in cultured astrocytes and neurons.[6] In astrocytes, TNTs are primarily established between stressed and unstressed cells, and their formation seems to be mainly controlled by the transcription factor p53. In stressed neurons and astrocytes, p53 stimulation induces both caspase-3 upregulation and TNT formation. It has been proposed that oxidative insults cause p53 activation that, in turn, upregulates Epidermal Growth Factor Receptor (EGFR) expression, activates the Akt/PI3K/mTOR pathway, and induces M-Sec overexpression.[6] M-Sec, first described as tumor necrosis factor-alpha-induced protein 2,[23] in cooperation with the RalA small GTPase and the exocyst complex, trigger F-actin polymerization that induces the initiation of membrane protrusions for TNT formation from the cell membrane.[23,24] Another pathway involved in TNTs formation and TNT-mediated transfer of cargoes between neurons may be the Wnt/Ca^{2+} pathway, an intracellular cascade involved in actin cytoskeleton remodeling. In this pathway, the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is identified as a key molecule in modulating the TNT-mediated transfer of cargoes.[25] The calcium-binding protein S100A4 and its receptor RAGE are other critical elements for the formation of stable protrusions and for guiding TNT growth between astrocytes and neurons. Caspase-3 cleaves intracellular S100A4, decreasing its concentration. This creates a chemical gradient from a low concentration in initiating cells toward a high concentration in target cells, which is followed by the cell finding the target. Interestingly, whereas for TNT formation between astrocytes, S100A4 seems to be sufficient for cell targeting, neuronal activation is also required for the TNT formation between astrocytes and neurons, suggesting that healthy target cells are needed to successfully build nanotubes.[26] TNTs between stressed and unstressed cells were also observed by Kretschmer and coworkers in prostate cancer cells (PCa) as well as from stressed PCa and osteoblasts;[27] they also occur in T cells triggered by apoptosis by FasL.[28,29]

4 | INTERCELLULAR EXCHANGE OF CELLULAR COMPONENTS

TNTs transport a variety of cellular components, including organelles, proteins, calcium ions, viruses, and bacteria. Mitochondria are important trafficked entities through TNTs. Transcellular mitochondria transfer enables their incorporation into the endogenous network of recipient cells, changing the bioenergetic profile and other functional properties of the recipient.[30]

Miro1 (mitochondrial Rho-GTPase 1, synonym: RhoT1), is a calcium-sensitive adaptor protein that connects the mitochondrion and the KIF5 motor protein through additional proteins, like Miro2, TRAK1, TRAK2, and Myo19, seems to be an important player in mitochondrial transfer. Miro1 overexpression causes a more efficient mitochondrial transfer from mesenchymal

stem cells (MSCs) to damaged neural cells improving survival and recovery.[31,32] Conversely, knocking down Miro1 inhibits TNTs generation and MSCs rescue efficacy.[33] Miro1 also enhances the transfer of mitochondria from MSCs to neuron-like PC12 cells experiencing ischemic damage,[32] improving recovery and cell proliferation.

Lysosomes have been identified as another important cargo within the TNTs structures. Lysosomes are membrane-bound organelles whose primary function is to digest macromolecules and degrade cellular waste. The mechanism of lysosome transfer can be both diffusive and active, the latter involving ATP and motor proteins like dyneins and kinesins, which regulate the movement of lysosomes along microtubules.[34] Lysosomes were initially observed in TNTs connecting macrophage cells[13] and then reported in other cells connected through TNTs.[34] The study of the intercellular transport of lysosomes revealed that TNTs can also facilitate the transport of viruses and proteins, with the latter being linked to various neurodegenerative diseases.[35,36] Calcium ion flux has also been documented to be relevant to TNT physiology. Calcium ion flux is mediated through Cx42 gap junctions (GJs) and by IP3 which stimulates calcium ion release from the endoplasmic reticulum.[37]

Other cargoes transported by TNTs include microRNA (miRNA), lipids, proteins, and likely many other cell products. miRNA transfer has been investigated mainly in cancer cells and has been identified as a mechanism for tumor cells to spread novel resistance gene mutations.[38] Recently, the presence of lipid droplets within TNTs has been demonstrated in endothelial cells by Astanina and coworkers;[39] they reported that under angiogenic conditions the number of lipid droplets and TNTs formation increased significantly.

