ISSN 0006-2979, Biochemistry (Moscow), 2015, Vol. 80, No. 5, pp. 542-548. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © E. Y. Plotnikov, V. A. Babenko, D. N. Silachev, L. D. Zorova, T. G. Khryapenkova, E. S. Savchenko, I. B. Pevzner, D. B. Zorov, 2015, published in Biokhimiya, 2015, Vol. 80, No. 5, pp. 642-650.

REVIEW

Intercellular Transfer of Mitochondria

E. Y. Plotnikov[1]*, V. A. Babenko[2], D. N. Silachev[1], L. D. Zorova[3], T. G. Khryapenkova[1], E. S. Savchenko[4], I. B. Pevzner[1,2], and D. B. Zorov[1]

[1]*Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, 119991 Moscow, Russia; fax: +7 (495) 939-0338; E-mail: plotnikov@genebee.msu.ru*
[2]*Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, 119991 Moscow, Russia*
[3]*Lomonosov Moscow State University, International Laser Center, 119991 Moscow, Russia*
[4]*Lomonosov Moscow State University, Biological Faculty, 119991 Moscow, Russia*

Received December 2, 2014

Abstract—Recently described phenomenon of intercellular transfer of mitochondria attracts the attention of researchers in both fundamental science and translational medicine. In particular, the transfer of mitochondria results in the initiation of stem cell differentiation, in reprogramming of differentiated cells, and in the recovery of the lost mitochondrial function in recipient cells. However, the mechanisms of mitochondria transfer between cells and conditions inducing this phenomenon are studied insufficiently. It is still questionable whether this phenomenon exists *in vivo*. Moreover, it is unclear, how the transfer of mitochondria into somatic cells is affected by the ubiquitination system that, for example, is responsible for the elimination of "alien" mitochondria of the spermatozoon in the oocyte during fertilization. Studies on these processes can provide a powerful incentive for development of strategies for treatment of mitochondria-associated pathologies and give rise a new avenue for therapeutic approaches based on "mitochondrial transplantation".

DOI: 10.1134/S0006297915050041

Key words: mitochondria, stem cells, axonal transport, tunneling nanotubes, differentiation

TRANSFER OF MITOCHONDRIA WITHIN THE CELL

Intracellular transfer of mitochondria for the first time was found in neurons having long axons [1]. The mitochondria in these cells were shown to relocate from the cell body into its periphery, into the region of synaptic contacts (anterograde transport) and backward from the cell periphery into the perinuclear zone (retrograde transport). Such a transfer of mitochondria is believed to provide energy to regions of high-energy expenses. Acute changes in sodium, potassium, and calcium ions concentrations in the cytoplasm of neurons and other electrically excitable cells require large amounts of energy for functioning of the ion transporting systems and for the nerve impulse transmission [2].

In elongated cells, the area best provided with substrates and oxygen (the cell areas adjacent to the blood capillaries) can be located rather far from places of the highest energy consumption. In this case, the problem of energy transfer can be solved by two ways. In the first mode, elongated mitochondria acting as electric cables can transmit the energy accumulated during respiration in form of transmembrane potential [3]. Alternatively, mitochondria themselves can be translocated acting as mobile electric power stations.

Axonal transfer of mitochondria is realized using a system of microtubules allowing the mitochondria to move to the synapses and backward exploiting a set of adaptor and motor proteins [4].

The translocation of mitochondria is highly important for the normal functioning of neurons and mitochondria themselves [5]. The transport of mitochondria not only delivers them into zones with high rates of metabolism, but using this mechanism it also eliminates impaired mitochondria (in particular, damaged due to excessive production of reactive oxygen species) which have to be delivered to autophagosomes.

Disorders in axonal transport are very often associated with various neurological diseases, in particular, with Alzheimer's disease [6].

Abbreviations: iPS, induced pluripotent stem cells; MMSC, multipotent mesenchymal stromal cell; mtDNA, mitochondrial DNA; TNT, tunneling nanotube.

* To whom correspondence should be addressed.

INTERCELLULAR TRANSFER OF MITOCHONDRIA: MECHANISMS

It is noteworthy that active studies of the intercellular transfer of mitochondria were accompanied by a discovery of tunneling nanotubes (TNTs), i.e. of those intercellular structures which role in mechanisms of transporting the organelles between the cells was described in detail. Moreover, some researchers [7] suggest that the presence of TNTs is a requisite for the transfer of mitochondria, as it will be described below.

