Osteoarthritis and Cartilage

Mitochondrial Biogenesis: A Potential Therapeutic Target for Osteoarthritis

--Manuscript Draft--

Manuscript Number:	
Article Type:	Invited Editorial
Section/Category:	
Keywords:	mitochondria, osteoarthritis, mitophagy, inflammation
Corresponding Author:	Francisco J. Blanco, MD INIBIC-Hospital Universitario A Coruña A Coruña, SPAIN
First Author:	Francisco J. Blanco, MD
Order of Authors:	Francisco J. Blanco, MD
	Mercedes Fernandez-Moreno
Manuscript Region of Origin:	SPAIN
Abstract:	Mitochondrial dysfunction of human articular chondrocytes is considered a hallmark of cartilage degradation and OA pathogenesis. Due to the huge number of cellular processes in which mitochondria is implicated, even in the closed context of cellular respiration, the term mitochondrial function can refer to a variety of features which include fusion and fission, turnover (biogenesis and mitophagy), and plasticity. M itochondrial biogenesis and mainly mitochondrial fusion and reduced mitophagy, contribute to the metabolic disorder and inflammation that occurs during OA. Reduced MFN2 and increased PARKIN expression represent potential therapeutic targets for the treatment of joint cartilage degradation during the OA process

Mitochondrial Biogenesis: A Potential Therapeutic Target for Osteoarthritis

Francisco J. Blanco (1) and Mercedes Fernández-Moreno (2)

Authors

1) Grupo de Investigación en Reumatología. Instituto de Investigación Biomédica de A Coruña (INIBIC). Agrupación estratégica CICA-INIBIC. Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas. Department of Medicine, Universidade de A Coruña (UDC), Spain.
2) Centro de investigación biomédica en Red, Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN).

Keywords: Mitochondrial dynamics, Mfn2, Osteoarthritis,

Address correspondence to:

Francisco J. Blanco
INIBIC-Hospital Universitario A Coruña, Xubias, 84,
15006 A Coruña,
Spain.
E-mail address: fblagar@sergas.es

Osteoarthritis (OA), the most frequent joint disease, is a heterogeneous disorder. Genetic, ageing, biomechanical, metabolic and inflammatory factors are involved in its pathogenesis (1). Mitochondrial dysfunction of human articular chondrocytes is considered a hallmark of cartilage degradation and OA pathogenesis (2-5).

Due to the huge number of cellular processes in which mitochondria is implicated, even in the closed context of cellular respiration, the term mitochondrial function can refer to a variety of features which include fusion and fission, turnover (biogenesis and mitophagy), and plasticity (6). The consideration of these mitochondrial capabilities has advanced our understanding of how the cell may respond to metabolic disturbances such as oxidative stress, inflammation, and gluco- and lipotoxicity (7).

Mitochondrial fission and fusion control number, distribution, and morphology of these organelles in the cell and therefore play important roles for diverse mitochondrial functions such as energy production, metabolism, intracellular signaling and apoptosis (8, 9). Fission is needed to created new mitochondria and it is mediated by dynamic-related peptide 1 (*Drp1*) and fission protein 1 (*Fis1*) (8). Mitochondrial fusion is controlled by GTPase enzymes; namely mitofusins 1 and 2 (*Mfn1* and *Mfn2*), that control outer mitochondrial membrane fusion, and optic atrophy 1 (*Opa1*) that governs inner mitochondrial membrane fusion (9). Fusion has several essential roles inside the cells, for example, it helps mitigate cellular stress by removing damaged mitochondria through mitophagy (23).

The balance between fission and fusion is a key mechanism in the regulation of mitochondrial structure, function and cellular health (8, 11). Mitochondrial dynamics are altered in several illnesses such as insulin-resistance (12), type 2 diabetes (13), several neurodegenerative diseases (14) including Alzheimer's disease (15), cancer (16), cardiovascular alterations (17, 18) and ischemic and dilated cardiomyopathy (19, 20). Given the relevance of mitochondrial morphology in the regulation of multiple cell functions, and its potential connection with several pathologies, the importance of a detailed knowledge of the molecules/mechanisms that govern mitochondrial fusion and fission processes seems clear.