5 | TUNNELING NANOTUBES AND MESENCHYMAL STEM CELLS

MSCs are stromal cells, nonhematopoietic in nature, which can differentiate into multiple cell types. MSCs can be isolated from dental tissue, bone marrow, adipose tissue, umbilical cord, placenta, and other sources. MSCs possess the capacity to form TNTs and gap junctions, and transfer mitochondria and other constituents to target cells. This occurs under both physiological and pathological conditions, leading to changes in cell energy metabolism and functions. Spees and coworkers described the mitochondrial donation from MSCs initially in 2006.[40] The authors demonstrated that cocultivation of human bone marrow MSCs (hMSCs)

and A549p cells, which were incapable of aerobic respiration and growth because of defective mtDNA, fostered the cells to acquire functional mitochondria provided by the donor hMSCs. Accordingly, Liu and coworkers demonstrated that MSCs repaired postischemic endothelial cells with dysfunctional mitochondria by transferring functional mitochondria from healthy MSCs via TNTs in vitro.[41] In a murine model of lipopolysaccharide (LPS)-induced acute lung injury, mitochondrial transfer from MSCs to pulmonary alveolar epithelial cells occurred after establishing gap junctions with the injured lung epithelial cells; this resulted in the regeneration of the damaged alveoli.[42]

The therapeutic efficacy of MSCs via TNTs-mediated transfer of mitochondria has also been shown in preclinical studies related to ischemia, oxidative stress, mitochondrial damage, inflammation, and respiratory illness; likewise, they form gap-junction, secrete immunomodulatory factors, growth factors, and release exosomes/microvesicles.[43] Because of these effects, MSCs have been frequently evaluated for their regenerative applications. MSCs have been shown to influence immunity,[44] increase epithelial cell proliferation,[45] and support recovery of ischemic tissues.[46] MSCs can protect cells from injury and promote tissue repair,[47] and when injected into the myocardium after infarction can reduce the incidence of scar formation.[48]

It remains unclear, however, how MSCs mediate their rescue efficacy. Four potential but nonexclusive mechanisms have been proposed: (i) direct cell-to-cell signaling; (ii) paracrine signaling with soluble secreted factors such as hormones and proteins; (iii) homing of released exosomes or microvesicles that contain immunoregulatory molecules and other molecules including RNA; and (iv) mitochondrial trafficking via TNTs or microvesicles.[49] It is believed that, as a strategy that can restore the bioenergetic needs of damaged cells, mitochondrial transfer is by itself an effective reparative means to rescue different types of damaged cells. Below is discussed the potential role of mitochondrial transfer through MSCs after an ischemic insult and how it can aid in repairing and regenerating injured and damaged cells.

6 | TUNNELING NANOTUBES, MESENCHYMAL STEM CELLS, AND ISCHEMIA

Research on TNTs and ischemia has mainly been related to nanotube building by MSCs when applied as a therapeutic tool for stroke in the brain and after myocardial infarction. During an ischemic insult, the lack of blood flow causes irreversible damage to neurons