TNTs are thin cytoplasmic extensions bordered by plasma membrane and connecting the cells. These structures are 100-800 nm in diameter and up to 100 μm long [8]. During their formation, TNTs initially resemble phyllopodia, but while reaching the neighboring cell, they fuse with its membrane and produce straight non-branching filaments separated from the substrate. TNTs are found to contain F-actin and motor proteins [8, 9], and this implies the possibility of active transfer using these structures. TNTs were initially described in pheochromocytoma PC12 cells [9, 10], but later they have been found in kidney cell lines [11], various immune cells [12-14], hematopoietic stem cells [15], and between endothelial progenitor cells and cardiomyocytes [16]. We have also demonstrated the formation of TNTs between cardiomyocytes and multipotent mesenchymal stromal cells (MMSCs) [17] and then between MMSCs and epitheliocytes of kidney tubules [18]. By now, structures characterized as TNTs have been described for many types of intercellular interactions, but in the majority of studies a stem cell was taken as a partner of bilateral communication [7].

The possibility of transporting organelles including mitochondria through TNTs has been shown in many studies [7, 9, 10, 19]. In some cases, the traffic of mitochondria has been detected directly inside of nanotubes [16, 17]. The transfer of mitochondria can be observed either in formed TNTs or at their formation, when initially an extension of a donor cell is formed which later elongates and fuses with the membrane of a recipient cell, resulting in a TNT [7-9]. It has been already mentioned that the presence of TNTs is thought to be a requisite for transporting mitochondria. The necessity of TNT for transfer of organelles was confirmed by several studies, when conditions were established preventing the formation of these structures, such as intensive vibration of the cell culture or, in the presence of chemical inhibitors of TNT formation, e.g. cytochalasin D [9-11]. Under such conditions, the transfer of mitochondria was absent or decreased in parallel with the decrease in the number of TNTs. The mandatory role of TNT for the organelles transfer was demonstrated under conditions of cooling the cultural medium when all other possible transfer mechanisms (endocytosis, exocytosis, phagocytosis) were suppressed, but nanotubes were preserved providing the transfer [9, 11].

Although mechanisms of mitochondria transport through TNTs are poorly studied, it has been suggested that within each nanotube these organelles can move unidirectionally – from the cell initializing production of this TNT to the cell receiving it, but not in the opposite direction [9]. The organelles are transported by the mechanism involving interaction of myosin with the F-actin cytoskeleton inside of the nanotube. Some studies suggest that inhibition of myosin activity can prevent the translocation of organelles; however, the transfer can be increased due to inhibition of the retrograde movement powered by myosin [11]. This indicates that there is a complicated cross-talk between mechanisms of anterograde and retrograde transport of the organelles towards the recipient cell. Although a complete picture of the regulation of mitochondrial transport is still absent, some proteins involved in this process have been revealed. Thus, it is well known that Rho GTPases participate in the movement of mitochondria [20, 21], whereas β-catenin and E-cadherin seem to be absent within TNTs [22]. Proteins responsible for attaching the organelles to myosin or supporting their movement within a nanotube are not yet identified, but there is already established "tunneling nanotube proteome" consisting of ~275 proteins [23].

An activation of intercellular transport of mitochondria observed under various types of stresses [24, 25] seems to be associated with changes in TNT formation rather than with influence on the transport mechanisms. The majority of researchers have observed an increase in TNT formation in response to medium acidification, hyperglycemia, serum deprivation, or addition of toxic agents [24]. Formation of nanotubes was increased in cultures of hippocampal neurons and astrocytes after activation of p53-dependent signaling pathways, and the direction of their growth depended on the difference in contents of the S100A4 protein. The amount of this protein decreased due to caspases activity in damaged neurons, which became TNT-producing cells, whereas cells with a high level of S100A4 became recipient cells [25].

TNTs are not a purely *in vitro* phenomenon; such structures and, consequently, the transport of mitochondria through them, are also observed *in vivo*. Nanotubes are found in the mouse heart between cardiomyocytes and fibroblasts [26], in some human tumors [27], and in dendritic cells of the eye cornea [28]. So far, *in vivo* transport of mitochondria has been demonstrated only in a few studies. The transfer of mitochondria from MMSCs into the alveolar epithelium cells in the case of inflammatory damage was associated with TNTs formation and depended on activities of a number of associated proteins, in particular, Rho GTPases [21, 29]. However, in another study mitochondria were transferred from a melanocyte into a keratinocyte without involvement of a TNT but through formation by the melanocyte of mitochondria-containing vesicles, which were high-jacked by the keratinocyte [30]. Thus, apparently, in the organism there are some other

mechanisms of intercellular transport of mitochondria different from those described *in vitro*.

More examples of the mitochondria transport beyond the cell border should be mentioned. In particular, in the protozoan *Tetrahymena thermophila* intact mitochondria are ejected from the cell in functional state under heat shock conditions [31]. The mechanism of this ejection is unclear, but it obviously is calcium-dependent. Similar ejections of mitochondria from cells under various stressed conditions were first described in [32]: reticulocytes were shown to release damaged mitochondria. Later, removal of mitochondria or their fragments was demonstrated during apoptosis of fibroblasts [33] and in HeLa cells treated by uncouplers of oxidative phosphorylation and by respiratory inhibitors [34].

However, ejection of mitochondria is more likely associated with the system of mitochondria elimination during mitoptosis and is based on other mechanisms than the intercellular transport of mitochondria.

