

## NEIGHBORHOOD WATCH

# Platelets fuel mesenchymal stem cells by providing live mitochondria

Zilong Zhao<sup>1</sup> | Jianning Zhang<sup>1</sup> | Jing-fei Dong<sup>2,3</sup> 

<sup>1</sup>Department of Neurosurgery, Tianjin Institute of Neurology, Tianjin Medical University General Hospital, Tianjin, China

<sup>2</sup>BloodWorks Research Institute, Seattle, WA, USA

<sup>3</sup>Division of Hematology, Department of Medicine, University of Washington, School of Medicine, Seattle, WA, USA

**Correspondence:** Jing-fei Dong, BloodWorks Research Institute, 1551 Eastlake Avenue East, 98102 Seattle, WA, USA.

Email: jfdong@BloodWorksNW.org

## Funding information

National Institutes of Health, Grant/Award Number: HL152200; National Natural Science Foundation of China, Grant/Award Number: 82022020 and 81930031

Platelets are the smallest enucleated cells circulating in blood.<sup>1</sup> Their primary function is to stop bleeding at the site of vascular injury in a process called hemostasis. Platelets are therefore considered to be acute-phase reactants that are rapidly mobilized in response to injury. However, platelets have also been increasingly recognized for their participation in processes beyond bleeding arrest, such as inflammation, immune response, angiogenesis, and wound healing.<sup>2–5</sup> These diverse activities require not only adhesion receptors on platelets, but also molecules released from platelet storage granules and extracellular vesicles (EVs) shed from activated platelets. Wound healing exemplifies synergistic actions among these platelet activities, from initiating hemostasis and inflammatory and immune reaction at the site of injury during the acute phase to promoting angiogenesis and tissue regeneration during the repair phase. Growth factors and angiogenic molecules released from activated platelets are most commonly studied for roles of platelets in tissue repairs. For example, these factors have been studied in the context of platelet-mediated improvement of the viability of mesenchymal stem cells (MSCs).<sup>6</sup>

In the February issue of *Cell Metabolism*, Levoux et al.<sup>7</sup> reported that platelets can also promote wound healing by donating metabolically competent mitochondria to MSCs to enhance their angiogenic activity and thus promote tissue repair. In mouse models, the authors showed that activated platelets release mitochondria that are taken up by MSCs through dynamin-dependent, clathrin-mediated endocytosis. These platelet-derived respiratory-competent mitochondria change the metabolic dynamics of recipient MSCs to increase de

novo fatty acid synthesis. It has been previously shown that de novo fatty acid synthesis stimulates proliferation and angiogenesis of endothelial cells and cancer stem cells. The study by Levoux et al. now documents that de novo fatty acid synthesis in MSCs is enhanced by metabolically competent mitochondria released from activated platelets to fuel the pro-angiogenic activity of these stem cells. While platelets have been extensively shown to promote wound healing, especially in the form of platelet-rich plasma (PRP), this study demonstrates a novel mechanism by which platelets modulate MSCs to enhance their capability of promoting wound healing. This study is important in several respects.

First, the authors reported that platelets activated by calcium overload release respiratory-competent mitochondria into the extracellular space.<sup>7</sup> These extracellular mitochondria are released as either free organelles or embedded in the plasma membrane, consistent with a previous report.<sup>8</sup> The authors did not provide details regarding how mitochondria are released from activated platelets, raising several important questions: (1) Do platelets release mitochondria through exocytosis or by self-disruption? The former is a regulated process that requires fusion between mitochondria and the plasma membrane of host cells. If this is the case, it will be interesting to study the underlying pathway of mitochondrial exocytosis of platelets as well as other cells. The latter is a passive process that occurs during tissue injury or after apoptosis. We have shown that traumatically injured brain cells release metabolically competent mitochondria into circulation,<sup>9</sup> likely through this passive process. Platelet activation has been considered by some as the process of apoptosis,<sup>10,11</sup> but it is less likely in this case because platelets release functional mitochondria, instead