and the loss of numerous neuronal networks. Cells directly affected by the lack of glucose and oxygen in the ischemic core die by necrosis. In the surrounding penumbra, neurons, astrocytes, microglia, oligodendrocytes, pericytes, and endothelial cells respond in different ways to the damaging events caused by excitotoxicity, oxidative stress, and inflammation. The fate of neurons is multifactorial, and survival relies on communication among all the different players. Neurons are connected to each other forming an extensive communication network through synaptic transmission; failures at the synaptic level cause neuronal disintegration and transsynaptic degeneration ending in severe neuronal dysfunction.[50] Glial cells, neurons, and blood vessels are strictly interconnected to maintain cerebral blood flow.[51] Moreover, the integrity and functionality of the blood–brain barrier are critical features for the preservation and recovery of neural tissues.[52] The work of this "neurovascular unit"[51] seems to involve several factors, including TNTs.[53] Babenko and coworkers observed the formation of TNTs in cocultures of MSCs with either rat astrocytes or neuron-like PC12 pheochromocytoma cells exposed to oxygen-glucose deprivation. Their study demonstrated that mitochondria transported through TNTs restored cell proliferation and respiration affected by the ischemic insult.[32] A recent in vivo study demonstrated that the infusion of bone marrow MSCs to ischemic rats caused an effective TNTs-mediated mitochondrial transfer to promote recovery from ischemic stroke.[54]

Published reports documented similar results in cardiac stroke. Thus, TNTs–mediated mitochondrial transfer from bone marrow MSCs to H9c2 cells rescued cardiomyocytes from ischemia/reperfusion damage.[55] Similarly, a study by Figeac et al. demonstrated that mouse cardiomyocytes triggered human adipose MSCs to release several soluble factors related to cardiac protection through TNTs formation.[56] A protective role of TNTs was also observed between human bone marrow MSCs and human umbilical cord vein endothelial cells subjected to oxygen and nutrient deprivation.[41]

7 | MELATONIN: A POSSIBLE NEW ROLE IN MSCS COMMUNICATION?

Melatonin, produced by the pineal gland and secreted in blood and cerebrospinal fluid, has numerous biological functions. Other cells also synthesize melatonin, but they release it into the extracellular space but not in the blood.[57–59] Many melatonin's actions are mediated by membrane receptors, MT1 and MT2; these are members of the G-protein coupled receptor superfamily.

Melatonin may also interact with other intracellular molecules, including the so-called MT3 receptor, the nuclear receptor ROR/RZR, and calmodulin.[60,61] Some functions of melatonin, such as its direct radical scavenging actions, are receptor independent.

There is a large amount of evidence confirming that melatonin protects tissues from ischemia/reperfusion injury;[62–65] the protective actions involve several mechanisms including a reduction of oxidative damage,[63,66,67] inhibition of the mitochondrial permeability transition pore (MPTP), and activation of uncoupling proteins (UCPs).[68] Melatonin also regulates the inflammatory reaction by modulating pro- and anti-inflammatory cytokines[69,70] and preventing the expression of cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS).[71]

Robust data have recently documented the role of melatonin in mitochondrial function.[72–74] Melatonin is synthesized in, taken up by, and concentrated in mitochondria, and the presence of the MT1 receptor on the mitochondrial membranes isolated from brain lysates has been reported.[75] Mitochondria are highly dynamic and energy-producing organelles that act as a signal transduction hub to regulate physiological activities such as intracellular calcium homeostasis, apoptosis, and autophagy.[76,77] Mitochondria are also the primary source of reactive oxygen species (ROS) as a by-product of oxidative phosphorylation; moreover, ROS accumulation and oxidative stress are elevated during mitochondrial dysfunction.[78,79] Oxidative stress alters the mitochondrial electron transport chain activity and the cardiolipin structure; these changes facilitate the opening of the mitochondrial permeability transition pore, promoting apoptosis, autophagy, and eventually cell death.[80,81] Melatonin is a powerful antioxidant and decreases ROS levels by directly scavenging free radicals and stimulating the activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX),[74,82,83] and by regulating the activity of the complex I and III, which reduce ROS generation.[84]