CONSEQUENCES OF MITOCHONDRIAL TRANSFER

Increase in cell viability under stressed conditions. The intercellular transfer of mitochondria has been shown in many models associated with cell damage [24, 25]. The most demonstrative data were obtained in a model of lung adenocarcinoma A549 cell line deprived of mitochondria as a result of treatment with ethidium bromide for two weeks. The loss of mitochondria allowed these cells to proliferate only in a special medium supplemented with pyruvate and uridine. During the co-cultivation of such cells with MMSCs, the latter donated their mitochondria to the A549 cells. This resulted in the recovery of aerobic respiration in the mitochondria-free A549 cells and of their ability to proliferate in standard cultural medium [35]. Thus, normally functioning mitochondria were donated by MMSCs to the mitochondria-free A549 cells, which began to produce ATP not only due to glycolysis but also through oxidative phosphorylation. Moreover, the cells provided synthesis of pyrimidine nucleotides, which cannot be synthesized without mitochondria.

In another study, the transfer of mitochondria from MMSCs into lung epithelium cells afforded protection of these cells against endotoxin-induced death, retained the normal level of ATP production, and prevented lung damage *in vivo*. It was demonstrated that the positive effects were associated just with the transfer of functioning mitochondria, because mitochondrial dysfunction abolishes the afforded protection against the lung damage. Note that *in vivo*, virtually the whole population of cell's mitochondria was sometimes transferred from MMSCs into lung epithelial cells that was accompanied by recovery of epithelium functions [29]. These data were firmly supported by a recent work [21] that indicated not only the dependence of the lung epithelial cells recovery on the delivery of mitochondria from the MMSCs, but also revealed the most likely mechanism of such transport through TNTs. This study revealed a direct dependence of both the transfer of mitochondria and positive effects of MMSCs on the alveolar epithelium from functioning of Miro1 – a Rho GTPase responsible for the movement of mitochondria along the cytoskeleton of nanotubes.

Several studies have shown that transport of mitochondria increases under stressed conditions applied to cells. Thus, Yasuda et al. found that on co-cultivation of endothelial HUVEC cells and endothelial progenitor cells about 17% of the endothelial cells received mitochondria from the progenitor cells after 24 h of the cultivation. However, if the HUVEC cells were subjected to the toxic action of adriamycin, the number of cells receiving mitochondria increased twofold [36]. Hippocampal neurons and astrocytes were found to initiate formation of TNTs, which seemed to transfer mitochondria only after removal of serum from the medium or after treatment with hydrogen peroxide [25]. In the study of Cho et al. [37], it was shown that mesenchymal stem cells transferred their mitochondria to osteosarcoma cells only when the recipient cells have been completely deprived of mitochondria after treatment with ethidium bromide. In this case, the transfection of the recipient cells with a mutant mtDNA leading to a partial dysfunction of mitochondria did not induce transfer of mitochondria. Thus, in the majority of cases a virtually complete elimination of mitochondria in the recipient cell is needed for transfer of mitochondria.

Finally, it has recently been shown that MMSCs obtained from induced pluripotent cells (iPS) were able to transfer their mitochondria to lung epithelium cells and decrease their damage caused by cigarette smoke [38]. Moreover, the efficiency of prevention of lung epithelium damage seemed to be associated with the transfer of mitochondria. MMSCs obtained from iPS more efficiently transferred mitochondria to the epithelium than MMSCs from adult donors, and the same cells injected into animals provided more pronounced protection against alveolar destruction.

Although the majority of studies have shown positive effects of mitochondrial transfer, in some cases donor mitochondria can manifest a toxic effect. Otsu et al. [39] found that MMSCs could induce apoptosis of endothelium after their co-cultivation. This process was accompanied by the transfer of mitochondria from MMSCs into the endothelium. The authors suggested that the transfer of mitochondria might be an important component of the cytotoxicity of MMSCs in a model of suppression of angiogenesis.

Reprogramming of cells. We were among the first who demonstrated that the intercellular transfer of mitochondria mediated differentiation of stem cells [17, 18]. In a co-culture of MMSCs with cardiomyocytes, we demonstrated a directed transport of mitochondria associated with the

expression of cardio-specific proteins, in particular, of the heavy chain of β-myosin in the MMSCs. Similar studies on co-cultures of kidney epithelium and MMSCs also revealed a directed transport of mitochondria and initiation of MMSC differentiation toward epitheliocytes.

Changes in MMSCs were also observed after their co-cultivation with smooth muscle cells [40]. In this case, the transport of mitochondria from smooth muscle cells enhanced MMSC proliferation, whereas inhibition of nanotube formation abolished this effect. The proliferation did not increase after the transfer of non-functional mitochondria received by pretreatment of the cells with ethidium bromide.

