MitoFit Preprint Arch (2019 MiP/MitoEAGLE School Coimbra2019
2019-06-17 doi:10.26124/mitofit:ea19.MiPSchool.0008

Interplay between metformin and miR-378a-3p in cells under hyperglycaemia

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http://www.mitofit.org/index.php/Machado_2019_MitoFit_Preprint_Arch_EA

Editor MitoFit Preprint Archives: Gnaiger E

Introduction

Metformin is commonly used for the treatment of Type 2 Diabetes Mellitus (T2DM) being known for its role in the impairment of hepatic gluconeogenesis. However, it has been associated with the upregulation of microRNAs (miRNAs) involved in T2DM and cancer. Recently, it was reported that metformin promoted the expression of miR-378a-3p in HepG2 cells [1]. This miRNA is embedded in the *Ppargc1b* gene and has been reported to be an important player in the amelioration of obesity through the activation of the pyruvate-phosphoenolpyruvate (PEP) futile cycle in skeletal muscle [2]. Additionally, this miRNA has also been implicated in the regulation of autophagy in skeletal muscle [3] suggesting that it may also be involved in the removal of damaged mitochondria through mitochondria. In order to study this relation, *Sesn2* KD cells were generated given that SESN2 is a stress-inducible protein and it was reported to be crucial in the induction of mitophagy [4]. Additionally, in the current study we explore the importance of miR-378a-3p in the improvement of mitochondrial function in C2C12 cells exposed to hyperglycaemia and demonstrate a possible interplay between metformin and the miRNA in these conditions.

Methods

To mimic hyperglycaemic conditions, C2C12 myoblasts were incubated for 3 days in 25 mM of glucose media, supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic, after which they were incubated with 10 mM and 25 mM of metformin for 24 h. The total RNA, including miRNA, was extracted using the miRNeasy Mini Kit (Qiagen) and the expression of miR-378a-3p was analysed by RT-qPCR using specific TaqMan probes (Applied Biosystems). In order to evaluate the function of miR-378a-3p on mitophagy, C2C12 *Sesn2* knockdown (KD) cells were generated using lentiviral particles containing a pGIPZ plasmid with an shRNA to target and silence *Sesn2* (Dharmacon). Furthermore, for the functional assays the cells were successfully

transfected with *mir*Vana miR-378a-3p mimics and inhibitors using the Neon Transfection System (Invitrogen). The mitochondrial function and oxidative capacity were evaluated using the Seahorse XF Cell Mito Stress Test kit (Agilent). Lastly, mitophagy was accessed using the Mitophagy Detection Kit (Dojindo).

Results and discussion

C2C12 myoblasts and *Sesn2* KD cells were exposed to 3 days of hyperglycaemia-mimicking conditions and were incubated with 10 mM and 25 mM of metformin in order to study the possible role of metformin in the upregulation of miR-378a-3p. Our results clearly demonstrate that miR-378a-3p expression isn't affected by metformin 10 mM, but it's elevated upon treatment with a higher dose of metformin (25 mM), in both C2C12 myoblasts and *Sesn2* KD cells (Figure 1). Furthermore, preliminary data show that the overexpression of miR-378a-3p in cells exposed to 3 days of hyperglycaemia lead to an increased consumption of oxygen after the addition of the uncoupler FCCP, suggesting that it increases the maximal mitochondrial respiration of the cells. Additionally, the inhibition of this miRNA in cells treated with metformin impaired its effect on the maximal mitochondrial respiration. Lastly, C2C12 myoblasts and *Sesn2* KD cells exposed to 25 mM of glucose were transfected with miR-378a-3p negative controls (NC) and mimics (Mimic), and mitophagy was evaluated by using probes that target lysosomes (Lyso Dye) and damaged mitochondria that are being degraded (Mtphagy Dye). We report that the overexpression of miR-378a-3p in C2C12 myoblasts increases the fluorescence intensity and the colocalization of both probes in comparison with the control in C2C12 myoblasts and *Sesn2* KD cells (Figure 2a and b).

In the current study, we succeeded to demonstrate that metformin is able to increase the expression of miR-378a-3p in C2C12 myoblasts although this is dependent of metformin's concentration. The activation of the miRNA by metformin could be relying on the induction of *Ppargc1b* expression that was previously demonstrated to be co-expressed with miR-378a-3p [5]. miR-378a-3p may be involved at some extent in metformin's mechanism of action. Additionally, miR-378a-3p seems to be an important player in the prevention of mitochondrial dysfunction caused by metabolic stress induced by hyperglycaemic conditions. The overexpression of miR-378a-3p was demonstrated to improve the mitochondrial respiration and to favour and restore mitophagy, that is important in the prevention of the accumulation of damaged mitochondria typically observed in the context of metabolic diseases. This finding is in agreement with the previous reported role of this miRNA in the promotion of autophagy in skeletal muscle [4].