Manuscript handled by: Ton Lisman

Final decision: Ton Lisman, 10 March 2021

© 2021 International Society on Thrombosis and Haemostasis

of condensed and damaged mitochondria found during apoptosis. Detailed knowledge of how mitochondria are released from activated platelets is critical to understanding one of the fundamental processes of cell-cell communications, even though it may not be relevant for studying the efficacy of PRP in improving wound healing. (2) Is PRP more efficacious for promoting wound healing when a platelet agonist is added, as shown in this study? Alternatively, platelets may be rapidly activated upon being applied to a wound by extracellular matrix proteins (e.g., collagen) exposed at the site of injury. (3) Do the mitochondria released from activated platelets differ structurally from those inside platelets? The mitochondria released from injured brains maintain their metabolic activity, but also express the anionic phospholipid cardiolipin on their surface.<sup>9</sup> Cardiolipin is located exclusively in the inner membrane of intracellular mitochondria during homeostasis,<sup>12</sup> suggesting structural changes of the mitochondrial membrane. A similar structural change may also occur to some or all platelet-derived mitochondria. Cardiolipin exposed on these extracellular mitochondria could trigger local coagulation to generate fibrin,<sup>13</sup> which is also known to promote wound healing.

Second, the authors showed that mitochondria are transferred to MSCs through dynamin-dependent, clathrin-mediated endocytosis, which is blocked by dynasore.<sup>7</sup> Dynamin is a GTPase that belongs to a large dynamin superfamily. It dimerizes to regulate the process of membrane budding and fission.<sup>14</sup> Dynasore inhibits the GTPase activity of dynamin to prevent endocytosis.<sup>15</sup> Interesting questions related to this pathway include: (1) whether the mitochondrial dynamin-like GTPase (e.g., OPA1) is also involved in the process and (2) if there is a loading limitation regarding how many mitochondria each MSC can take. In this study, the authors show that MSCs exposed to platelets have more and hyper-fused mitochondria defined by transmission electron microscopy,<sup>7</sup> suggesting potential integration or fusion between donor and host mitochondria.

Third, the authors showed that platelet-derived mitochondria endocytosed by MSCs remain metabolically active in the host environment and promote the angiogenic activity of MSCs without enhancing the rates of proliferation and differentiation of these cells.<sup>7</sup> The authors showed that MSCs have an enhanced rate of respiration measured by oxygen concentration when they are incubated with respiratory-competent platelets, but not when they are incubated with respiratory-suppressed platelets (i.e., rotenone and antimycin A treated). Respiratory-competent platelets increase citrate-dependent fatty acid synthesis (i.e., the tricarboxylic acid cycle). Exogenous citrate restores the pro-angiogenic activity of MSCs incubated with respiration-suppressed platelets. In contrast, respiratory-suppressed platelets enhance glycolysis, which converts glucose to pyruvate enzymatically. Both processes are energy dependent, but they appear to be differentially regulated by platelets or platelet-derived mitochondria. These findings should lead to further investigation into their underlying mechanisms because fatty acids are synthesized from acetyl coenzyme A, which comes from carbohydrates via the glycolytic pathway. Nevertheless, this study demonstrates that MSCs with adopted mitochondria change their

rates of fatty acid and sugar metabolism differently. It reveals a new link between fatty acid synthesis and angiogenesis, but the molecular mechanism of this link is not discussed. Questions also remain regarding: (1) whether MSCs with adopted mitochondria synthesize and release more pro-angiogenic factors to promote angiogenic activity and (2) how long adopted mitochondria survive in MSCs and how these mitochondria avoid the intracellular degradation machineries such as autophagy.