The effect was opposite in the case of mouse cardiomyocytes and human MMSCs [41]. In co-culture of mature cardiomyocytes with stem cells isolated from fat or bone marrow, the transfer of mitochondria into the cardiomyocytes led to their partial dedifferentiation. The cardiomyocytes acquired the more progenitor phenotype because of fusion with the stem cells and homing of their mitochondria. Note that this effect disappeared upon treatment of MMSCs with ethidium bromide, i.e. it was observed only with functional mitochondria.

Finally, there is data on transfer of mitochondria between tumor cells [22, 27]; thus, it seems that such transfer can also modulate the proliferation or differentiation of tumor cells, but this hypothesis has to be further investigated.

Control of mitochondrial exchange – ubiquitination. On considering the phenomenon of intercellular transfer of mitochondria, the following question arises: how often does it occur *in vivo*, and is the exchange of mitochondria observed *in vitro* just an artifact of cell cultivation? On the other hand, whether the intercellular exchange of mitochondria really occurs in many cells of the organism, why are there no obvious consequences of this transfer, such as a continuous exchange of mitochondria between very distinct cell types, maintaining a constant level of mtDNA heteroplasmy, the absence of mitochondria segregation in various tissues. However, the segregation of mitochondria, especially during embryogenesis, has been described [42], and many diseases associated with damage of mitochondria (e.g. various ischemic pathologies) do not really demonstrate ability of tissue cells to recover due to donation of mitochondria by healthy cells to the damaged ones.

These discrepancies could be resolved by a system responsible for maintaining the constant composition of mitochondria in the cell, and this is the most clearly exemplified by the existence of maternal mitochondrial inheritance in all mammals. It is known that the majority of animals inherit mitochondria only maternally (except rare cases of "paternal inheritance leakage" [43, 44]), and the descendants' cells never contain paternal mitochondria. However, during fertilization some paternal mitochondria enter the oocyte. Although the number of these mitochondria is small (about a hundred in humans), it nevertheless seems to be sufficient for manifestation of paternal mtDNA in the developing embryo cells. Note that just the transfer of a spermatozoon's mitochondria during fertilization is the most widespread case of intercellular transfer of mitochondria that was known long before transfer of mitochondria through TNTs was described. Consequences of such transfer are also generally known: the paternal mitochondria are entirely eliminated from all cells of the embryo during the period from the first to the fourth divisions of the zygote (depending on the animal species) [45]. This elimination is executed through tagging of the paternal mitochondria with ubiquitin and directing them into proteasomes or autophagosomes. In the study of Sutovsky et al. [46, 47] on rhesus-macaques, cows, and mice, the spermatozoa's mitochondria were shown to be ubiquitinated and degraded immediately after their entry into the oocyte's cytoplasm. The degradation mechanism included, on one hand, directing the ubiquitinated mitochondria into 26S proteasomes and, on the other hand, the formation of phagophores and then autophagosomes containing the mitochondria, their fusion with lysosomes, and the proteolytic cleavage of the mitochondria [45]. In case of autophagosomal degradation of mitochondria, ubiquitin conjugates to the mitochondrial proteins, acting as a signal for elimination, and acquires a number of proteins responsible for the degradation by a cascade mechanism.

It is interesting that proteins of the spermatozoon's mitochondria were ubiquitinated also during spermatogenesis [47, 48] when a significant fraction of the initial mitochondria of the spermatogonium was eliminated in the polar body. Moreover, in some cases the paternal mitochondria after entry into an oocyte already had ubiquitin on their proteins. Thus, on entering the oocyte, the spermatozoon's mitochondria could already carry a "black label" that instantly determined their fate [47, 49].

However, it was shown that in interspecies mating in cows the spermatozoon's mitochondria were not ubiquitinated in the oocyte [46] and could be observed up to the 8-cell stage, when usually all paternal mitochondria were already eliminated [49, 50]. Paternal mtDNA in the cells of interspecies hybrids in mice was also found [51]. Thus, the mechanism of mitochondria elimination includes species-specific elements, as well as sex-specific elements, since the system of degradation selectively attacks the paternal mitochondria not touching mitochondria of the oocyte.

Ultimately, we must take into account the reports about the exchange of mitochondria in cell culture with the involvement of TNTs and also in other examples, e.g. associated with the cell fusion, which is often observed for stem and differentiated cells. On the one hand, the exchange of mitochondria in cell culture can be explained by a difference of the stem cell mitochondria from mitochondria of other somatic cells, e.g. their ubiquitination sites can be somehow masked and be unavailable for attacking by ubiquitin ligase. In this connection, it should

be noted that virtually all reports about the transfer of mitochondria are results of experiments when a stem or a progenitor cell has been used as a partner. Alternatively, for co-cultivation of post-mitotic cells conventionally different animal species were used as a source, so the mechanism of the donors' mitochondria retention in this case may be similar to that observed in the case of interspecies fertilization.

