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PGC-1α driven mitochondrial biogenesis in stromal cells underpins mitochondrial trafficking to leukemic blasts

Christopher R Marlein[1], Lyubov Zaitseva[1], Rachel E Piddock[1], Livia Raso-Barnett[2], Michael A Scott[2], Christopher J Ingham[3], Angela Collins[4], Kristian M Bowles[1,4*], Stuart A Rushworth[1*]

[1]Department of Molecular Haematology, Norwich Medical School, The University of East Anglia, Norwich Research Park, NR4 7TJ, United Kingdom
[2]Haematopathology and Oncology Diagnostic Service, Addenbrooke's Hospital, Hills Rd, Cambridge, CB2 0QQ, United Kingdom
[3]Department of Trauma and Orthopaedic Surgery, Norfolk and Norwich University Hospitals NHS Trust, Colney Lane, Norwich, NR4 7UY, United Kingdom
[4]Department of Haematology, Norfolk and Norwich University Hospitals NHS Trust, Colney Lane, Norwich, NR4 7UY, United Kingdom

* denotes joint corresponding authors

email: s.rushworth@uea.ac.uk / k.bowles@uea.ac.uk

Department of Molecular Haematology,
Norwich Medical School,
University of East Anglia,
Norwich Research Park,
Norwich, NR4 7TJ,
United Kingdom: Tel: 01603 591802

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Acute myeloid leukemia (AML) is a disease known to be heavily reliant on its bone microenvironment (BMM) to survive and proliferate [1, 2]. We have previously shown that AML disease progression is enabled by the transfer of functional mitochondria to the malignant cell from bone marrow stromal cells (BMSC) [3, 4]. This process was shown to be stimulated by superoxide generated by NADPH oxidase-2 (NOX2) on the AML blast [3]. However, beyond the stimulation of reactive oxygen species in BMSC, the mechanisms controlling mitochondrial transfer in BMSC have yet to be elucidated.

There are no apparent adverse effects on BMSC after donation of mitochondria to AML blasts, implying the presence of a mechanism whereby the BMSC can recover their metabolic potential. The master regulator of mitochondrial biogenesis [5], peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), has been implicated in cancer progression and metabolism [6, 7]. In these studies, PGC-1α is up-regulated and causes an increased accumulation of functional mitochondria. Here, we investigate the effect AML has on the mitochondrial mass, bio-energetic potential and PGC-1α expression in BMSC.

First, using MitoTracker Green staining and flow cytometry, we determined the mitochondrial levels in primary BMSC (n=8) after co-culture with AML blasts. In Figure 1A, we found significantly elevated mitochondrial levels in BMSC after co-culture, implying that AML blasts stimulate BMSC to produce more mitochondria. We next wanted to see if this increase in mitochondrial mass caused an increase in mitochondrial based metabolism. To do this we analysed BMSC oxygen consumption rate using the Seahorse extracellular flux assay. Increased mitochondrial respiration was observed in BMSC (n=4) after co-culture with AML blasts (Figure 1B and C). Moreover, in Figure 1D, we show that BMSC from patients with AML had increased mitochondrial respiration compared to BMSC from healthy individuals (n=3). Together, these results show that BMSC from patients with AML have increased mitochondrial mass and functional bio-energetic consequence in BMSC metabolism.

As the transcription factor PGC-1α is known to cause increased mitochondrial biogenesis [5], we examined PGC1α expression in BMSC after co-culture with AML blasts. RNA expression of PGC-1α in BMSC (n=5) was increased in BMSC after co-

culture with AML blasts compared to BMSC cultured alone (Figure 1F). Next, we showed that total PGC-1α protein was elevated in BMSC after AML co-culture compared to control (Figure 1F). Moreover, we show that BMSC have increased nuclear levels of PGC-1α after co-culture with AML compared to BMSC cultured alone (Figure 1F), an effect which was reversed upon the addition of N-acetylcysteine to the co-culture (Supplementary figure 1). Previous studies have shown that AMPK can be stimulated by ROS [8] and in turn can stimulate PGC-1α [9]. Therefore as NOX-2 derived ROS stimulates mitochondrial transfer to AML [3], we assessed whether AMPK is activated in BMSC after culture with AML blasts. We found increased phosphorylation of Thr182 in AMPK from BMSC after co-culture with AML blasts (Supplementary figure 2). Together, results from RNA and Western blotting highlight that AML blasts cause an increase in PGC-1α expression and localization in BMSC suggesting that PGC-1α becomes activated via AMPK, in response to AML co-culture.

We next wanted to determine if elevated PGC-1α expression and nuclear localization and subsequent mitochondrial biogenesis was required for the mitochondrial transfer from BMSC to AML blasts. Figure 2A shows that mitochondrial transfer occurs from BMSC to the primary AML blasts. Next, we knocked down (KD) PGC-1α in BMSC with shRNA (Figure 2B). A MitoTracker Green based staining assay was then used to analyse the levels of mitochondrial transfer to AML blasts cultured on control KD and PGC-1α KD BMSC. Figure 2C shows that mitochondrial transfer from BMSC to AML is impaired when cultured on PGC-1α KD BMSC compared with control KD BMSC (Figure 2C). This data shows that PGC-1α activation is prerequisite for pro-tumoral mitochondrial transfer from BMSC to blasts in AML.