In the current issue of Osteoarthritis and Cartilage, Xu and co-workers investigate the effect of mitochondrial fusion on metabolic changes during aging and OA. They

define the impact of *Mfn2* on OA progression both *in vitro* and *in vivo* models. Authors tested the expression of OPA1, MFN1 and MFN2, the most relevant proteins involved in mitochondrial fusion, and results showed a significant increase in *Mfn2* expression during aging, as well as OA, suggesting that MFN2 contributed to mitochondrial dynamics impairment in chondrocytes during aging. To ascertain whether MFN2 regulated metabolic changes of aging cells, they knocked down *Mfn2* with small interfering RNA and found that basal and maximal oxygen consumption rates (OCR) were downregulated while glucose uptake was upregulated, suggesting a metabolic shift from oxidative phosphorylation to glycolysis.

These results disagree with other authors reporting that *Mfn2* knockdown suppresses mitophagy in different cell types, such as cardiomyocytes or neurons, resulting in the accumulation of damaged mitochondria and pathological conditions (21). Similarly, age-related *Mfn2* depletion in muscles was identified as a link between an inhibition of mitophagy, and the subsequent accumulation of dysfunctional mitochondria, to sarcopenia (21). *Mfn2* expression has also been found to be reduced in obesity and type I diabetes, two diseases associated with OA. MFN2 down-regulation activates the JNK pathway, favoring the formation of lipid intermediates that lead to insulin resistance in both skeletal muscle and liver (22).

MFN1 and MFN2 are homologous proteins that belong to the large family of mitochondrial trans-membrane GTPases and share some functions in the mitochondrial fusion process. Mfn1 plays a more relevant role in mitochondrial fusion, whereas MFN2 mainly affects mitochondrial metabolism by regulating several mitochondrial functions such as membrane potential, fuel oxidation and the OXPHOS system (23). A fused, continuous network is associated to a higher ATP production, likely due to optimized exchanges of metabolites within their matrix. However, Xu et al. found declined, rather than elevated, ATP production by *Mfn2* knockdown.

We agree with the authors' proposal to explain this discrepancy, as well as the differences found compared to cardiomyocytes and neurons and the data reported in diabetes mellitus and obesity. They argue that these previous results were obtained in models with a metabolism predominantly based on oxidative phosphorylation, while the metabolism of the chondrocyte *in situ* is mainly glycolytic. As a result, any action that promotes an increase in the oxidoreductive metabolism, such as mitochondrial fusion,

would generate higher levels of ATP, ROS and inflammation mediators, causing damage to the chondrocyte and to cartilage.

This hypothesis is supported by their experiments using lentiviruses to establish *Mfn2* overexpression/knockdown models. Results strongly suggested that *Mfn2* overexpression played a pro-inflammatory role in DMM models by upregulating the expression of inflammation-related genes, including COX2 and MMP13, via NF-κB pathway, and that knockdown of *Mfn2* had a protective effect against OA progression. In contrast, Chen Y et al. reported that *Mfn2* expression was decreased in human nucleus pulposus tissues during intervertebral disc degeneration. In addition, these authors showed that *Mfn2* knockdown aggravated the impairment of autophagic flux, mitochondrial dysfunction and cellular apoptosis in rat nucleus pulposus cells, while *Mfn2* overexpression significantly reversed these alterations (24).

Thus, both increased and decreased *Mfn2* expression have been found in different disease experimental models (human and animal). All these data support the idea that MFN2 expression differs between organs and tissues, much like bioenergetic efficiencies and mechanisms of adaptation to nutrient availability.

Mitochondria biogenesis (fission, fusion and mitophagy) represent an attractive therapeutic target to treat multiple diseases such as Alzheimer (15), age-related skeletal muscle alteration (255) or several cancers (26). Pharmacological inhibition of fission may only be protective in the short-term, because chronic inhibition would be detrimental to the organs as fission is critical to maintaining a healthy mitochondrial network (27, 28). In the case of OA, according to the results reported, the therapeutic target must be focused on reducing mitochondrial fusion and *Mfn2* expression. In this sense, increasing the levels of ubiquitin E3 ligase Parkin (PARKIN) could be a good option. The process for culling damaged mitochondria is mediated via PTEN-induced putative kinase 1 (PINK1) which is stabilized on the mitochondria membrane and recruits the PARKIN that, in turn can ubiquitinate MFN2. The poly-ubiquitination of MFN2 leads to the engulfment of mitochondria in the autophagosome via P62/Beclin 1, LC3 activation and fusion with lysosomes (29, 31. Data reported so far support this hypothesis, loss of PARKIN function contributes directly to the pathogenesis of OA (32) and the elevated expression of MFN2 in OA cartilage is a result of the declined levels of PARKIN.