In particular, in our experiments using mesenchymal stem cells co-cultivated with cardiomyocytes [17] or with the kidney epithelial cells [18], an interspecies transfer of mitochondria took place. Since for revealing MMSCs in these co-cultures we used human antigen (available antibodies were to human cells nuclei (Human Nuclei; Millipore, USA)), for the co-cultivation experiments we chose human stem cells and rat cardiomyocytes or kidney cells.

Possibilities of therapeutic application of the exchange of mitochondria. The knowledge of mitochondrial transfer between cells seems helpful in the development of treatment of various pathologies. Many diseases, including rather severe ones, are known to be associated with impairment of mitochondria and disorders in their functions. We have considered in detail the spectrum of these pathologies in our recent review [52], and here we only would like to note that manifestations of pathological symptoms can be caused not only by genetic disorders in the functions of mitochondria, but also by their damage made by an external factors. In such cases, recovery of the functions of damaged mitochondria is one of the most seeking solution to use in therapy. Therefore, pharmacological mitochondria-targeted agents are now being intensively designed and explored [53, 54]. The replacement of disfunctional mitochondria in cells in a damaged tissue by delivery of functional mitochondria from donor cells can become a new approach in the therapy of mitochondria-associated diseases.

It should be noted again that in the majority of given examples the transfer of mitochondria between somatic cells is associated with using stem or progenitor cells. This opens a new page in the history of studies on mechanisms of translation of therapeutic effects of these cells and, on the other hand, allows us to consider such cells as the most promising candidates for development of approaches for therapeutic transplantation of mitochondria. The known features of stem cells, such as homing, a directed migration into a damaged tissue or an inflammatory focus, make these cells as a vehicle for delivery of healthy mitochondria into target organs.

The problem becomes more complicated in the case of a genetically determined dysfunction of mitochondria, because mitochondria of virtually all cells of the organism are the subject for replacement. However, because the most severe consequences of such diseases are associated with individual organs, especially with the brain and heart, therapeutic transplantation of mitochondria can become an important component if not a cure, at minimum decreasing pathological symptoms of the disease.

The replacement of a mitochondrial population in early embryogenesis can be an approach to the prenatal prevention of genetically determined mitochondrial diseases as a component of assisted reproductive technologies. If attempts of the mechanical replacement of mitochondria [55] are without an instant result, attention should be redirected to biological modes of the mitochondrial transfer. In particular, based on data on the mitochondrial transfer in co-cultures, it is reasonable to expect a transfer after co-cultivation of stem cells and the zygote or blastocyst during early steps of division. It is most important that there is a necessity to halt the elimination of the donor's mitochondria, which is the main problem to be solved in a way to exclude a domination of maternal inheritance of mitochondria.

Finally, the most recent data aimed increasing the therapeutic efficiency of MMSCs by affecting the system of mitochondrial transport [21] suggests that intercellular transfer of mitochondria can be enforced pharmacologically. This would provide the possibility of the replacement of nonfunctional mitochondria in a damaged tissue through delivery of mitochondria from adjacent healthy cells. This would be at least a partial correction of acquired mitochondrial pathologies, such as stroke or infarction, when the dying cells are located close to undamaged ones containing functional mitochondria.

Obviously, the intercellular transport of mitochondria is an important component of cell-to-cell cross-talk, especially under stress conditions. To reveal the possibility of using this phenomenon in clinical practice, further studies are required on the mitochondrial transport mechanisms and factors activating or suppressing them and also a detailed description is necessary of the full spectrum of consequences of the mitochondria transfer for the donor and recipient cells.

The authors congratulate V. P. Skulachev on his anniversary and wish him many years of fruitful activities in domestic and world science.

This work was supported by the Russian Scientific Foundation (the project 14-15-00107).

REFERENCES

1. Weiss, P. A., and Mayr, R. (1971) Neuronal organelles in neuroplasmic ("axonal") flow. I. Mitochondria, *Acta Neuropathol.*, **5**, Suppl. 5, 187-197.
2. Zorov, D. B., Isaev, N. K., Plotnikov, E. Y., Zorova, L. D., Stelmashook, E. V., Vasileva, A. K., Arkhangelskaya, A. A., and Khryapenkova, T. G. (2007) The mitochondrion as Janus bifrons, *Biochemistry (Moscow)*, **72**, 1115-1126.
3. Amchenkova, A. A., Bakeeva, L. E., Chentsov, Y. S., Skulachev, V. P., and Zorov, D. B. (1988) Coupling membranes as energy-transmitting cables. I. Filamentous mito-

