Atherosclerosis 275 (2018) 58–67

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Loss of CLOCK under high glucose upregulates ROCK1-mediated endothelial to mesenchymal transition and aggravates plaque vulnerability

Hanfei Tang [a, 1], Mengjiao Zhu [b, 1], Gefei Zhao [a], Weiguo Fu [a], Zhenyu Shi [a], Yong Ding [a], Xiao Tang [a, **], Daqiao Guo [a, *]

[a] Department of Vascular Surgery, Institute of Vascular Surgery, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Department of Orthodontics, School & Hospital of Stomatology, Tongji University, Shanghai Engineering Research Center of Tooth Restoration and Regeneration, Shanghai, 200072, China

ARTICLE INFO

Article history:
Received 15 December 2017
Received in revised form
19 May 2018
Accepted 23 May 2018
Available online 24 May 2018

Keywords:
Endothelial-to-mesenchymal transition
CLOCK
Atherosclerosis
Plaque stability
Endothelial cells
Rho-associated coiled-coil-containing protein kinase 1

ABSTRACT

Background and aims: Carotid atherosclerotic plaque is one of the main sources of ischemic stroke, and endothelial-to-mesenchymal transition (EndMT) is a major feature of atherosclerosis. Rho-associated coiled-coil-containing protein kinase 1 (ROCK1) activation, stimulated by high glucose, plays an important role in EndMT, and *circadian locomotor output cycles protein kaput* (*Clock*) deficiency leads to hyperglycemia and enhanced atherosclerosis in $Clock^{\Delta 19/\Delta 19}$ *apolipoprotein E* $(ApoE)^{-/-}$ mice. These findings point to a mechanism whereby CLOCK exerts a protective effect against EndMT and atherosclerotic plaque accumulation.

Methods: Cultured human umbilical vein endothelial cells (HUVECs) were stimulated with 66 mM glucose for 120 h to induce EndMT. The expression of CLOCK and ROCK1 was assayed, as were their effects on EndMT. We also conducted molecular and morphometric examination of carotid artery plaques from patients with carotid artery stenosis to assess the clinical relevance of these findings.

Results: Upon EndMT, HUVECs exhibited decreased CLOCK expression and increased ROCK1 expression. Notably, *CLOCK* silencing increased high glucose-induced EndMT, migration ability, and ROCK1 activation, while overexpressing *CLOCK* attenuated these characteristics. Moreover, inhibition of ROCK1 largely blocked EndMT induced by high-glucose or transforming growth factor (TGF)-β1 but failed to rescue the reduced CLOCK expression. The vulnerability of human carotid artery plaque was strongly correlated with loss of CLOCK expression, activation of TGF-β/ROCK1 signaling, and the extent of EndMT.

Conclusions: The data indicate that loss of protective endothelial CLOCK expression aggravates TGF-β/ROCK1-modulated EndMT progression, which contributes to the vulnerability of human carotid plaque.

1. Introduction

Carotid atherosclerotic plaque is one of the main sources of ischemic stroke and is associated with systemic risk factors such as hypercholesterolemia, hyperglycemia, smoking, and age [1]. Phenotypic cell switching through EndMT, a process through which endothelial cells (ECs) transit into mesenchymal stem cells, is a major feature of atherosclerosis [2]. In addition, recent studies have reported a strong correlation between the presence of EndMT and the severity of atherosclerosis [3].

Rho-associated coiled-coil-containing protein kinase 1 (ROCK1), as a downstream effector of TGF-β, has been identified as an important player in driving EndMT progression [4,5]. Abnormal activation of ROCK1 was found to be associated with endothelial dysfunction and contributes to the development of many cardiovascular diseases [6]. Several studies have shown that ROCK1 is an

* Corresponding author. Department of Vascular Surgery, Institute of Vascular Surgery, Zhongshan Hospital, Fudan University, 180 Fenglin Rd, Shanghai, 200032, China.
** Corresponding author. Department of Vascular Surgery, Institute of Vascular Surgery, Zhongshan Hospital, Fudan University, 180 Fenglin Rd, Shanghai, 200032, China.
E-mail addresses: tang.xiao@zs-hospital.sh.cn (X. Tang), guo.daqiao@zs-hospital.sh.cn (D. Guo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.atherosclerosis.2018.05.046
0021-9150/

