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Rapid communication

NEDDylation promotes endothelial dysfunction: A role for HDAC2

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ABSTRACT

Emerging evidence strongly supports a role for HDAC2 in the transcriptional regulation of endothelial genes and vascular function. We have recently demonstrated that HDAC2 reciprocally regulates the transcription of Arginase2, which is itself a critical modulator of endothelial function via eNOS. Moreover HDAC2 levels are decreased in response to the atherogenic stimulus OxLDL via a mechanism that is apparently dependent upon proteasomal degradation. NEDDylation is a post-translational protein modification that is tightly linked to ubiquitination and thereby protein degradation. We propose that changes in NEDDylation may modulate vascular endothelial function in part through alterations in the proteasomal degradation of HDAC2. In HAEC, OxLDL exposure augmented global protein NEDDylation. Pre-incubation of mouse aortic rings with the NEDDylation activating enzyme inhibitor, MLN4924, prevented OxLDL-induced endothelial dysfunction. In HAEC, MLN enhanced HDAC2 abundance, decreased expression and activity of Arginase2, and blocked OxLDL-mediated reduction of HDAC2. Additionally, HDAC2 was shown to be a substrate for NEDD8 conjugation and this interaction was potentiated by OxLDL. Further, HDAC2 levels were reciprocally regulated by ectopic expression of NEDD8 and the de-NEDDylating enzyme SENP8. Our findings indicate that the observed improvement in endothelial dysfunction with inhibition of NEDDylation activating enzyme is likely due to an HDAC2-dependent decrease in Arginase2. NEDDylation activating enzyme may therefore be a novel target in endothelial dysfunction and atherogenesis.

1. Introduction

The considerable burden of atherosclerotic and other vascular diseases on health, cost, and productivity in both western and developing countries continues to fuel investigation into pathogenic mechanisms that are amenable to modulation or ablation by new therapies [1]. The presence of proteasomal degradation across the spectrum of cellular, tissue, and organ system contexts has ignited a focus on modulation of proteasome dynamics for a wide variety of clinical applications ranging from cancer to autoimmune diseases [2]. Recent studies highlight the importance of NEDDylation for a subset of ubiquitin-dependent protein degradation [3], thereby offering a therapeutic target that narrows the spectrum of target proteins and limits impact on off-target protein turnover events.

We have previously defined a specific role for HDAC2 in the transcriptional suppression of Arginase2 — an uncoupling inhibitor of eNOS. We have also shown that OxLDL decreases the level of HDAC2 protein in vascular endothelium, and demonstrated that the expression of HDAC2 defends vascular function in the setting of oxidative injury [4]. We report here that the NEDDylation activating enzyme (NAE) inhibitor MLN4924 blocks OxLDL-mediated vascular dysfunction via upregulation of HDAC2 abundance. These data suggest that NEDDylation is a key regulatory input for HDAC2 and vascular homeostasis.

2. Materials and methods

Detailed methods are included in the on-line supplement.

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3. Results

3.1. Inhibition of NEDDylation provides protection against OxLDL-mediated vascular endothelial dysfunction

To better understand the link between NEDDylation and vascular function, we performed ex-vivo vascular reactivity experiments in isolated mice aortas incubated with MLN4924 (1 μM) prior to OxLDL (50 μg/mL) exposure. Impairment of endothelial-dependent vascular relaxation by OxLDL was assessed using acetylcholine dose response curves (Fig. 1A). Substantial rescue of endothelial function was demonstrated in aortas pretreated with MLN4924 prior to OxLDL stimulation, whereas MLN4924 alone had negligible effect (Fig. 1A). In contrast, endothelial-independent vascular relaxation as assessed using sodium nitroprusside was unaffected by either OxLDL or MLN4924 (Fig. 1B).

We next showed that exposure of HAEC to OxLDL (50 μg/mL) for 24 h increased global protein NEDDylation (Fig. 1C). Furthermore, pretreatment of OxLDL-exposed HAEC with either the NEDDylation inhibitor-MLN4924 or the proteasome inhibitor-MG132 rescued HDAC2 levels (Fig. 1D). These findings suggest that oxidative injury to the vascular endothelium triggers HDAC2 degradation via NEDDylation and ubiquitination.

Our laboratory has identified Arg2 as a major culprit in vascular endothelial dysfunction induced by OxLDL, and the dominant arginase isoform in aortic endothelium [5]. OxLDL-induced increases in the abundance and activity of endothelial arginase were abolished with MLN4924 and MG132 (Figs. 1D and E). MLN4924 was then used to determine whether HDAC2 and arginase levels were modulated by NEDDylation in HAEC in the absence of OxLDL. A robust dose-dependent increase in HDAC2 protein expression was observed (Fig. 1F), as was decreased arginase activity (Fig. 1G). We also observed that MLN4924 did not rescue vascular endothelial dysfunction that resulted from the inhibition of class I HDACs (Supplemental Fig. 1). The NAE inhibitor MLN4924 attenuated the quantity of NEDDylation conjugates in a dose-dependent fashion in HAEC that expressed ectopic HA-tagged-NEDD8 (Fig. 1H).