chondria in fibroblasts and mitochondrial clusters in cardiomyocytes, *J. Cell Biol.*, **107**, 481-495.
4. Leterrier, J. F., Rusakov, D. A., Nelson, B. D., and Linden, M. (1994) Interactions between brain mitochondria and cytoskeleton: evidence for specialized outer membrane domains involved in the association of cytoskeleton-associated proteins to mitochondria *in situ* and *in vitro*, *Microsc. Res. Tech.*, **27**, 233-261.
5. Chang, D. T., and Reynolds, I. J. (2006) Mitochondrial trafficking and morphology in healthy and injured neurons, *Prog. Neurobiol.*, **80**, 241-268.
6. Baloyannis, S. J. (2006) Mitochondrial alterations in Alzheimer's disease, *J. Alzheimer's Dis.*, **9**, 119-126.
7. Rogers, R. S., and Bhattacharya, J. (2013) When cells become organelle donors, *Physiology* (*Bethesda*), **28**, 414-422.
8. Gerdes, H. H., Bukoreshtliev, N. V., and Barroso, J. F. (2007) Tunneling nanotubes: a new route for the exchange of components between animal cells, *FEBS Lett.*, **581**, 2194-2201.
9. Rustom, A., Saffrich, R., Markovic, I., Walther, P., and Gerdes, H. H. (2004) Nanotubular highways for intercellular organelle transport, *Science*, **303**, 1007-1010.
10. Bukoreshtliev, N. V., Wang, X., Hodneland, E., Gurke, S., Barroso, J. F., and Gerdes, H. H. (2009) Selective block of tunneling nanotube (TNT) formation inhibits intercellular organelle transfer between PC12 cells, *FEBS Lett.*, **583**, 1481-1488.
11. Gurke, S., Barroso, J. F., Hodneland, E., Bukoreshtliev, N. V., Schlicker, O., and Gerdes, H. H. (2008) Tunneling nanotube (TNT)-like structures facilitate a constitutive, actomyosin-dependent exchange of endocytic organelles between normal rat kidney cells, *Exp. Cell Res.*, **314**, 3669-3683.
12. Onfelt, B., Nedvetzki, S., Yanagi, K., and Davis, D. M. (2004) Cutting edge: membrane nanotubes connect immune cells, *J. Immunol.*, **173**, 1511-1513.
13. Schiller, C., Huber, J. E., Diakopoulos, K. N., and Weiss, E. H. (2013) Tunneling nanotubes enable intercellular transfer of MHC class I molecules, *Hum. Immunol.*, **74**, 412-416.
14. Galkina, S. I., Molotkovsky, J. G., Ullrich, V., and Sud'ina, G. F. (2005) Scanning electron microscopy study of neutrophil membrane tubulovesicular extensions (cytonemes) and their role in anchoring, aggregation and phagocytosis. The effect of nitric oxide, *Exp. Cell Res.*, **304**, 620-629.
15. Freund, D., Bauer, N., Boxberger, S., Feldmann, S., Streller, U., Ehninger, G., Werner, C., Bornhauser, M., Oswald, J., and Corbeil, D. (2006) Polarization of human hematopoietic progenitors during contact with multipotent mesenchymal stromal cells: effects on proliferation and clonogenicity, *Stem Cells Dev.*, **15**, 815-829.
16. Koyanagi, M., Brandes, R. P., Haendeler, J., Zeiher, A. M., and Dimmeler, S. (2005) Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes, *Circ. Res.*, **96**, 1039-1041.
17. Plotnikov, E. Y., Khryapenkova, T. G., Vasileva, A. K., Marey, M. V., Galkina, S. I., Isaev, N. K., Sheval, E. V., Polyakov, V. Y., Sukhikh, G. T., and Zorov, D. B. (2008) Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture, *J. Cell Mol. Med.*, **12**, 1622-1631.
