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# Nuclear envelope proteins Nesprin2 and LaminA regulate proliferation and apoptosis of vascular endothelial cells in response to shear stress

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ABSTRACT

The dysfunction of vascular endothelial cells (ECs) influenced by flow shear stress is crucial for vascular remodeling. However, the roles of nuclear envelope (NE) proteins in shear stress-induced EC dysfunction are still unknown. Our results indicated that, compared with normal shear stress (NSS), low shear stress (LowSS) suppressed the expression of two types of NE proteins, Nesprin2 and LaminA, and increased the proliferation and apoptosis of ECs. Targeted small interfering RNA (siRNA) and gene overexpression plasmid transfection revealed that Nesprin2 and LaminA participate in the regulation of EC proliferation and apoptosis. A protein/DNA array was further used to detect the activation of transcription factors in ECs following transfection with target siRNAs and overexpression plasmids. The regulation of AP-2 and TFIID mediated by Nesprin2 and the activation of Stat-1, Stat-3, Stat-5 and Stat-6 by LaminA were verified under shear stress. Furthermore, using Ingenuity Pathway Analysis software and real-time RT-PCR, the effects of Nesprin2 or LaminA on the downstream target genes of AP-2, TFIID, and Stat-1, Stat-3, Stat-5 and Stat-6, respectively, were investigated under LowSS. Our study has revealed that NE proteins are novel mechano-sensitive molecules in ECs. LowSS suppresses the expression of Nesprin2 and LaminA, which may subsequently modulate the activation of important transcription factors and eventually lead to EC dysfunction.



## 1. Introduction

Vascular endothelial cells (ECs) covering the inner surfaces of blood vessels are constantly exposed to fluid shear stress created by blood flow. Homeostasis of ECs is required for maintaining vascular physiological functions and protection against pathophysiological changes, such as atherosclerosis [1]. Atherosclerosis develops preferentially at branches and curvatures of arterial trees, where blood flow patterns are low or disturbed [2]. ECs respond to changes in local shear stress, which subsequently lead to the alterations in gene expression, the metabolism of biochemical substances or cellular functions, such as proliferation, apoptosis, differentiation and migration [2]. The exposure of ECs to shear stress activates multiple mechanosensors, including membrane glycocalyx [3], membrane proteins, such as integrins [4], G proteins and G protein-coupled receptors [5], receptor tyrosine kinases [6] and $Ca^{2+}$ channels [7]. It has also become clear that the cytoskeleton forming the internal scaffold can sense and absorb the mechanical loading of the cell [8]. However, it remains unclear, besides mechanosensors located on membrane structures and cytoskeleton, whether nuclear skeletal elements, such as nuclear envelope (NE) proteins, are mechano-sensitive and affect EC behaviors.

Our previous proteomic analysis revealed that LaminA, which is a major protein constituent of the mammalian nuclear lamina, may participate in modulating EC proliferation and migration during shear stress application, suggesting that NE proteins may be mechano-responsive molecules that contribute to vascular remodeling [9]. The nucleus is the largest and stiffest organelle in the cell, and it is the site of transcriptional regulation and contains the genome [10]. NE proteins maintain the skeletal structure of the nucleus, but their roles in mechano-sensing and gene expression regulation are still unclear.

The NE is composed of the outer nuclear membrane (ONM), which is continuous with the endoplasmic reticulum, the inner nuclear membrane (INM), and the nuclear lamina underlying the INM [11]. Nesprin proteins at the ONM contain an N-terminal actin-binding domain in the cytoplasm and a C-terminal transmembrane domain, which interacts with the INM, in the perinuclear space [12]. The nuclear lamina, which is composed of LaminA and C (A-type lamins) and LaminB1

*Abbreviations:* AP-2, activator protein-2; ECs, endothelial cells; HRP, horseradish peroxidase; IFNG, interferon-gamma; INM, inner nuclear membrane; IPA, ingenuity pathway analysis; LINC, linker of nucleoskeleton and cytoskeleton; LowSS, low shear stress; NC, negative control; NE, nuclear envelope; NSS, normal shear stress; ONM, outer nuclear membrane; PET, polyethylene terephthalate; siRNA, small interfering RNAs; TAFs, TBP-associated factors; TBP, TATA-box binding protein; TEM, transmission electron microscope; TF, transcription factors

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