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SDF-1/CXCL12 modulates mitochondrial respiration of immature blood cells in a bi-phasic manner

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ABSTRACT

SDF-1/CXCL12 is a potent chemokine required for the homing and engraftment of hematopoietic stem and progenitor cells. Previous data from our group has shown that in an SDF-1/CXCL12 transgenic mouse model, lineage$^-$ Sca-1$^+$ c-Kit$^+$ (LSK) bone marrow cells have reduced mitochondrial membrane potential versus wild-type. These results suggested that SDF-1/CXCL12 may function to keep mitochondrial respiration low in immature blood cells in the bone marrow. Low mitochondrial metabolism helps to maintain low levels of reactive oxygen species (ROS), which can influence differentiation. To test whether SDF-1/CXCL12 regulates mitochondrial metabolism, we employed the human leukemia cell line HL-60, that expresses high levels of the SDF-1/CXCL12 receptor, CXCR4, as a model of hematopoietic progenitor cells in vitro. We treated HL-60 cells with SDF-1/CXCL12 for 2 and 24 h. Oxygen consumption rates (OCR), mitochondrial-associated ATP production, mitochondrial mass, and mitochondrial membrane potential of HL-60 cells were significantly reduced at 2 h and increased at 24 h as compared to untreated control cells. These biphasic effects of SDF-1/CXCL12 were reproduced with lineage negative primary mouse bone marrow cells, suggesting a novel function of SDF-1/CXCL12 in modulating mitochondrial respiration by regulating mitochondrial oxidative phosphorylation, ATP production and mitochondrial content.

1. Introduction

Stromal cell-derived factor 1α (SDF-1), also known as CXCL12, is an important member of the CXC family of chemokines. SDF-1/CXCL12 is expressed in a wide array of different tissues and cell types, including immune cells, endothelial cells, stromal cells, fibroblasts, and cancer cells [1]. The gene encoding SDF-1/CXCL12 is located at 10q11.1 and has 6 exons encoding 68 amino acids. It has a molecular weight of 8 kDa, and its promoter contains binding sites for transcription factors such as SP1 [2]. Signal transduction induced by SDF-1/CXCl12 is mediated through the chemokine receptor CXCR4 [3–6]. Knockout of SDF-1/CXCL12 is perinatal lethal and mice lacking SDF-1/CXCL12 have severe defects in gastrointestinal vascularization, cerebral development, and hematopoietic defects [7–9]. CXCR4 knockout studies reveal a strikingly similar phenotype to that of SDF-1/CXCL12 knockout mice, suggesting that the SDF-1/CXCL12 and CXCR4 signaling axis is non-promiscuous [10].

SDF-1/CXCL12 is a potent chemotactic factor for hematopoietic stem (HSCs) and progenitor (HPCs) cells [11,12]. It plays an essential role in the maintenance of HSCs, including homing, engraftment and repopulating activity, as well as HSC quiescence and retention in the bone marrow [13–17]. It has been shown to enhance the survival of HSCs and HPCs, an effect increased in synergy with other cytokines [5,18–20]. Treatment of mouse bone marrow cells and human cord blood HPCs with soluble SDF-1/CXCL12 enhanced their replating efficiency, and bone marrow cells from mice expressing a human SDF-1/CXCL12 transgene exhibited increased replating capacity of single macrophage-and multipotent progenitor-derived colonies [21].

SDF-1/CXCL12 appears to be a key regulator of HSCs in the bone marrow microenvironment [22]. The niche provides signals regulating HSC functions, such as self-renewal and long term repopulating capability, as well as the ability to undergo multiline age differentiation. Several groups have shown in genetic studies that mesenchymal progenitor, endothelial, and stromal cell populations play a critical role in the maintenance of HSCs in the niche and depending on which niche cells HSCs interact with, help to define the specific "sub-niche" in which HSCs may reside [23–30]. Deletion of SDF-1/CXCL12 from different types of niche cells leads to the reduction in HSC numbers, competitive repopulation, and increases in splenic HSCs, all of which indicate an essential role for SDF-1/CXCL12 in HSC function in the bone marrow microenvironment [23,25,27,29,30].

Despite work from several groups describing the role of SDF-1/CXCL12 in the maintenance of HSCs and HPCs in the various niches in the bone marrow [23,25,27,29,30], there is a paucity of information on

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the mechanism by which SDF-1/CXCL12 functions at the molecular level for immature blood cell function in the bone marrow. Regulation and restriction of mitochondrial metabolism has been shown to be critical in maintaining the quiescent state of HSCs in the bone marrow by preventing mitochondrial produced reactive oxygen species (ROS), which can promote differentiation and HSC attrition and potential dysfunction [31–36]. Recent work from our group has shown that SDF-1/CXCL12 can modulate mitochondrial activity and mitochondrial mass in murine bone marrow cells expressing a human SDF-1/CXCL12 transgene [37]. We therefore hypothesized that SDF-1/CXCL12 regulates mitochondrial respiration in early hematopoietic cells.

2. Materials and methods

2.1. Oxygen consumption rates

Basal oxygen consumption rates (OCR) and mitochondrial-associated ATP production were obtained using the Seahorse Bioscience XF96 Extracellular Flux Analyzer from Seahorse Bioscience, and measurements were performed according to the manufacturer's instructions and as described previously [37–39]. Mitochondrial-associated ATP production is the difference between the basal OCR and oligomycin-A repressed OCR [40].