Acknowledgments

This work was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER-000012-HealthyAging2020, the Portugal 2020 - Operational Programme for Competitiveness and Internationalisation, and the Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, I.P.: project POCI-01-0145-FEDER-016770, as well as by UID/NEU/04539/2013 (CNC.IBILI Consortium strategic project). JST is a recipient of an FCT grant (SFRH/BPD/94036/2013).

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Figures

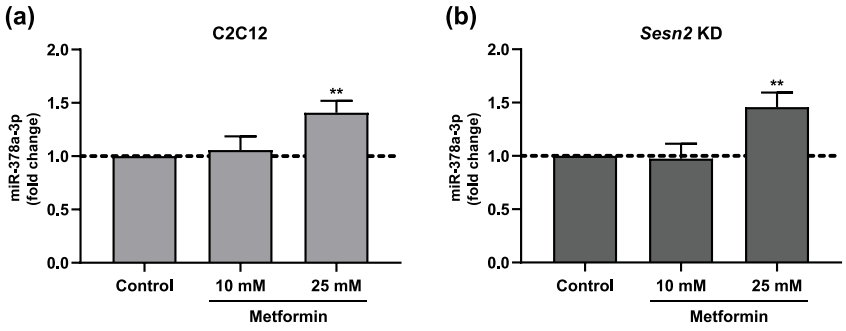

Figure 1. Metformin 25 mM upregulates miR-378a-3p in C2C12 myoblasts previously exposed to 3 days of hyperglycaemia. (a) miR-378a-3p expression in C2C12 myoblasts, and in (b) *Sesn2* KD cells. All data is given as mean ± S.E.M. (n = 5). ***P* < 0.007 *versus* Control.

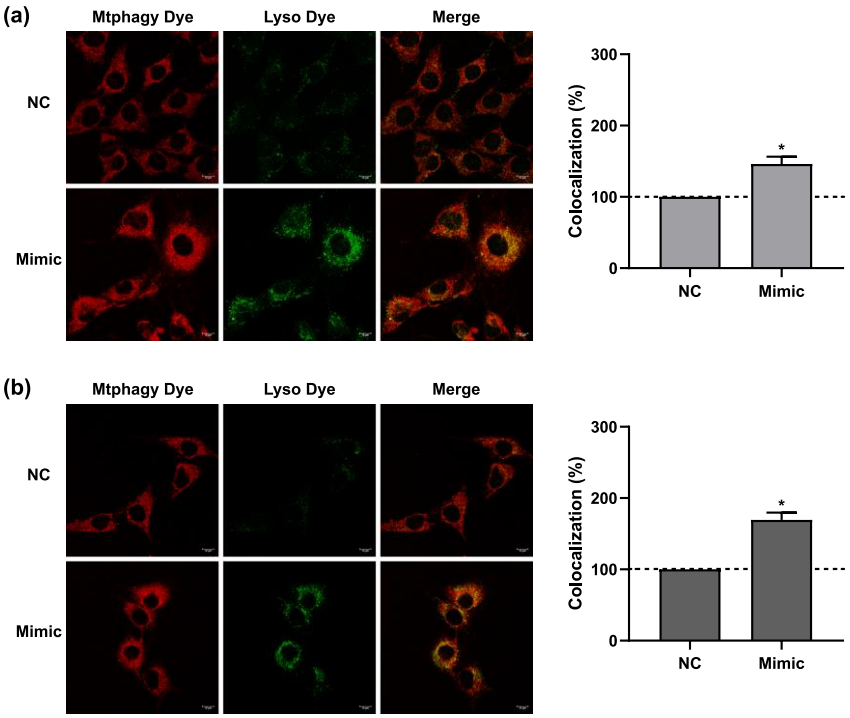

Figure 2. Mitophagy detection in C2C12 cells. (a) C2C12 myoblasts and (b) *Sesn2* KD cells were exposed to 3 days of hyperglycaemia (upper panel) and transfected with miR-378a-3p mimics (lower panel). The bar graphs represent the colocalization between Mtphagy Dye and Lyso Dye. All data is given as mean ± S.E.M. (n=2). **P* < 0.05 *versus* NC.