To investigate the effect of PGC-1α on ROS and oxidative stress in BMSC, ROS levels in PGC-1α KD and control KD BMSC were analysed with and without AML co-culture. Basal ROS levels were elevated in BMSC when PGC-1α was knocked down (Supplementary figure 3A), however upon the addition of AML reduced ROS levels compared with control KD BMSC were observed (Supplementary figure 3B). Therefore, AML blasts are unable to stimulate ROS in PGC-1α KD BMSC to the same extent as control KD BMSC, which would account for the reduced mitochondrial transfer observed.

Finally, we wanted to examine the effect PGC-1α KD in BMSC has on the disease progression of AML. To do this we used an NSG mouse model whereby we transplanted BMSC and AML blasts subcutaneously. Using this model, OCI-AML3 cells tagged with a luciferase construct [10] and then subcutaneously injected with BMSC (into the right flank) and without BMSC (into the left flank), only proliferate in the presence of BMSC (Supplementary Figure 1). We modified this model for use in the PGC-1α KD study, where we injected OCI-AML3 or MV4-11 luciferase cells with control KD BMSC (left flank) or PGC-1α KD BMSC (right flank). Figure 2D and 2E show that AML combined with PGC-1α KD BMSC has reduced tumor volume compared with animals with control KD BMSC. Figure 2F shows the bioluminescence from live animal imaging matches with excised tumors, where the tumors are reduced in the PGC-1α KD flank. Histologic analysis showed no difference between the AML tumors grown with PGC-1α KD BMSC compared to those that developed with control KD BMSC; with respect to the type or frequency of inflammatory cells or other non-malignant cells (Supplementary figure 5). Overall it was observed that PGC-1α KD in BMSC has a negative effect on AML disease progression *in vivo*.

In conclusion, this study provides a novel insight into the mechanisms controlling pro-tumoral mitochondrial transfer in AML. We have shown that AML increases oxidative stress in the BMSC [3], and this causes an increase in PGC-1α expression and mitochondrial biogenesis in BMSC. This process is prerequisite for the pro-tumoral mitochondrial transfer from BMSC to leukemic blasts observed in AML. Inhibition of PGC-1α in BMSC reduces the trafficking of mitochondria and thus limits the proliferative capacity of the tumor. As pro-tumoral mitochondrial transfer is increasingly recognised as part of the malignant phenotype in multiple cancers [11-13], this study provides a novel mechanistic insight as to how PGC-1α may be targeted in the microenvironment as a means to limiting mitochondrial transfer to cancer. Treatments inhibiting mitochondrial metabolism and function in AML blasts, including IDH1/2 mutant inhibitors [14] and the Bcl-2 inhibitor venetoclax, have recently been shown to be clinically effective [15]. This study also provides an important step in understanding the complex nature of tumor metabolism, not only in the malignant cell, but also within the microenvironment which supports it.

Conflict of interest

All authors declare no conflict of interest.

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Authorship contributions

CRM, KMB and SAR designed the research; CRM performed the research; CRM and REP carried out *in vivo* work; LZ, LRB, MAS, CJI, AC and KMB provided essential knowledge and reagents; CRM, KMB and SAR wrote the paper

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Figure legends

Figure 1. AML stimulates mitochondrial biogenesis in BMSC through PGC-1α

(A) BMSC were cultured with AML blasts for 24 hours, then BMSC (n=8) were stained with 200 nM MitoTracker Green FM. Mitochondrial levels were analysed using MitoTracker Green mean fluorescence intensity by flow cytometry. (B) A representative plot of oxygen consumption rate from the Seahorse MitoStress assay. Oligomycin (O), FCCP (F) and rotenone (R) were injected periodically and oxygen consumption rate was measured. (C and D) Basal and maximum mitochondrial respiration in BMSC cultured with and without AML blasts (C) and from AML and healthy patients (D) (n=3). (E) RNA qPCR analysis of BMSC (n=5) with and without co-culture with AML blasts. (F) Western blot analysis of nuclear, cytosolic and total PGC-1α protein from BMSC (n=2) cultured with and without AML blasts (n=2).

Figure 2. PGC-1α is crucial for mitochondrial transfer and AML disease progression.

(A) MitoTracker Green based transfer assay showing that AML blasts, used in this study, have acquired mitochondria from BMSC. (B) PGC-1α RNA expression is significantly reduced in BMSC after specific lentiviral targeting. (C) Mitochondrial transfer levels to AML blasts are reduced when cultured on PGC-1α KD BMSC. (D) Schematic representation of the NSG mouse model used. (E) Bioluminescent live animal images showing OCI-AML3/MV4-11 AML disease progression, when injected subcutaneously with control KD or PGC-1α BMSC. (F) Quantification of bioluminescent images seen in E (OCI-AML3; n=5. MV4-11; n=4). (G) Excised tumors were measured using calipers.