Mitochondria form a dynamic network that continuously changes size, shape, and location. Mitochondrial dynamics is a critical part of cell quality control and is managed through several mechanisms, including biogenesis, fission, and fusion.[85–87] Mitochondrial fission is characterized by the division of a mitochondrion into two daughter mitochondria; this process is regulated by dynamin-related protein 1 (DRP1), mitochondrial fission protein 1 (FIS1), and mitochondrial fission factor (Mff).[88] In contrast, mitochondrial fusion is the union of two mitochondria forming a large new mitochondrion. This is generally regulated by mitofusin-1 (MFN1), mitofusin-2 (MFN2), and optic atrophy 1 (OPA1), three

GTPase-dependent proteins in the dynamin superfamily.[86,89,90] The balance between fission and fusion is crucial to ensure a steady energy supply and cellular homeostasis. Mitochondrial fission occurs during cardiolipin oxidation, promoting the mitochondrial apoptosis pathway, and is always accompanied by mitophagy, an autophagic response specifically targeting damaged mitochondria.[91,92] Mitochondrial fusion, on the other hand, permits the content exchange between two different mitochondria, which maintain biochemical and genetic uniformity via the elimination of ROS and mutated DNA, and allows for the repolarization of membranes.[93]

Mitochondrial dynamics are critical for MSCs to acquire the optimal morphology, enabling cells to quickly and adaptively respond to environmental stresses, affecting their self-renewal, differentiation, and fate.[94-96] Melatonin has important effects on mitochondrial dynamics and dysfunctions. Melatonin can improve mitochondrial biogenesis in ischemia/reperfusion-induced myocardial injury, affect fission/fusion dynamics in the diabetic retina,[97] regulate

mitochondrial dynamics and alleviate neuronal damage in prion diseases,[98] and improve mitochondrial dynamics and functions in an experimental model of obesity and associated diabetes[99] (Figure 2).

Using cultured hippocampal neurons (HT22 cells) subjected to oxygen/glucose deprivation (OGD), we recently reported that the addition of melatonin to the medium in which the cells were grown reduced ROS formation and restored the mitochondrial fusion/fission dynamics affected by OGD, thereby preventing mitochondrial dysfunction. Notably, while the stress induced by OGD by itself increased TNTs formation in HT22 cells, the presence of melatonin in the medium resulted in an increased number of TNT connections between cells. In addition, in the presence of melatonin, we found a higher number of mitochondria containing TNTs compared to the OGD and the control condition.[100] The improved mitochondrial biogenesis and dynamics and the increased donation of mitochondria may support mitochondrial respiration in suffering cells and may foster cell recovery via anastasis.[11] Yip and colleagues
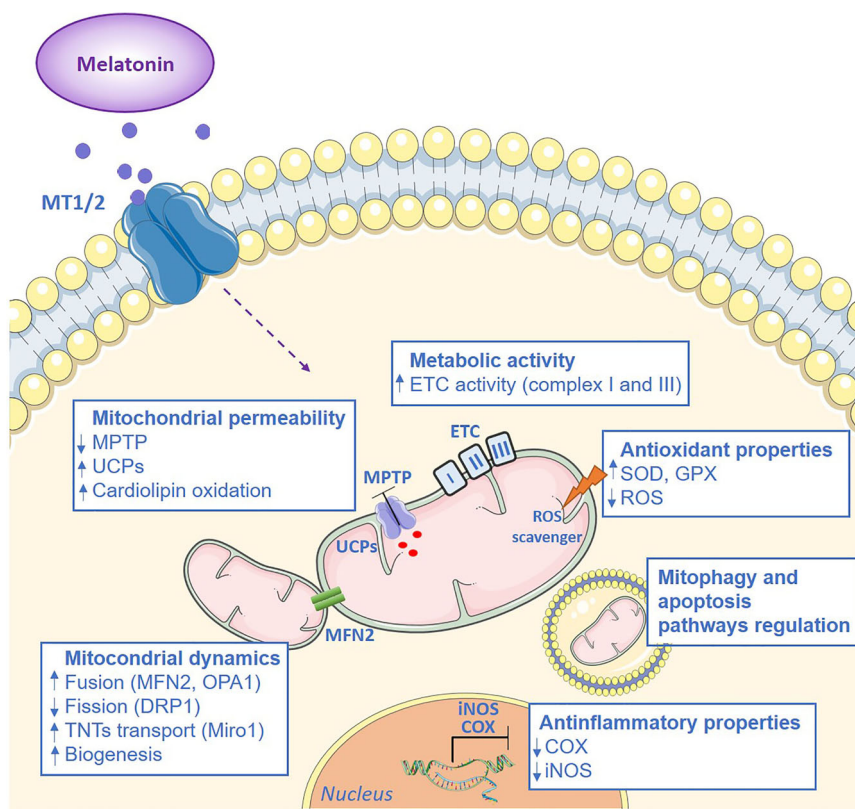

