Dysregulation of DDAH1/ADMA impairs mitophagy in endothelial cells and determining differentiation cell fate of BMSCs via delivering miRNA-19b-2-5p by exosomes

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INTRODUCTION: DDAH1 (Dimethylarginine Dimethylaminohydrolase 1) regulates vascular endothelial function and bone homeostasis through the degradation of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA). Decreased DDAH1 expression in endothelial cells (ECs) during aging is associated with bone loss, but the exact mechanism has not been clarified. The aim of this study was to elucidate how DDAH1 deficiency leads to H-type vessels reduction and osteoporosis development through H-type endothelial cells dysfunction, mitochondrial autophagy abnormalities, and exosome-mediated intercellular communication.

METHODS: The number of H-vessels ($CD31^+$ $EMCN^+$) and bone microstructural changes were analyzed by immunofluorescence staining and micro-CT (Micro-CT) using endothelial cell-specific DDAH1 knockout mice (DDAH1-CDH5cre). The effects of DDAH1 knockdown on tube-forming capacity and wound healing were assessed in human umbilical vein endothelial cells (HUVECs). The effects of DDAH1 deletion on mitochondrial function were explored by combining transcriptome analysis, transmission electron microscopy observation and mitochondrial autophagy-related proteins (PINK1, Parkin, LC3B) detection. The role of exosome-borne microRNA-19b-2-5p in osteogenic-lipogenic differentiation was resolved by exosome high-throughput sequencing and bone marrow mesenchymal stem cells (BMSCs) differentiation experiments.

RESULTS: In our study, the data was shown that ADMA levels were significantly elevated in endothelial cells of Ddah1fl/fl;Cdh5-cre mice, the number of H-vessels was reduced by almost 60%, bone mineral density was decreased by almost 50%, and the volume (BV/TV) and number (Tb.N) of bone trabeculae were also decreased. In vitro, Ddah1 deficiency led to a decrease in the tube-forming ability of HUVECs by 15%, and a slowing down of wound healing by 20%. Analyzed by transcriptomics assay, we found that mitochondrial autophagy-related genes were down-regulated in *Ddah1* deficiency of ECs, particularly Bnip3-mediated pathway. Meanwhile, the proportion of mitochondrial vacuolization was increased, whereas the mitochondria-targeting drug MA-5 reversed the above impairment. Knocking out of Ddah1 increased the level of miRNA-19b-2-5p in exosomes secreted by ECs, which is a miRNA that, by inhibiting the expression of mitochondrial autophagy-related genes in BMSCs, leads to the inhibition of osteogenesis but promotion of adipogenesis. Finally, the engineered exosomes intervention significantly promoted bone repair in the bone fracture model and bone defect model.

DISCUSSION: This study reveals that DDAH1 coordinates the homeostasis of endothelial cells and bone metabolism through the regulation of mitochondrial autophagy and exosomal miRNA delivery, and *Ddah1* deficiency leads to the reduction of H-type vasculature and the inhibition of osteogenesis in BMSCs, which suggests that targeting the DDAH1-mitochondrial autophagy axis or exosomal miRNAs may provide a new strategy for intervention in bone loss and bone repair. The study provides an important theoretical basis for understanding the vascular-bone metabolism coupling mechanism.
SIGNIFICANCE/CLINICAL RELEVANCE:

1. exosome-mediated intercellular metabolic crosstalk as a novel mechanism controlling bone regeneration and repair.
2. It provides a valuable framework for developing engineered endothelial exosomes as therapeutic approach for skeletal disorders.