The Journal of Physiology

JOURNAL CLUB

# Mitochondrial-derived vesicles: a new player in cardiac mitochondrial quality control

**Luiz H. M. Bozi[1], Luiz R. G. Bechara[1], Ancély F. dos Santos[2] and Juliane C. Campos[1,3]**

[1] *Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil*

[2] *Department of Biochemistry, Chemistry Institute, University of Sao Paulo, Sao Paulo, Brazil*

[3] *Cedars-Sinai Heart Institute, Cedars-Sinai Medical Center, Los Angeles, USA*

Email: luizbozi@usp.br

Mitochondria are critical organelles involved in adenosine triphosphate (ATP) synthesis, reactive oxygen species generation, ion homeostasis, aldehyde metabolism and programmed cell death. Loss of mitochondrial integrity is sufficient to establish pathological conditions such as cardiovascular diseases. In an attempt to ensure the maintenance of mitochondrial functionality, eukaryotic cells developed an integrated quality control system. This mitochondrial quality control machinery works in different levels of surveillance: (1) the antioxidant enzymes protect the organelle against oxygen-mediated toxicity, (2) the ubiquitin–proteasome system as well as the mitochondrial proteases and chaperones ensure the proteostasis by refolding or degrading damaged mitochondrial proteins, and (3) the interconnected processes of mitochondrial dynamics (fusion and fission) and mitophagy controls mitochondrial size, shape and clearance (Sugiura *et al.* 2014). More recently, a study by Cadete *et al.* (2016) published in *The Journal of Physiology* provides evidence that mitochondrial-derived vesicles (MDVs) are also involved in the maintenance of cardiac mitochondrial homeostasis. Although the results are associative, the well-conducted experiments complemented by the relevant findings make this work attractive, opening a new field of investigation in cardiac mitochondrial physiology.

MDVs are generated by selective incorporation of mitochondrial cargo into small vesicles (70–150 nm of diameter) which transit to the lysosome for subsequent degradation (Sugiura *et al.* 2014). Despite it being a conserved mechanism from bacteria to mammals, the presence of MDVs and their physiological relevance in various cells types, including cardiomyocytes, needs to be clarified. In an attempt to characterize the role of MDVs in heart physiology, Cadete *et al.* (2016) used both *in vitro* and *in vivo* approaches. The authors first showed the presence of MDVs in H9C2 myoblasts (a cardiac cell line). It is worth mentioning that their innovative approach of switching the cell energy substrate from glucose to galactose, in order to stimulate the mitochondrial metabolism, helped the authors to identify MDVs under normal conditions. MDVs were identified by their apparent size and selective enrichment for mitochondrial markers from both matrix (PDH – pyruvate dehydrogenase) and outer membrane (TOM20 – translocase of the outer membrane) at baseline. A further increase in MDVs along with a hyperfused mitochondrial network was detected upon mild oxidative stress. Under severe oxidative stress myoblasts accumulated both PDH-enriched vesicles and fragmented mitochondria. Using another stress condition, authors demonstrated that doxorubicin-induced stress increased MDV formation within 30 min without affecting mitochondrial morphology and bioenergetics in myoblasts. Interestingly, PDH and TOM20-enriched vesicles gradually declined in the following 6 h, while the mitochondrial network became fragmented. Therefore, the authors suggest that MDV formation (1) occurs in cardiac cells, (2) is responsive to specific mitochondrial stress conditions and (3) precedes mitochondrial dysfunction.

Despite H9C2 myoblasts having been widely studied as a cardiac cell line, they are phenotypically and metabolically different from cardiomyocytes. In order to verify whether MDV formation occurs in cardiomyocyte-like cells, H9C2 cells undergoing differentiation were analysed. At baseline, a 3-fold increase in both PDH- and TOM20-enriched vesicles were detected in differentiated H9C2 cells compared to myoblasts. Moreover, differentiated cells increased MDVs upon mitochondrial stress. This scenario can be explained, at least in part, by the shift from glycolytic to oxidative metabolism along with an increasing number of mitochondria that occurs during cellular differentiation. Similarly, Soubannier *et al.* (2012) showed that elevated mitochondrial metabolism increases MDV transit to the lysosome upon stress in HeLa cells.

