ELSEVIER

Blood 142 (2023) 1205

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

N-Acetylneuraminic Acid Induces Vascular Endothelial Dysfunction through Targeting Mcu/SQSTM1 Pathway and Attenuates Atherosclerosis

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Atherosclerosis (AS) is a major cause of various fatal diseases, such as stroke, and ischemic heart failure worldwide. The risk factors of AS include hypertension, diabetes mellitus, dyslipidemia, smoking and obesity. Recently, several investigations have shown that metabolic disorder within plaque was characterized as one of the main causes for those risk factors. The increase of N-acetylneuraminic acid (Neu5Ac), a metabolite produced by hexosamine-sialic acid pathway branching from glucose metabolism, in blood was identified as a metabolic biomarker for AS progression. Our previous study revealed that Neu5Ac could promote endothelial inflammatory response and attenuates AS plaque progression in apoE $^{-/-}$ mice. Mitochondrial dysfunction was suggested to be involved for endothelial injury after Neu5Ac accumulation in circulation. However, the underlaying mechanisms have not been fully understood yet. Therefore, the aim of the study was to understand the mechanism of mitochondrial injury induced by Neu5Ac accumulation *in vivo* using apoE-/- mouse model and *in vitro* using the endothelial cell line, HUVECs.

Investigations have shown that impaired Ca $^{2+}$ homeostasis plays an interactive role in the progression of mitochondrial dysfunction. In our present study, we found that Neu5Ac induced Ca $^{2+}$ overload, increased ROS levels and promoted the expression of mitochondrial injury makers such as Parkin, TOMM20 and TIMM23. While pretreatment of HUVECs with BAPTA-AM *in vitro* could inhibit the increase of the intracellular Ca $^{2+}$ concentration and reversed the mitochondrial fragment induced by Neu5Ac, suggesting that Neu5Ac in circulation promoted mitochondrial Ca $^{2+}$ uptake and induced the mitochondrial homeostasis disorder.

Based on the reported results that mitochondrial Ca $^{2+}$ uniporter (MCU) was the key regulator of impaired Ca $^{2+}$ homeostasis, we then detected the expression of MCU both *in vivo* and *in vitro*. We found an increase of MCU both at protein and mRNA levels in HUVEC cell lines. Meanwhile, we also observed that MCU expression was upregulated in aortic arch of apoE $^{-/-}$ mice after Neu5Ac treatment. Silencing MCU *in vitro*, mitochondrial Ca $^{2+}$ concentrations were downregulated and the ability of endothelial adhesion to monocytes was reversed as well. These results suggested that Neu5Ac specifically targeted MCU and induced Ca $^{2+}$ uptake. Targeting MCU might be a potential strategy for preventing AS progression induced by Neu5Ac. Furthermore, mitochondrial Ca $^{2+}$ uptake was considered as an important determinant of cell fate and mitophagy/autophagy pathway. Our previous study has confirmed that Neu5Ac could activate SQSTM1/p62-mediated excessive autophagy and subsequently induced endothelial injury. To investigate whether MCU silencing could attenuate the autophagy mediated AS progression, we investigated the SQSTM1/p62 expression after silencing MCU in HUVECs. The present results confirmed that Neu5Ac increased mitochondrion-dependent autophagy, and the effects of which were largely relieved by MCU silencing.

In summary, impaired Ca $^{2+}$ homeostasis was involved in the endothelial injury induced by accumulated Neu5Ac in blood. Furthermore, MCU upregulation is responsible for Neu5Ac-induced pathological AS disorder. Our findings reveal a novel molecular mechanism regulating mitochondrial dysfunction and may open a new window for therapeutic targeting in the treatment of AS.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-189843