Finally, the results from this study add to a growing body of evidence that mitochondria can be released into the extracellular space and transferred between cells,<sup>9,16-18</sup> suggesting a common pathway of cell-to-cell communication through live mitochondria. This communication can regulate both the physiology and pathophysiology of cells, depending on the types of cells and the environment. For example, astrocyte-derived mitochondria are transferred to damaged neurons to upregulate pro-survival and anti-apoptotic signals through the calcium-dependent CD38 pathway in a mouse model of stroke.<sup>19</sup> Mitochondria derived from the bone-marrow-derived stromal cells are transferred to pulmonary alveoli to alleviate acute lung injury.<sup>20</sup> In addition to these protective activities, extracellular mitochondria have also been shown to have detrimental effects. For example, brain-derived mitochondria express the anionic phospholipid cardiolipin, which contributes to acute consumptive coagulopathy induced by traumatic brain injury.<sup>13</sup> Furthermore, these metabolically competent mitochondria are released into the circulation, where they become a major source of oxidative stress. Reactive oxygen species produced from mitochondria inside a cell are rapidly neutralized in the highly reducing environment of the cytoplasm, but those produced from mitochondria circulating in blood may persist for a prolonged period of time because blood is only mildly reducing.<sup>21-24</sup> Moreover, cell-free mitochondria bind to cells (platelets, leukocytes, and endothelial cells) to concentrate oxidative stress locally or evade the intrinsic antioxidants in blood. We have shown that cell-free mitochondria bind platelets through the lipid receptor CD36 to activate platelets in an oxidative stress-dependent fashion.<sup>25</sup> These cell-free mitochondria can also serve as the endogenous substrate for secreted phospholipase A2 IIA (sPLA2-IIA) to promote inflammation,<sup>8</sup> which is a key aspect of wound healing. Platelet-derived mitochondria have been reported to serve as endogenous antigens that contribute to the pathophysiology of systemic lupus erythematosus.<sup>26</sup> In addition, the transfer between cells can serve as a means to scavenge mitochondria, as it has been reported that mitochondria released from retinal neurons are endocytosed by astrocytes for disposal and recycling.<sup>27</sup> Macrophages take up dysfunctional mitochondria ejected from cardiomyocytes during homeostasis of the heart.<sup>28</sup> These dysfunctional mitochondria are then dismantled and recycled through autophagy in macrophages.

## 1 | FUTURE PERSPECTIVES

The findings reported by Levoux et al. offer future perspectives for both therapeutics and fundamental cell biology. For therapeutics,

platelet products in various forms (e.g., PRP, platelet-fibrin-rich plasma) have been extensively demonstrated to improve wound healing. Their therapeutic potential is consistent with their biological activities involving every aspect of tissue injury and repair. While studies in the past have been focused mostly on soluble factors released from platelets, this study shows that mitochondria as organelles can promote wound healing by enhancing MSC functions, potentially leading to a new path for cell-based therapies with far more applications. This mitochondria-based therapy will require further investigations to answer fundamental questions. For instance, are platelet-derived mitochondria specifically active in promoting wound healing? If not, can platelets, each of which contains four to five mitochondria,<sup>8</sup> be replaced by mitochondria-rich cells (e.g., leukocytes in the buffy coat) as better donors? Can extracellular mitochondria be produced and stored as adjuvant stem cell-based treatment for injury and tissue regeneration? If mitochondria improve MSCs by enhancing citrate-based fatty acid synthesis, can agents directly regulating this pathway be equally effective in enhancing MSC function?

The findings described in this report also offer new aspects of mitochondrial biology that differ from mitochondrial (dys)function within a cell and from molecules released from fragmented mitochondria (e.g., mitochondrial damage-associated molecular patterns [mDAMPs]). In this regard, there is sufficient evidence to suggest that mitochondrial transfer between cells can alter the biological activities of both donor and recipient cells. More importantly, results from this study suggest that mitochondria do not increase the energy production of host cells simply by adding more mitochondria, but rather by differentially regulating pathways of fatty acid synthesis and carbohydrate metabolism in a respiration-dependent manner. It will be important to learn how these pathways are regulated by respiration-competent mitochondria. Another question is whether adopted mitochondria are active transiently and degraded rapidly or become an integral part of host cells. If the latter is true, studying mitochondrial transfer could potentially lead to paradigm-shifting discoveries.

## ACKNOWLEDGMENTS

This work is supported by the Young Scientists Award 82022020 from the National Natural Science Foundation of China (ZLZ), a research grant from the National Natural Science Foundation of China 81930031 (JNZ), and the NIH grant HL152200 (JFD).

## CONFLICTS OF INTEREST

The authors claim no relevant conflicts of interest.

## AUTHOR CONTRIBUTIONS

NF, ABM, MvdB, KM, and EHTME designed the study. NF, MBS, and EHTME performed the experimental work. FPJvA helped with the development of Mass Spectrometry experiments. NF, ABM, MvdB, KM, and EHTME analysed and interpreted data. NF and EHTME made the figures. NF, ABM, MvdB, KM, and EHTME wrote the manuscript.

