H ave you seen?

Miro1: New wheels for transferring mitochondria

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Mesenchymal stem cells (MSC) are capable of protecting cells harboring mitochondrial damage. This protection is associated with the transfer of mitochondria through tunneling nanotubes (TNT) from MSC to the injured cells. In this issue of *The EMBO Journal*, the group of Anurag Agrawal shows that mitochondrial transfer is dependent on the levels of Miro1, a mitochondrial Rho-GTPase that regulates intercellular mitochondrial movement. Miro1 is the first protein shown to accelerate mitochondrial transfer. Amplifying the mitochondrial transfer phenomenon may allow for the study of the mechanisms that regulate it and contribute to our understanding of its role in disease and aging.

See also: T Ahmad et al (May 2014)

The number of diseases in which impaired mitochondrial function is thought to contribute to pathogenesis is constantly increasing. While the main strategy employed in the treatment of mitochondrial dysfunction involves targeting mitochondrial genes encoded by the nuclear genome, our current capacity to supplement, repair, or replace mitochondrial DNA in somatic cells is very limited. Mitochondrial DNA is located in the matrix at up to thousands of copies per cell. It encodes 13 proteins that are essential for proper respiratory chain function. Those proteins are highly hydrophobic and therefore cannot be transferred to the mitochondria from the cytosol; they have to be synthesized in the mitochondria. The absence of an efficient way to transduce mitochondria with exogenous DNA means that common gene or protein delivery approaches, such as viral or transfection, cannot be used to address the accumulation of mutated or deleted mitochondrial DNA.

Yet, an exciting discovery by Spees et al has opened the door for a potential approach to treat mitochondrial diseases. By co-culturing somatic cells deleted of their mitochondrial DNA with either human mesenchymal stem cells (MSC) or skin fibroblasts, they were able to rescue respiration via the transfer of mitochondria from the healthy cells to the respiratory-deficient cells (Spees et al, 2006). Later studies have shown the capacity of MSC to transfer mitochondria to other cell types harboring mitochondrial damage (though not all type of mitochondrial damage [Cho et al, 2012]), including to cardiomyocytes, which responded to the transfer by reprogramming (Acquistapace et al, 2011), and to cancer cells, which responded to the transfer by increasing their chemoresistance (Pasquier et al, 2013). The implications of these discoveries were demonstrated in an *in vivo* model consisting of inducing acute lung injury by administrating LPS to mice via airways followed by the administration of MSC. Mitochondrial transfer from MSC protected from acute lung injury and was found to be associated with mitochondrial transfer (Islam et al, 2012).

The molecular mechanism mediating mitochondrial transfer remains unclear. Treatment of cells harboring damaged mitochondria with isolated mitochondria does not rescue respiratory capacity function (Spees et al, 2006), nor does supernatant of stem cells, indicating that mitochondria are not transferred through endocytosis and uptake of vesicles. The transfer of mitochondria from one cell to the other seems to be mediated by actin-based extensions named tunneling nanotubes (TNT) and by gap junctions containing connexin 43 (Islam et al, 2012; Pasquier et al, 2013).

In a study published in *The EMBO Journal*, Ahmad et al show that Miro1 (mitochondrial Rho-GTPase 1), a calcium-sensitive adaptor protein that drives the movement of mitochondria along microtubules (Fransson et al, 2006; Saotome et al, 2008; Macaskill et al, 2009), is a key mediator of mitochondrial transfer (Ahmad et al, 2014). The authors developed an *in vitro* system of co-culture of MSC and epithelial cells (EC) as well as an *in vivo* system of mice treated with MSC via the trachea. In both systems, the transfer of mitochondria from MSC was increased in response to the pre-incubation of the acceptor cells with the mitochondrial complex I inhibitor, rotenone, suggesting that impairment of respiratory function may be required for the generation of an acceptor cell. The transfer of mitochondria was associated with a significant recovery of rotenone-induced impairment of mitochondrial bioenergetics function. Co-culturing with MSC reversed the effects of rotenone treatment on ATP levels and complexes I and IV activities and decreased mitochondrial ROS production and cytochrome c release.

Importantly, mitochondrial transfer and the rescue of EC *in vitro* and *in vivo* were blocked when MSC were themselves treated with rotenone. To determine the cause for this blockage, Ahmad et al examined the levels of various proteins known to be associated with mitochondrial intracellular transport. While Miro2, TRAK1, KHC, Myo19 were not affected by rotenone, Miro1 was decreased. An association between Miro1 levels and mitochondrial transfer was also observed when comparing different cell types with different mitochondrial transfer
capacity. MSC, which are potent mitochondrial donors, expressed high levels of Miro1 as compared to lung epithelial cells (LA-4) and fibroblasts (3T3), which are poorer donors. To determine the significance of Miro1, Ahmad et al generated MSC in which Miro1 was knocked down (MSCmiroLo) and MSC in which Miro1 was overexpressed (MSCmiroHi). Compared to control MSC, the transfer of mitochondria by MSCmiroLo to injured EC was reduced. This reduction was not due to a decrease in TNT formation, but to decrease in mitochondrial motility through the nanotubules, which is consistent with Miro1’s role in mitochondrial movement. The impaired transfer of mitochondria from MSCmiroLo also reduced the rescue potential from rotenone toxicity. Miro1 overexpression, in contrast, increased mitochondrial transfer efficiency. MSCmiroHi were also more efficient than control in decreasing airway hyperresponsiveness, decreasing pro-inflammatory cytokines, and restoring ATP levels.

To conclude, Ahmad et al have shown the involvement of Miro1 in the process of mitochondrial transfer (Fig 1). While the signaling pathways initiating mitochondrial transfer in the acceptor and donor cells remain elusive, the discovery of an essential downstream player may allow for the generation of robust experimental systems in which the missing components can be identified. Moreover, the observation that Miro1 overexpression in itself can increase mitochondrial transfer in different models of epithelial lung injury suggests Miro1 as a potential target for enhancing mitochondrial transfer capacity for therapeutic purposes.

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Conflict of interest
The authors declare that they have no conflict of interest.

References

Figure 1. Mitochondrial transfer to the rescue.
The mitochondrial Rho-GTPase, Miro1, enhances intercellular mitochondrial transfer via tunneling nanotubes (TNT) from mesenchymal stem cells (MSC) to epithelial cells (EC) harboring compromised mitochondria, thereby restoring mitochondrial function and preventing epithelial cell death.