In summary, mitochondrial biogenesis and mainly mitochondrial fusion and reduced mitophagy, contribute to the metabolic disorder and inflammation that occurs during OA. Reduced MFN2 and increased PARKIN expression represent potential therapeutic targets for the treatment of joint cartilage degradation during the OA process.

Author contributions

FJB and MFM researched data for the article, made substantial contributions to discussions of the content, wrote the article and contributed to reviewing and editing of the manuscript before submission.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

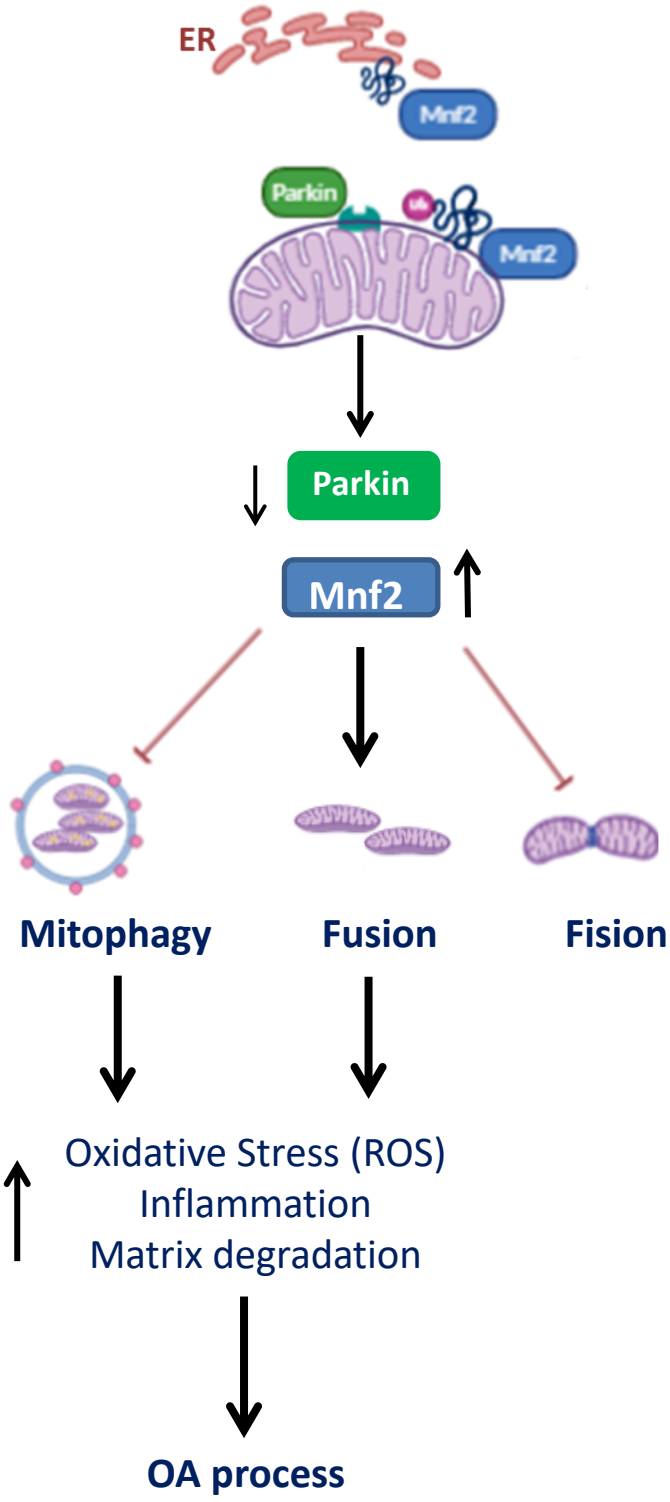
The authors' work is supported by Fondo de Investigacion Sanitaria (grants CIBERCB06/01/0040-Spain, RETIC-RIER-RD16/0012/0002,PRB2-ISCIII-PT13/0001, integrated in the National Plan for Scientific Program, Development and Technological Innovation 2013-2016 and funded by the ISCIII-General Subdirection of Assessment and Promotion of Research-European Regional Development Fund (FEDER) “A way of making Europe”.