FIGURE 2 The protective role of melatonin on mitochondrial functions. Melatonin exerts several protective actions on mitochondria that involve its antioxidant properties, the inhibition of mitochondrial permeability, and the enhancement of metabolic activity. In addition, melatonin regulates mitochondrial dynamics and promotes tunneling nanotube formation. COX, cyclooxygenase; ETC, electron transport chain; DRP1, dynamin-related protein 1; GPX, glutathione peroxidase; iNOS, inducible nitric oxide synthase; MT1/2, melatonin receptors 1 or 2; I, II, III, mitochondrial complexes; MPTP, mitochondrial permeability transition pore; MFN2, mitofusin-2; OPA1, optic atrophy 1; Miro1, mitochondrial Rho-GTPase 1; ROS, reactive oxygen species; SOD, superoxide dismutase; UCPs, uncoupling proteins

published almost simultaneously similar results.[101] These authors using both in vitro and in vivo experimental approaches showed that in the injured area TNTs transfer mitochondria from healthy to apoptotic cells allowing them to recover. In the in vivo MCAO rat model, purified healthy mitochondria pretreated with melatonin were injected 60 min after reperfusion into the infarcted site. The injection reduced mitochondrial DNA damage, oxidative stress levels, apoptotic neurons, and the infarct volume, and enhanced the number of intact and functional mitochondria in the damaged neurons. Using mitochondrial trackers, the authors showed that the injected healthy mitochondria were transferred via TNTs. Our findings[100] and those of Yip and colleagues[101] underpin the importance of melatonin in helping to maintain the function of damaged mitochondria and potentially supporting cell recovery. The molecular mechanisms by which melatonin enhances TNT formation and functionality remain unknown and deserve intensive study. A modulatory effect of melatonin on the cytoskeleton has been reported in different types of cells[102–104] and cytoskeletal rearrangements seem to protect the neuro-cytoskeleton from damage caused by free radicals, which, in turn, contributes to cell survival.

Melatonin is also involved in exosome biogenesis and release.[105] Exosomes are small microvesicles of endosomal origin harboring biomolecules with the ability to alter the function of target cells often distant from the original host cell.[106] A recent publication has shown that exosomes of bone marrow MSCs pre-conditioned with melatonin had benefits in MSCs via paracrine effects and improved their therapeutic efficacy on I/R induced acute renal failure.[107] In line with these observations, Pournaghi et al. demonstrated that melatonin alters exosomal size and production in bovine granulosa cells in a dose-dependent manner.[108] Liu and co-workers examined a potential correlation between melatonin receptors and exocytosis. The authors demonstrated the involvement of the MT2 receptor and the GSK-3β/CRMP2-axis in the exocytosis rat hippocampal neurons suggesting that this pathway may be relevant for the release of extracellular vesicles.[109] Several authors reported that melatonin treatment alters the unsaturated/saturated fatty acid ratio by increasing the flexibility of the cell membrane by increasing the release of exosomes and their delivery to the target cells.[108]