In a second arm of the study, the authors used ultrastructural analysis to determine the physiological relevance of MDV formation *in vivo*. In fact, single and double membrane MDVs measuring 50–200 nm in diameter, without any evident cristae, were identified in isolated mice hearts perfused with antimycin A (a mitochondrial complex III inhibitor). A better characterization of MDVs was performed using electron tomography analysis, where MDVs could be distinguished from sarcoplasmic reticulum or small mitochondria undergoing fission or fusion. Corroborating the *in vitro* data, MDVs were also observed in healthy mice hearts. Furthermore, mice acutely treated with doxorubicin also displayed increased MDVs (number and diameter) along with early signs of cardiac remodelling, mitochondrial dysfunction and mitophagy. Interestingly, autophagosomes containing mitochondria were fewer in number in doxorubicin-treated hearts compared to MDVs, while no autophagosomes were found in control mice hearts. Based on these findings, the authors suggest that MDVs are an active and physiologically relevant mitochondrial quality control element in cardiac cells.

The mechanisms underlying MDV formation, transport and delivery to the lysosome are still poorly understood. For this reason, the physiological role of MDVs is difficult to determine. There are two pieces of evidence that support the authors' claim about the involvement of MDV transit to the lysosome in the mitochondrial quality control system. First, MDV protein cargo is selectively incorporated based on the nature of mitochondrial stress. For example, global cellular oxidative stress induces MDVs carrying outer mitochondrial membrane proteins. On the other hand, oxidative stress inside the organelle leads to MDVs carrying mitochondrial complex III subunits (Sugiura *et al.* 2014). The second piece of evidence is that MDV transit to the lysosome requires the mitophagy-related

 DOI: 10.1113/JP273124

proteins PINK1 and Parkin, which have been shown to be involved in mitophagy (Sugiura *et al.* 2014). Since PINK1 and Parkin are associated with both mechanisms, it is difficult to sort out the role of MDVs in cardiac mitochondrial quality control. It has been demonstrated that PINK1 and Parkin are crucial for the maintenance of heart physiology. In fact, Parkin-deficient mice are more sensitive to myocardium infarction (Kubli *et al.* 2013), while PINK1 deficiency leads to impaired mitochondrial bioenergetics and cardiac dysfunction (Billia *et al.* 2011). Interestingly, protein levels of PINK1 are reduced in the myocardium of end-stage heart failure patients (Billia *et al.* 2011). Therefore, considering that both MDV and mitophagy share common regulation pathways and that cardiac MDV formation precedes mitochondrial fragmentation (required for mitophagy), it is reasonable to suggest MDV transit to the lysosome as an early stage of the cardiac mitochondrial quality control system. However, direct evidence will be required to clarify how MDV transit to the lysosome contributes to the maintenance of heart physiology.

In summary, Cadete *et al.* (2016) provided evidence that cardiac cells generate MDV transit to the lysosome in response to mitochondrial stress. Moreover, MDVs seem to be a new component of mitochondrial quality control in the heart. However, the contribution of MDVs to cardiac physiology and pathophysiology is still unknown.

## References

Billia F, Hauck L, Konecny F, Rao V, Shen J & Mak TW (2011). PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci USA* **108**, 9572–9577.

Cadete VJ, Deschenes S, Cuillerier A, Brisebois F, Sugiura A, Vincent A, Turnbull D, Picard M, McBride H & Burelle Y (2016). Formation of mitchondrial-derived vesicles is an active and physiologically relevant mitochondrial quality control process in the cardiac system. *J Physiol* **594**, 5343–5362.

Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, Jimenez R, Petrosyan S, Murphy AN & Gustafsson AB (2013). Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem* **288**, 915–926.

Soubannier V, McLelland GL, Zunino R, Braschi E, Rippstein P, Fon EA & McBride HM (2012). A vesicular transport pathway shuttles cargo from mitochondria to lysosomes. *Curr Biol* **22**, 135–141.

Sugiura A, McLelland GL, Fon EA & McBride HM (2014). A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. *EMBO J* **33**, 2142–2156.

## Additional information

### Competing interests

None declared.

### Funding

L.H.M.B. and L.R.G.B. hold scholarships from Conselho Nacional de Pesquisa e Desenvolvimento (CNPq). A.F.S and J.C.C hold scholarships from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

### Acknowledgements

We would like to acknowledge Dr Julio C. B. Ferreira for his critical review.