References

1. Kraus VB, Blanco FJ, Englund M, Karsdal MA, Lohmander LS. Call for standardized definitions of osteoarthritis and risk stratification for clinical trials and clinical use. Osteoarthritis Cartilage. 2015;23(8):1233-41.
2. Blanco FJ, Rego I, Ruiz-Romero C. The role of mitochondria in osteoarthritis. Nat Rev Rheumatol. 2011;7(3):161-9.
3. Wu L, Liu H, Li L, Cheng Q, Li H, Huang H. Mitochondrial pathology in osteoarthritic chondrocytes. Curr Drug Targets. 2014;15(7):710-9.
4. Blanco FJ, June RK, 2nd. Cartilage Metabolism, Mitochondria, and Osteoarthritis. J Am Acad Orthop Surg. 2019.
5. Liu H, Li Z, Cao Y, Cui Y, Yang X, Meng Z, et al. Effect of chondrocyte mitochondrial dysfunction on cartilage degeneration: A possible pathway for osteoarthritis pathology at the subcellular level. Mol Med Rep. 2019;20(4):3308-16.
6. Koliaki C, Roden M. Alterations of Mitochondrial Function and Insulin Sensitivity in Human Obesity and Diabetes Mellitus. Annu Rev Nutr. 2016;36:337-67.
7. Galloway CA, Yoon Y. Mitochondrial morphology in metabolic diseases. Antioxid Redox Signal. 2013;19(4):415-30.
8. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science. 2012;337(6098):1062-5.
9. Liesa M, Palacin M, Zorzano A. Mitochondrial dynamics in mammalian health and disease. Physiol Rev. 2009;89(3):799-845.
10. Schrepfer E, Scorrano L. Mitofusins, from Mitochondria to Metabolism. Mol Cell. 2016;61(5):683-94.
11. Chan DC. Fusion and fission: interlinked processes critical for mitochondrial health. Annu Rev Genet. 2012;46:265-87.
12. Bernhardt D, Muller M, Reichert AS, Osiewacz HD. Simultaneous impairment of mitochondrial fission and fusion reduces mitophagy and shortens replicative lifespan. Sci Rep. 2015;5:7885.
13. Dube JJ, Collyer ML, Trant S, Toledo FGS, Goodpaster BH, Kershaw EE, et al. Decreased mitochondrial dynamics is associated with insulin resistance, metabolic rate, and fitness in African Americans. J Clin Endocrinol Metab. 2019.
14. Rovira-Llopis S, Banuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, Victor VM. Mitochondrial dynamics in type 2 diabetes: Pathophysiological implications. Redox Biol. 2017;11:637-45.
15. Elfawy HA, Das B. Crosstalk between mitochondrial dysfunction, oxidative stress, and age related neurodegenerative disease: Etiologies and therapeutic strategies. Life Sci. 2019;218:165-84.
16. Sita G, Hrelia P, Graziosi A, Morroni F. Back to The Fusion: Mitofusin-2 in Alzheimer's Disease. J Clin Med. 2020;9(1).
17. Srinivasan S, Guha M, Kashina A, Avadhani NG. Mitochondrial dysfunction and mitochondrial dynamics-The cancer connection. Biochim Biophys Acta Bioenerg. 2017;1858(8):602-14.
18. Hoppel CL, Lesnefsky EJ, Chen Q, Tandler B. Mitochondrial Dysfunction in Cardiovascular Aging. Adv Exp Med Biol. 2017;982:451-64.
19. Wu NN, Zhang Y, Ren J. Mitophagy, Mitochondrial Dynamics, and Homeostasis in Cardiovascular Aging. Oxid Med Cell Longev. 2019;2019:9825061.
20. Chen L, Gong Q, Stice JP, Knowlton AA. Mitochondrial OPA1, apoptosis, and heart failure. Cardiovasc Res. 2009;84(1):91-9.
21. Tang Y, Mi C, Liu J, Gao F, Long J. Compromised mitochondrial remodeling in compensatory hypertrophied myocardium of spontaneously hypertensive rat. Cardiovasc Pathol. 2014;23(2):101-6.
22. Ong SB, Hausenloy DJ. Mitochondrial Dynamics as a Therapeutic Target for Treating Cardiac Diseases. Handb Exp Pharmacol. 2017;240:251-79.
23. Filadi R, Pendin D, Pizzo P. Mitofusin 2: from functions to disease. Cell Death Dis. 2018;9(3):330.
24. Liesa M, Shirihai OS. Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. Cell Metab. 2013;17(4):491-506.
25. Bach D, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, et al. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem. 2003;278(19):17190-7.
26. Chen KH, Dasgupta A, Ding J, Indig FE, Ghosh P, Longo DL. Role of mitofusin 2 (Mfn2) in controlling cellular proliferation. Faseb j. 2014;28(1):382-94.
27. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature. 2008;456(7222):605-10.