## ORCID

Jing-fei Dong  <https://orcid.org/0000-0001-6379-4133>

## REFERENCES

1. Thon JN, Italiano JE. Platelets: production, morphology and ultra-structure. *Handb Exp Pharmacol*. 2012;210:3-22.
2. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood*. 2014;123(18):2759-2767.
3. Italiano JE Jr, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. *Blood*. 2008;111(3):1227-1233.
4. Kisucka J, Butterfield CE, Duda DG, et al. Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. *Proc Natl Acad Sci USA*. 2006;103(4):855-860.
5. Opneja A, Kapoor S, Stavrou EX. Contribution of platelets, the coagulation and fibrinolytic systems to cutaneous wound healing. *Thromb Res*. 2019;179:56-63.
6. Hersant B, Sid-Ahmed M, Braud L, et al. Platelet-rich plasma improves the wound healing potential of mesenchymal stem cells through paracrine and metabolism alterations. *Stem Cells Int*. 2019;2019:1234263.
7. Levoux J, Prola A, Lafuste P, et al. Platelets facilitate the wound-healing capability of mesenchymal stem cells by mitochondrial transfer and metabolic reprogramming. *Cell Metab*. 2020;33(3):688-690.
8. Boudreau LH, Ducheze AC, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood*. 2014;124(14):2173-2183.
9. Zhao Z, Zhou Y, Li M, Zhang J, Dong JF. Extracellular mitochondria in traumatic brain injury induced coagulopathy. *Semin Thromb Hemost*. 2020;46(2):167-175.
10. Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. *J Thromb Haemost*. 2006;4(12):2656-2663.
11. Leung R, Gwozdz AM, Wang H, et al. Persistence of procoagulant surface expression on activated human platelets: involvement of apoptosis and aminophospholipid translocase activity. *J Thromb Haemost*. 2007;5(3):560-570.
12. Hovius R, Lambrechts H, Nicolay K, de Kruijff B. Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane. *Biochim Biophys Acta*. 1990;1021(2):217-226.
13. Zhao Z, Wang M, Tian Y, et al. Cardiolipin-mediated procoagulant activity of mitochondria contributes to traumatic brain injury-associated coagulopathy in mice. *Blood*. 2016;127(22):2763-2772.
14. Ferguson SM, De Camilli P. Dynamin, a membrane-remodelling GTPase. *Nat Rev Mol Cell Biol*. 2012;13(2):75-88.
15. Macia E, Ehrlich M, Massol R, Boucrot E, Brunner C, Kirchhausen T. Dynasore, a cell-permeable inhibitor of dynamin. *Dev Cell*. 2006;10(6):839-850.
16. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci USA*. 2006;103(5):1283-1288.
17. Puhm F, Afonyushkin T, Resch U, et al. Mitochondria are a subset of extracellular vesicles released by activated monocytes and induce type I IFN and TNF responses in endothelial cells. *Circ Res*. 2019;125(1):43-52.
18. D'Acunzo P, Perez-Gonzalez R, Kim Y, et al. Mitovesicles are a novel population of extracellular vesicles of mitochondrial origin altered in Down syndrome. *Sci Adv*. 2021;7(7):eabe5085.
19. Hayakawa K, Esposito E, Wang X, et al. Transfer of mitochondria from astrocytes to neurons after stroke. *Nature*. 2016;535(7613):551-555.

20. Islam MN, Das SR, Emin MT, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med*. 2012;18(5):759-765.
21. Anderson ME, Meister A. Dynamic state of glutathione in blood plasma. *J Biol Chem*. 1980;255(20):9530-9533.
22. Mansoor MA, Svardal AM, Ueland PM. Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. *AnalBiochem*. 1992;200(2):218-229.
23. Jones DP. Redefining oxidative stress. *AntioxidRedoxSignal*. 2006;8(9-10):1865-1879.
24. Lash LH, Jones DP. Distribution of oxidized and reduced forms of glutathione and cysteine in rat plasma. *ArchBiochemBiophys*. 1985;240(2):583-592.
25. Zhao Z, Zhou Y, Hilton T, et al. Extracellular mitochondria released from traumatized brains induced platelet procoagulant activity. *Haematologica*. 2020;105(1):209-217.
26. Melki I, Allaey I, Tessandier N, et al. Platelets release mitochondrial antigens in systemic lupus erythematosus. *Sci Transl Med*. 2021;13(581):eaav5928.
27. Davis CH, Kim KY, Bushong EA, et al. Transcellular degradation of axonal mitochondria. *Proc Natl Acad Sci USA*. 2014;111(26):9633-9638.
28. Nicolas-Avila JA, Lechuga-Vieco AV, Esteban-Martinez L, et al. A network of macrophages supports mitochondrial homeostasis in the heart. *Cell*. 2020;183(1):94-109 e123.