



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

509. BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Antioxidants As a Novel Treatment to Revert Impaired Angiogenic Potential Driven By Metabolic Alterations Observed in Shwachman-Diamond Syndrome Derived Mesenchymal Stromal Cells

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Introduction Shwachman-Diamond Syndrome (SDS) is a rare bone marrow (BM) failure disorder. SDS BM biopsies show tortuous and collapsed vessels, highlighting angiogenic abnormalities. Our group previously demonstrated that SDS BM mesenchymal stromal cells (MSCs) have *in vitro* and *in vivo* impaired angiogenic potential compared to healthy donors (HD)-MSCs.

Interestingly, recent evidence underlines that pathological angiogenesis could be accompanied by altered metabolism and that antioxidants could represent a novel strategy to target angiogenic defects.

Aims We aimed to characterize the metabolic status of SDS-MSCs and to investigate if the treatment with antioxidants may restore their impaired angiogenic potential.

Methods BM derived HD- and SDS-MSCs were stimulated with DMEM ± N-Acetylcysteine (NAC, 1mM) or dimethylsulfoxide (DMSO, 0.05% v/v) for 48h.

Oxidative phosphorylation (OxPhos) was assayed by oximetry and bioluminescent ATP synthesis assay. Spectrophotometric analyses were performed to evaluate ATP/AMP ratio, malondialdehyde (MDA) level, and Complex IV and lactate dehydrogenase (LDH) activity. The amount of reactive oxygen species (ROS) was quantified by flow cytometry.

The angiogenic capability was evaluated by performing *in vitro* Matrigel angiogenesis assay.

Results Firstly, we evaluated OxPhos oxygen consumption and ATP production (n=6). Concerning the I-III-IV mitochondrial complexes pathway, SDS-MSCs consumed 57% less oxygen (p=0.004) and produced 64% less ATP compared to HD-MSCs (p=0.002). Accordingly, the analysis of the II-III-IV complexes pathway demonstrated that the oxygen consumption was reduced by 62% (p=0.002) and the ATP synthesis was 67% lower than HD-MSCs (p=0.002). Furthermore, the P/O ratio, index of OxPhos efficiency, was significantly reduced in SDS-MSCs in both the electron transport chain pathways (p=0.002 for both). Therefore, we demonstrated that Complex IV activity was 61% lower in SDS- vs HD-MSCs (p=0.002), highlighting its role in the SDS OxPhos defect.

As for the energetic status, SDS-MSCs showed a low intracellular ATP/AMP ratio (mean=1.1, range=0.8-1.6 vs mean=3.6, range=3.1-4.0, in HDs; p=0.002) that was accompanied by 30% increase of LDH activity in SDS-MSCs (p=0.002), an expression of increased anaerobic glycolysis to compensate the mitochondrial defect.

The amount of ROS in SDS-MSCs was increased by 27% compared to HD-MSCs (n=5) and also the SDS-MSCs lipid peroxidation level was significantly higher over HDs (mean=12.7 μM of MDA/mg, range=10.5-14.1 μM/mg vs mean=6.6 μM/mg, range=5.9-7.4 μM/mg; p=0.002).

Then we treated SDS-MSCs for 48h with NAC, a broad-range antioxidant (n=5), or with DMSO, which acts at very low concentrations as scavenger, specifically on lipid peroxidation products (n=6).

Interestingly, NAC and DMSO stimulated SDS-MSCs increased by 75% the oxygen consumption and by 70% the ATP synthesis in both the electron transport chain pathways, thus resulting in levels comparable to HD-MSCs. The SDS OxPhos restoration was respectively associated to a 53% and 60% increase of Complex IV activity after NAC and DMSO stimulation ($p < 0.0001$ for both). Moreover, antioxidants corrected the SDS energetic defect by restoring ATP/AMP ratio and LDH activity to the levels of HDs. Importantly, SDS-MSCs lipid peroxidation level was drastically reduced after NAC and DMSO treatments (mean=12.7 μ M/mg, range=10.5-14.1 μ M/mg vs NAC mean=5.1 μ M/mg, range=4.3-5.4 μ M/mg and DMSO mean=6.2 μ M/mg, range=5.8-6.5 μ M/mg; $p < 0.0001$ for both).

Finally, we approached SDS-MSCs angiogenic defect and showed that SDS-MSCs defective capability to recreate a defined capillary-like network under angiogenic stimuli was completely restored after antioxidant stimulations ($n \geq 5$). As shown by *ImageJ Angiogenesis Analyzer* several angiogenic elements, including branches and segments, were significantly increased in NAC and DMSO stimulated SDS-MSCs that become comparable to HD-MSCs.

Conclusions We demonstrated that the altered OxPhos metabolism of SDS-MSCs significantly contributes to their angiogenic defect and, importantly, that antioxidants restored the metabolic alterations and the angiogenic potential in SDS-MSCs, paving the way for new therapeutic strategies.

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