MSCs transplantation has emerged as promising means for restoring tissue and organ functions. There are many types of MSCs, for example, cord blood mesenchymal stem cells (UCBMSCs), bone marrow mesenchymal stem cells (BMSCs), adipose-derived mesenchymal stem cells (ADMSCs), dental pulp MSCs, and limbal MSCs.[61] Recent work has shown that melatonin

increases MSC survival and induces a synergistic effect that alleviates inflammation, apoptosis, and oxidative stress.[110] This evidence is in line with the data that identified the melatonin receptors MT1 and MT2 on BMSC.[61,111] The injection of MSCs is a potentially effective treatment strategy in the developing injured brain, given their potent neuroregenerative properties and favorable immunological profile.[112] The possibility of obtaining large quantities of MSCs from birth-associated tissues, especially from the placenta and the umbilical cord, makes them excellent potential candidates for use in neonatal care. Transplantation of MSCs promotes functional neurologic recovery in various models of neonatal cerebral damage by promoting neurogenesis, oligodendrogenesis, and axonal remodeling and rewiring.[113,114] Donega et al., for example, found that after neonatal hypoxic-ischemic brain injury, intranasal MSCs transplantation improves behavioral outcomes and fosters neuronal and oligodendrocyte regeneration in mice.[115] It is important to consider that following the injection of MSCs many of them die or fail to proliferate due to reduced nutrition or growth factors. Recently, melatonin was found to improve the homing ability and decrease the apoptotic rate of MSC after transplantation.[110] Indeed, in the ischemic brain, melatonin pretreatment increased the survival of MSCs in vitro by reducing apoptosis after transplantation.[116] Melatonin also protects MSCs from senescence-associated mitochondrial dysfunction. A recent study by Lee and coworkers showed that treatment with melatonin rescued replicative senescent MSCs and improved functional recovery in a murine hindlimb ischemia model by increasing blood flow perfusion and neovascularization, an effect that was associated with enhanced mitophagy and improved mitochondrial function.[117] Mitochondrial dysfunction and excessive ROS production are primary causes of senescence in MSCs;[118,119] between senescent cells there is a high prevalence of intercellular bridges recognized as membrane-bound TNTs containing both actin and tubulin, which allow the transfer of large cargo including mitochondria between senescence cells.[120]