2.2. Cell culture and lineage negative bone marrow cell isolation

HL-60 cells (ATCC CCL-240) were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Iscove's Modified Dulbecco's Medium (IMDM) with 20% FBS. HL-60 cells were incubated in IMDM + 20% FBS with and without 50 ng/ml SDF-1 R&D, Minneapolis, Minnesota for two and 24 h, respectively. This concentration of SDF-1 has been shown to elicit optimal responses in numerous of our assays [12,18,19,21,41]. C57Bl/6 strain mice were used to isolate lineage negative bone marrow cells. The Indiana University Committee on Use and Care of Animals approved the mouse studies. Mouse lineage negative cells were isolated using the Miltenyi Biotech, San Diego, California mouse Lineage Cell Depletion Kit. After lineage depletion, lineage negative cells were incubated in IMDM + 10% FBS and stimulated with or without 50 ng/ml SDF-1 (R&D, Minneapolis, MN) for two and 24 h, respectively.

2.3. Reagents and instruments

Anti-human CXCR4 APC conjugated antibody (Clone 12G5) and anti-human CXCR7 FITC conjugated antibody (Clone 358426) were from R&D, Minneapolis, Minnesota. Mitotracker Green FM and Mitotracker Red CMXRos were from Molecular Probes, Eugene, Oregon. Flow cytometry was performed with a FACS Calibur flow cytometer (BD Biosciences, Franklin Lakes, NJ). Flow cytometry data were analyzed using FlowJo, Ashland, Oregon. Oligomycin-A was purchased from Sigma-Aldrich, St. Louis, Missouri and AMD3100 was a kind gift from AnorMed, Langley, British Columbia, Canada.

2.4. Statistical analysis

Data were statistically analyzed and plotted using GraphPad Prism 6 (San Diego, CA). Differences were assessed with a 2-tailed Student t-test or one-way ANOVA with Tukey's post-hoc correction. P values ≤0.05 were considered significant.

3. Results and discussion

To investigate the potential role that SDF-1/CXCL12 plays in controlling mitochondrial respiration, we employed the human promyelocitic

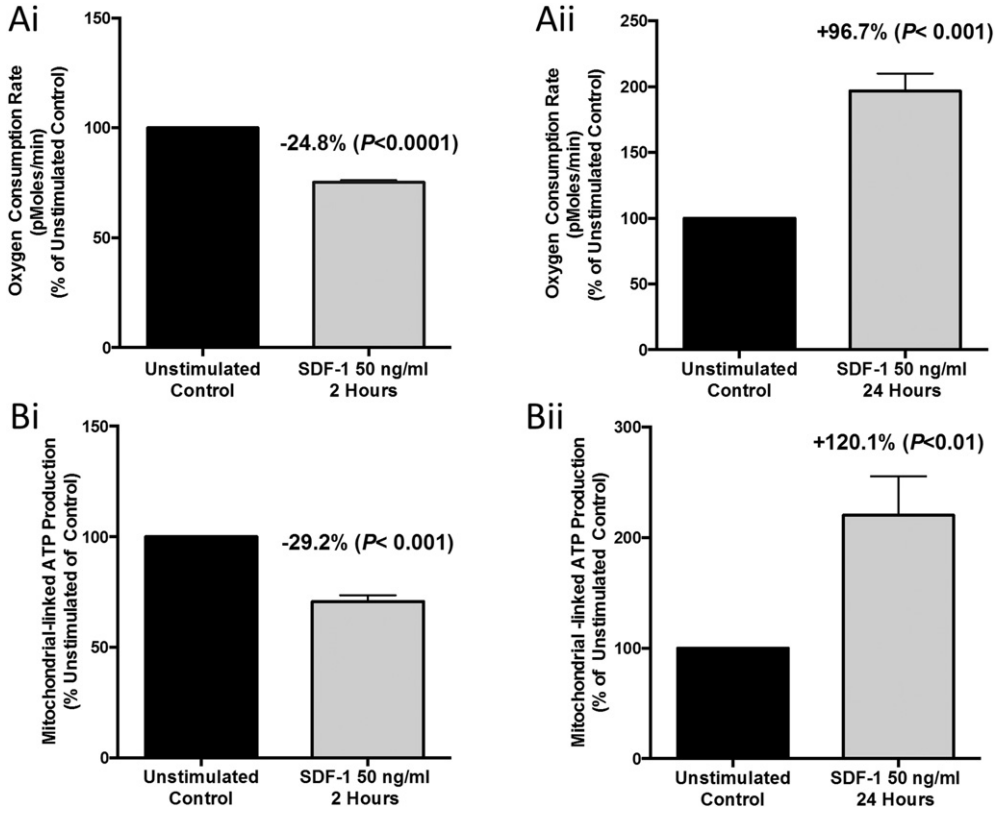

Fig. 1. SDF-1/CXCL12 regulates mitochondrial respiration of HL-60 cells in a biphasic manner. The human leukemia cell line, HL-60, was treated with 50 ng/ml of SDF-1 for 2 and 24 h respectively. After each time point, cells were collected and their oxygen consumption rates (OCR) was measured (A) on the Seahorse Bioscience Extracellular Flux Analyzer. (B) Mitochondrial-linked ATP production of SDF-1 treated HL-60 cells was also measured using the Seahorse Bioscience Extracellular Flux Analyzer. Results are the mean ± SEM of four independent experiments with significant differences as compared to unstimulated control shown in the figure.