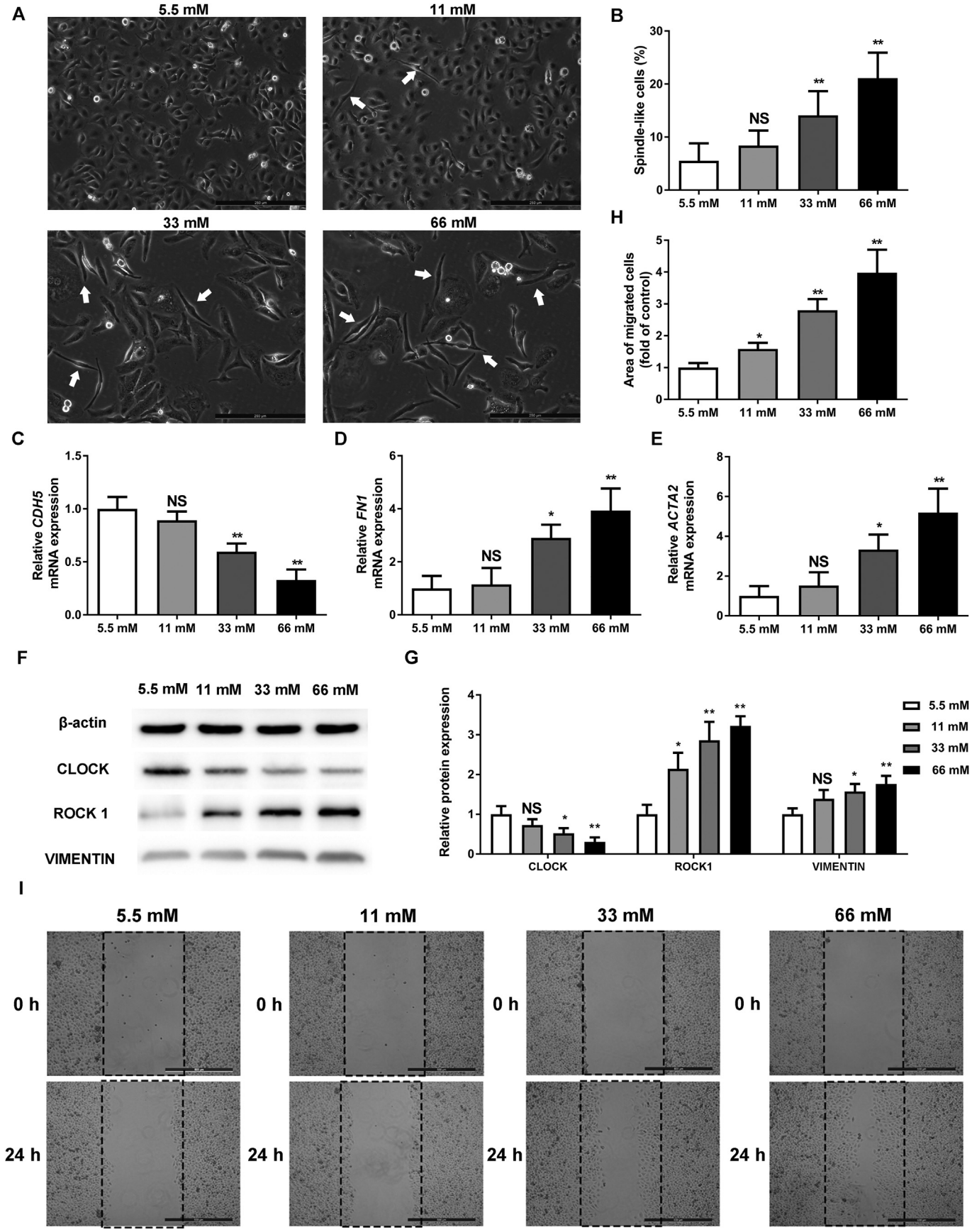

Fig. 1. High glucose induces EndMT in cultured HUVECs.
HUVECs were cultured with glucose (5.5, 11, 33, or 66 mM) and mannitol (60.5, 55, 33, or 0 mM) as an osmotic control for 120 h. (A) HUVEC morphology was imaged by phase contrast microscopy. Representative images are shown. Scale bar, 250 μm. White arrows denote spindle-like endothelial cells. (B) The number of spindle-like cells was counted using a hemocytometer (n = 10). (C–E) *CDH5*, *FN1*, and *ACTA2* mRNA levels were assayed using real-time PCR (n = 3). (F, G) CLOCK, VIMENTIN, and ROCK1 protein levels were assayed by western blotting. (H, I) HUVEC monolayers were scratched, and cell migration into the wounded area was quantified 24 h later. Scale bar, 500 μm. Values represent the mean ± SD. $^{*}p < 0.05$ *vs.* 5.5 mM group. $^{**}p < 0.01$ *vs.* 5.5 mM group. NS = not significant.