It is obviously crucial to clarify the effects of melatonin on MSCs transplantation and how they improve stem cell survival. Possible mechanisms include modulation of the inflammatory microenvironment, secretion of survival-promoting neurotrophic factors, stimulation of the plasticity and neural activity in damaged host tissue, and renewing synaptic transmission.[121] These combined actions of melatonin may rescue from senescence and anastasis and enhance in vivo stem cells' capacity to survive and interact with different cell types in the microenvironment,[122] displaying the ability to connect to target cells through TNTs development (Figure 3).
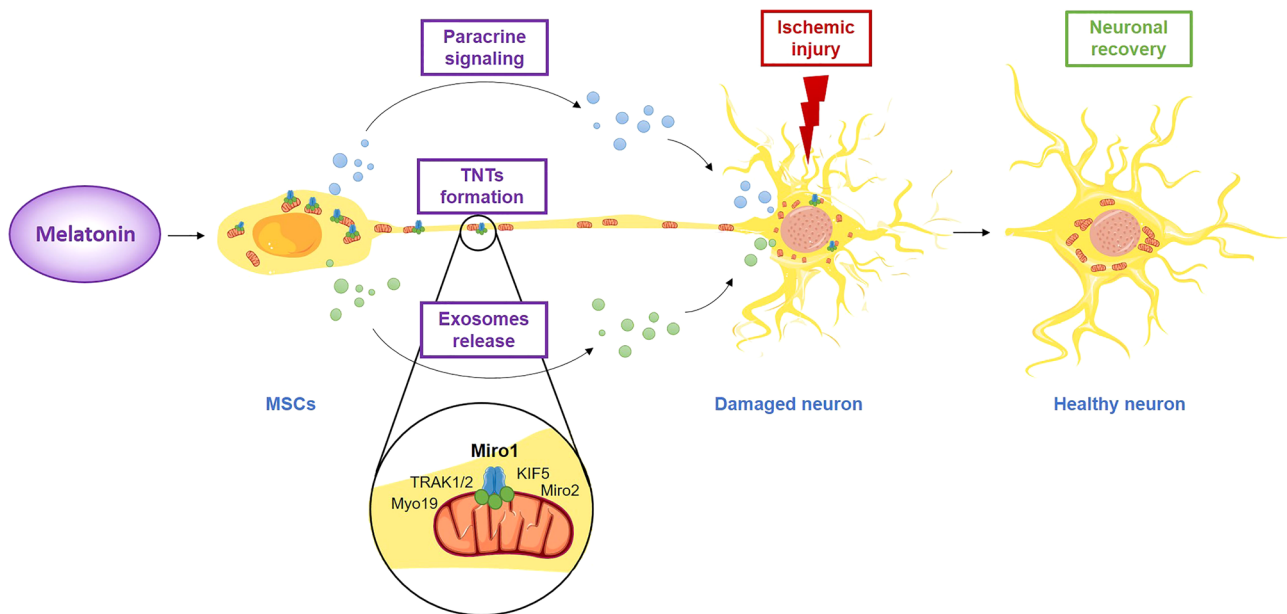
FIGURE 3 Melatonin as a potential modulator in mesenchymal stem cell communication and neuronal recovery. Melatonin has a variety of effects on MSCs, which include paracrine signaling through modulation of the inflammatory microenvironment or secretion of survival-promoting neurotrophic factors, exosomes release, and TNTs formation. TNTs transport a variety of cellular components, including organelles, proteins, calcium ions, viruses, and bacteria. This representation highlights the new role of melatonin as a promising agent able to promote mitochondrial transfer via TNTs in MSCs leading to neuronal recovery. KIF5, kinesin-related protein 5; Miro1, mitochondrial Rho-GTPase 1; Miro2, mitochondrial Rho-GTPase 2; MSCs, mesenchymal stem cells; Myo19, myosin XIX; TNTs, tunneling nanotubes; TRAK1/2, trafficking kinesin-binding protein 1 and 2

8 | CONCLUSIONS

Research progress has led to the identification of several actions of melatonin that would be beneficial for its use in tissue regeneration. MSCs represent the new frontier of regenerative medicine. The regenerative capacity of MSCs involves several processes: (i) direct cell-to-cell signaling; (ii) paracrine signaling with soluble secreted factors such as hormones and proteins; (iii) homing of released exosomes or microvesicles that contain immunoregulatory agents and other molecules including RNA; and (iv) mitochondrial trafficking via TNTs or microvesicles. The transfer of mitochondria via TNTs has been identified as a new mechanism for mitochondrial movement. This transfer of critical cellular components allows the recipient cells to regain viability and develop normal cellular functions. In this scenario, particular attention should be directed at the newly-described capacity of melatonin to promote both the TNT formation and the release of microvesicles. The modulation of these new routes of intercellular communication by melatonin could play a key role in increasing the therapeutic potential of MSCs in a large number of diseases, including neurological and immune disorders, cerebral and cardiac ischemia, diabetes, and bone and cartilage diseases. As summarized in this review, the findings suggest that melatonin's protective effects are attributed not only to its capacity to prevent mitochondrial damage in injured cells but also to promote anastasis, a natural cell recovery phenomenon that rescues cells from the brink of death.[123] Thus, we speculate that an important function of melatonin is to stimulate the transfer of highly-functional mitochondria through TNTs from healthy to apoptotic cells with faltering mitochondria, allowing the recipient cells to recuperate from apoptotic processes and return to a normal functional state.

AUTHOR CONTRIBUTIONS

Francesca Luchetti, Silvia Carloni, Maria G. Nasoni, and Walter Balduini contributed to the collection, interpretation, and writing of the manuscript. Russel J. Reiter critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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