3.2. NEDDylation of HDAC2 hastens its clearance from vascular endothelium

The effect of OxLDL on HDAC2 protein stability was assessed with a cycloheximide chase experiment that demonstrated a larger drop

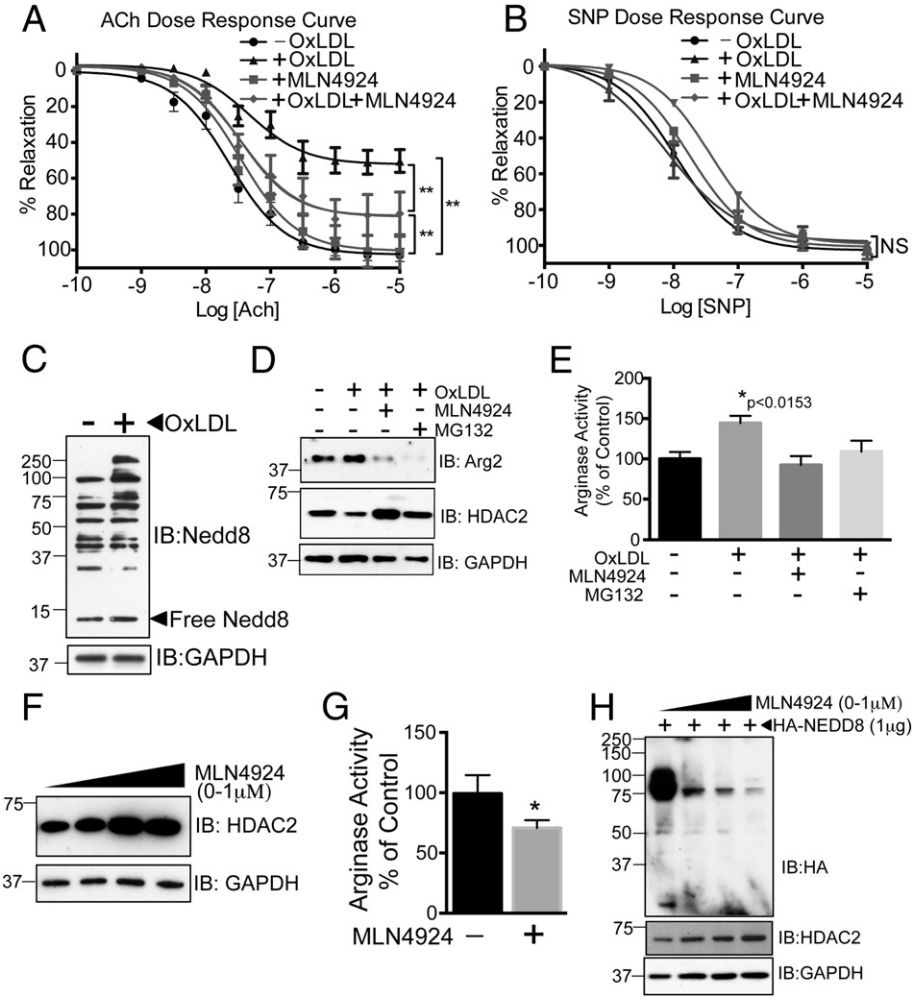

Fig. 1. Inhibition of NEDDylation by MLN4924 improves OxLDL-mediated vascular endothelial dysfunction via downregulation of Arginase2. Wire myography was used to measure vasorelaxation with acetylcholine (A, Ach) and sodium nitroprusside (B, SNP) in isolated mice aortas. Conditions included 24 h of OxLDL (50 μg/mL) or control medium preceded by 1 h with either vehicle (DMSO) or MLN4924 (1 μM). C) HAEC were exposed to OxLDL (50 μg/mL) for 24 h followed by 8 h of MG132 (10 μM) and lysates were immunoblotted for NEDD8 and GAPDH. D and E) HAEC were preincubated with DMSO, MG132 (10 μM), or MLN4924 (1 μM) for 8 h followed by stimulation with OxLDL (50 μg/mL) for 24 h and lysates were immunoblotted for Arg2, HDAC2 and GAPDH, or assayed for arginase activity. Note that native (and not transgene) HDAC2 protein levels are shown here. F) HAEC were incubated with DMSO or MLN4924 (0, 0.1, 0.3 or 1 μM) and lysates were immunoblotted for HDAC2 and GAPDH. G) HAEC were incubated with either vehicle (DMSO) or MLN4924 (1 μM) and arginase activity was measured in lysates. H) HAEC expressing HA-epitope-tagged NEDD8 were incubated with DMSO or MLN4924 (0, 0.1, 0.3 or 1 μM) and lysates were immunoblotted with HA, HDAC2 and GAPDH antibodies. Note that "0 μg HA-NEDD8" denotes an empty vector (pcDNA3.1) control. *Indicates $p < 0.05$ vs control; **indicates $p < 0.001$; $n = 6$ for vascular reactivity and arginase activity assays.