18. Plotnikov, E. Y., Khryapenkova, T. G., Galkina, S. I., Sukhikh, G. T., and Zorov, D. B. (2010) Cytoplasm and organelle transfer between mesenchymal multipotent stromal cells and renal tubular cells in co-culture, *Exp. Cell Res.*, **316**, 2447-2455.
19. Marzo, L., Gousset, K., and Zurzolo, C. (2012) Multifaceted roles of tunneling nanotubes in intercellular communication, *Front. Physiol.*, **72**, 1-14.
20. Arkwright, P. D., Luchetti, F., Tour, J., Roberts, C., Ayub, R., Morales, A. P., Rodriguez, J. J., Gilmore, A., Canonico, B., Papa, S., and Esposti, M. D. (2010) Fas stimulation of T lymphocytes promotes rapid intercellular exchange of death signals via membrane nanotubes, *Cell Res.*, **20**, 72-88.
21. Ahmad, T., Mukherjee, S., Pattnaik, B., Kumar, M., Singh, S., Kumar, M., Rehman, R., Tiwari, B. K., Jha, K. A., Barhanpurkar, A. P., Wani, M. R., Roy, S. S., Mabalirajan, U., Ghosh, B., and Agrawal, A. (2014) Miro1 regulates intercellular mitochondrial transport and enhances mesenchymal stem cell rescue efficacy, *EMBO J.*, **33**, 994-1010.
22. Lou, E., Fujisawa, S., Morozov, A., Barlas, A., Romin, Y., Dogan, Y., Gholami, S., Moreira, A. L., Manova-Todorova, K., and Moore, M. A. (2012) Tunneling nanotubes provide a unique conduit for intercellular transfer of cellular contents in human malignant pleural mesothelioma, *PLoS One*, **7**, e33093.
23. Kadiu, I., and Gendelman, H. E. (2011) Macrophage bridging conduit trafficking of HIV-1 through the endoplasmic reticulum and Golgi network, *J. Proteome Res.*, **10**, 3225-3238.
24. Domhan, S., Ma, L., Tai, A., Anaya, Z., Beheshti, A., Zeier, M., Hlatky, L., and Abdollahi, A. (2011) Intercellular communication by exchange of cytoplasmic material via tunneling nanotube-like structures in primary human renal epithelial cells, *PLoS One*, **6**, e21283.
25. Wang, Y., Cui, J., Sun, X., and Zhang, Y. (2011) Tunneling-nanotube development in astrocytes depends on p53 activation, *Cell Death Differ.*, **18**, 732-742.
26. He, K., Shi, X., Zhang, X., Dang, S., Ma, X., Liu, F., Xu, M., Lv, Z., Han, D., Fang, X., and Zhang, Y. (2011) Long-distance intercellular connectivity between cardiomyocytes and cardiofibroblasts mediated by membrane nanotubes, *Cardiovasc. Res.*, **92**, 39-47.
27. Lou, E., Fujisawa, S., Barlas, A., Romin, Y., Manova-Todorova, K., Moore, M. A., and Subramanian, S. (2012) Tunneling nanotubes: a new paradigm for studying intercellular communication and therapeutics in cancer, *Commun. Integr. Biol.*, **5**, 399-403.
28. Chinnery, H. R., Pearlman, E., and McMenamin, P. G. (2008) Cutting edge: membrane nanotubes *in vivo*: a feature of MHC class II+ cells in the mouse cornea, *J. Immunol.*, **180**, 5779-5783.
29. Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., Rowlands, D. J., Quadri, S. K., Bhattacharya, S., and Bhattacharya, J. (2012) Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury, *Nature Med.*, **18**, 759-765.
30. Wu, X. S., Masedunskas, A., Weigert, R., Copeland, N. G., Jenkins, N. A., and Hammer, J. A. (2012) Melanoregulin regulates a shedding mechanism that drives melanosome