28. Filadi R, Greotti E, Turacchio G, Luini A, Pozzan T, Pizzo P. Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling. Proc Natl Acad Sci U S A. 2015;112(17):E2174-81.
29. He C, Chen Y, Liu C, Cao M, Fan YJ, Guo XM. Mitofusin2 decreases intracellular cholesterol of oxidized LDL-induced foam cells from rat vascular smooth muscle cells. J Huazhong Univ Sci Technolog Med Sci. 2013;33(2):212-8.
30. McFie PJ, Ambilwade P, Vu H, Stone SJ. Endoplasmic reticulum-mitochondrial interaction mediated by mitofusin-1 or mitofusin-2 is not required for lipid droplet formation or adipocyte differentiation. Biochem Biophys Res Commun. 2016;478(1):392-7.
31. Narendra D, Tanaka A, Suen DF, Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol. 2008;183(5):795-803.
32. Chen Y, Dorn GW, 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science. 2013;340(6131):471-5.
33. Jin SM, Youle RJ. PINK1- and Parkin-mediated mitophagy at a glance. J Cell Sci. 2012;125(Pt 4):795-9.
34. Chen Y, Lin J, Chen J, Huang C, Zhang Z, Wang J, et al. Mfn2 is involved in intervertebral disc degeneration through autophagy modulation. Osteoarthritis Cartilage. 2020.
35. Sebastian D, Zorzano A. When MFN2 (mitofusin 2) met autophagy: A new age for old muscles. Autophagy. 2016;12(11):2250-1.
36. Yu M, Nguyen ND, Huang Y, Lin D, Fujimoto TN, Molkentine JM, et al. Mitochondrial fusion exploits a therapeutic vulnerability of pancreatic cancer. JCI Insight. 2019;5.
37. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010;121(18):2012-22.
38. Zhang N, Wang S, Li Y, Che L, Zhao Q. A selective inhibitor of Drp1, mdivi-1, acts against cerebral ischemia/reperfusion injury via an anti-apoptotic pathway in rats. Neurosci Lett. 2013;535:104-9.
39. Ikeda Y, Shirakabe A, Maejima Y, Zhai P, Sciarretta S, Toli J, et al. Endogenous Drp1 mediates mitochondrial autophagy and protects the heart against energy stress. Circ Res. 2015;116(2):264-78.
40. Miret-Casals L, Sebastian D, Brea J, Rico-Leo EM, Palacin M, Fernandez-Salguero PM, et al. Identification of New Activators of Mitochondrial Fusion Reveals a Link between Mitochondrial Morphology and Pyrimidine Metabolism. Cell Chem Biol. 2018;25(3):268-78.e4.
41. Ansari MY, Khan NM, Ahmad I, Haqqi TM. Parkin clearance of dysfunctional mitochondria regulates ROS levels and increases survival of human chondrocytes. Osteoarthritis Cartilage. 2018;26(8):1087-97.
42. Blanco FJ, Rego-Perez I. Mitochondria and mitophagy: biosensors for cartilage degradation and osteoarthritis. Osteoarthritis Cartilage. 2018.

Figure. MFN2 is involved in the mitochondrial dynamics, an essential process to eliminate damaged mitochondria in response of damage accumulation. This process is mediated via PINK1, who recruits Parkin, which can poly-ubiquitinate MFN2 leading the mitophagy process. However, MFN2 can block the mitophagy, damaged mitochondria are accumulated inside the cells causing an increase of apoptosis, oxidative stress, mitochondrial dysfunction, mitochondrial fragmentation. All these processes are related with the cartilage degradation during the OA process.



Figure