The Role of Mitochondria in Mesenchymal Stem Cell Osteogenesis During Aging

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Introduction: Mesenchymal stem cells (MSCs) are multipotent cells that reside in the marrow of long bones and give rise to various lineages, including osteoblasts, adipocytes and chondrocytes. In undifferentiated MSCs, glycolysis is the predominant mechanism of energy production while mitochondrial oxidative phosphorylation is inactive. During the process of osteogenic differentiation mitochondria are activated via yet unknown mechanism and oxidative phosphorylation becomes the major source of ATP. This bioenergetic switch is especially important for osteogenic differentiation of MSCs. Our goal is to determine the mechanism of disruption of mitochondrial function during osteogenic differentiation of MSCs during aging and find ways to manipulate this mechanism for the purposes of therapy and prevention. Preliminary data from our lab suggest that the mitochondrial permeability transition (MPT) is involved in the regulation of MSC bioenergetics and osteogenicity and in development of osteoporosis during aging. The MPT is a non-selective mitochondrial pore and a major sensor of oxidative stress [1,2]. Oxidative stress is currently coming into focus as a major pathogenic factor in aging-associated bone loss. The MPT has long been associated with cell demise during aging in various tissues [3-5]; however its role in aging bone has not been studied until now.

Methods: MSC culture; bone marrow (BM) MSC from young (3 mo.) and aged (13 mo.) C57BL (WT) and Cyclophilin D knockout (CypD KO) mice was used. Cells were cultured in MSC growth media. Osteogenic differentiation of MSCs; Cells were incubated in osteogenic media for 14 days. Cell bioenergetics; Mitochondrial function was assessed by measuring oxygen consumption rate (OCR). Mitochondrial function assay; MSC mitochondrial inner membrane potential (ΔΨm) was assessed by staining with tetramethylrhodamine ethyl ester (TMRE). Mitochondrial mass was assessed by staining with nonyl acridine (NAO). TMRE and NAO signals were detected by flow cytometry and data were analyzed with FlowJo. Bone Micro-CT and Histological Assessment; Bone quality parameters of tibia, femur and lumbar vertebra (L3) of young and aged wt and CypD KO mice were analyzed using Micro-CT. After micro-CT, the bones were decalcified, sectioned and stained. Stained sections were used for histomorphometric analysis.

Results: Our data indicate that the disruption of this bioenergetics switch leads to decreased osteogenicity of MSCs during aging (Fig.1) and may be one of the causes of osteoporosis (Fig. 3). Our data indicate that MSCs from aged mice with loss-of-function of the MPT due to Cyclophilin D knockout (CypD KO) have better mitochondrial function and osteogenicity than MSCs from aged wild type (wt) mice (Fig. 2). Importantly, aged CypD KO mice have significantly less osteoporosis when compared to the aged wt mice (Fig.3).

Discussion: Osteogenic differentiation and respiration are decreased in aged MSCs and CypD KO restores osteogenicity and respiration in aged MSCs. In aged CypD KO mice bone quality is significantly improved compared to aged WT mice. We, therefore, conclude the MPT is involved in disruption of mitochondrial function and osteogenic potential of MSCs during aging.

Significance: These findings have the potential to lead to development of new pharmacologic strategies for osteoporosis and fracture that will increase osteogenicity in MSCs by improving their bioenergetic function.

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Fig. 1: Activation of mitochondrial function during osteogenic differentiation is disrupted in aged MSCs. BM MSCs were isolated from young and aged wt mice and incubated in osteogenic media for 14 days. Basal respiration (A) and ATP linked respiration (B) were assessed. Data are Means ± SE (n=4). * indicates P<0.05 undifferentiated vs day 14 differentiated. # indicates P<0.05 young vs aged.

Fig. 2: Evidence that the MPT is involved in dysruption of mitochondrial function and osteogenic differentiation in aged MSCs. BM MSCs were isolated from young and aged wt mice aged CypD KO mice and incubated in osteogenic media for 14 days. ALP and Runx2 gene expression (A) were assessed using RT-PCR and cell respiration (B) was assessed using oxygen Clarke electrode. Data are Means ± SE (n=2). * indicates P<0.05 vs Day 0.

Fig. 3: In aged CypD KO mice bone quality is significantly improved, compared to WT mice. Tibia from young and aged wt mice and CypD KO mice were analyzed with micro-CT (A and B). Data are Means ± SE (n=3). * indicates P<0.05 in CypD KO aged vs wt aged.