transfer from melanocytes to keratinocytes, *Proc. Natl. Acad. Sci. USA*, **109**, 2101-2109.
31. Bisharyan, Y., and Clark, T. G. (2011) Calcium-dependent mitochondrial extrusion in ciliated protozoa, *Mitochondrion*, **11**, 909-918.
32. Simpson, C. F., and Kling, J. M. (1968) The mechanism of mitochondrial extrusion from phenylhydrazine-induced reticulocytes in the circulating blood, *J. Cell Biol.*, **36**, 103-109.
33. Nakajima, A., Kurihara, H., Yagita, H., Okumura, K., and Nakano, H. (2008) Mitochondrial extrusion through the cytoplasmic vacuoles during cell death, *J. Biol. Chem.*, **283**, 24128-24135.
34. Lyamzaev, K. G., Nepryakhina, O. K., Saprunova, V. B., Bakeeva, L. E., Pletjushkina, O. Y., Chernyak, B. V., and Skulachev, V. P. (2008) Novel mechanism of elimination of malfunctioning mitochondria (mitoptosis): formation of mitoptotic bodies and extrusion of mitochondrial material from the cell, *Biochim. Biophys. Acta*, **1777**, 817-825.
35. Spees, J. L., Olson, S. D., Whitney, M. J., and Prockop, D. J. (2006) Mitochondrial transfer between cells can rescue aerobic respiration, *Proc. Natl. Acad. Sci. USA*, **103**, 1283-1288.
36. Yasuda, K., Park, H. C., Ratliff, B., Addabbo, F., Hatzopoulos, A. K., Chander, P., and Goligorsky, M. S. (2010) Adriamycin nephropathy: a failure of endothelial progenitor cell-induced repair, *Am. J. Pathol.*, **176**, 1685-1695.
37. Cho, Y. M., Kim, J. H., Kim, M., Park, S. J., Koh, S. H., Ahn, H. S., Kang, G. H., Lee, J. B., Park, K. S., and Lee, H. K. (2012) Mesenchymal stem cells transfer mitochondria to the cells with virtually no mitochondrial function but not with pathogenic mtDNA mutations, *PLoS One*, **7**, e32778.
38. Li, X., Zhang, Y., Yeung, S. C., Liang, Y., Liang, X., Ding, Y., Ip, M. S., Tse, H. F., Mak, J. C., and Lian, Q. (2014) Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage, *Am. J. Respir. Cell Mol. Biol.*, **51**, 455-465.
39. Otsu, K., Das, S., Houser, S. D., Quadri, S. K., Bhattacharya, S., and Bhattacharya, J. (2009) Concentration-dependent inhibition of angiogenesis by mesenchymal stem cells, *Blood*, **113**, 4197-4205.
40. Vallabhaneni, K. C., Haller, H., and Dumler, I. (2012) Vascular smooth muscle cells initiate proliferation of mesenchymal stem cells by mitochondrial transfer via tunneling nanotubes, *Stem Cells Dev.*, **21**, 3104-3113.
41. Acquistapace, A., Bru, T., Lesault, P. F., Figeac, F., Coudert, A. E., le Coz, O., Christov, C., Baudin, X., Auber, F., Yiou, R., Dubois-Rande, J. L., and Rodriguez, A. M. (2011) Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer, *Stem Cells*, **29**, 812-824.
42. Mishra, P., and Chan, D. C. (2014) Mitochondrial dynamics and inheritance during cell division, development and disease, *Nature Rev. Mol. Cell Biol.*, **15**, 634-646.
43. Fontaine, K. M., Cooley, J. R., and Simon, C. (2007) Evidence for paternal leakage in hybrid periodical cicadas (Hemiptera: *Magicicada* spp.), *PLoS One*, **2**, e892.
44. Dokianakis, E., and Ladoukakis, E. D. (2014) Different degree of paternal mtDNA leakage between male and female progeny in interspecific *Drosophila* crosses, *Ecol. Evol.*, **4**, 2633-2641.
45. Song, W. H., Ballard, J. W., Yi, Y. J., and Sutovsky, P. (2014) Regulation of mitochondrial genome inheritance by autophagy and ubiquitin-proteasome system: implications for health, fitness, and fertility, *Biomed. Res. Int.*, **2014**, 981867; DOI: 10.1155/2014/981867.
46. Sutovsky, P., Moreno, R. D., Ramalho-Santos, J., Dominko, T., Simerly, C., and Schatten, G. (1999) Ubiquitin tag for sperm mitochondria, *Nature*, **402**, 371-372.
47. Sutovsky, P., Moreno, R. D., Ramalho-Santos, J., Dominko, T., Simerly, C., and Schatten, G. (2000) Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos, *Biol. Reprod.*, **63**, 582-590.
48. Sutovsky, P., Moreno, R., Ramalho-Santos, J., Dominko, T., Thompson, W. E., and Schatten, G. (2001) A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis, *J. Cell Sci.*, **114**, 1665-1675.
49. Sutovsky, P., Navara, C. S., and Schatten, G. (1996) Fate of the sperm mitochondria, and the incorporation, conversion, and disassembly of the sperm tail structures during bovine fertilization, *Biol. Reprod.*, **55**, 1195-1205.
50. Sutovsky, P., McCauley, T. C., Sutovsky, M., and Day, B. N. (2003) Early degradation of paternal mitochondria in domestic pig (*Sus scrofa*) is prevented by selective proteasomal inhibitors lactacystin and MG132, *Biol. Reprod.*, **68**, 1793-1800.
51. Shitara, H., Kaneda, H., Sato, A., Inoue, K., Ogura, A., Yonekawa, H., and Hayashi, J. I. (2000) Selective and continuous elimination of mitochondria microinjected into mouse eggs from spermatids, but not from liver cells, occurs throughout embryogenesis, *Genetics*, **156**, 1277-1284.
52. Zorov, D. B., Isaev, N. K., Plotnikov, E. Y., Silachev, D. N., Zorova, L. D., Pevszner, I. B., Morosanova, M. A., Jankauskas, S. S., Zorov, S. D., and Babenko, B. A. (2013) Perspectives of mitochondrial medicine, *Biochemistry (Moscow)*, **78**, 979-990.
53. Skulachev, M. V., Antonenko, Y. N., Anisimov, V. N., Chernyak, B. V., Cherepanov, D. A., Chistyakov, V. A., Egorov, M. V., Kolosova, N. G., Korshunova, G. A., Lyamzaev, K. G., Plotnikov, E. Y., Roginsky, V. A., Savchenko, A. Y., Severina, I. I., Severin, F. F., Shkurat, T. P., Tashlitsky, V. N., Shidlovsky, K. M., Vyssokikh, M. Y., Zamyatnin, A. A., Jr., Zorov, D. B., and Skulachev, V. P. (2011) Mitochondrial-targeted plastoquinone derivatives. Effect on senescence and acute age-related pathologies, *Curr. Drug Targets*, **12**, 800-826.
54. Smith, R. A., and Murphy, M. P. (2011) Mitochondria-targeted antioxidants as therapies, *Discov. Med.*, **11**, 106-114.
55. Takeda, K., Tasai, M., Akagi, S., Matsukawa, K., Takahashi, S., Iwamoto, M., Srirattana, K., Onishi, A., Tagami, T., Nirasawa, K., Hanada, H., and Pinkert, C. A. (2010) Microinjection of serum-starved mitochondria derived from somatic cells affects parthenogenetic development of bovine and murine oocytes, *Mitochondrion*, **10**, 137-142.