Journal of Molecular and Cellular Cardiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original Article

miR-200c-SUMOylated KLF4 feedback loop acts as a switch in transcriptional programs that control VSMC proliferation

Bin Zheng [a,*], Michel Bernier [b], Xin-hua Zhang [a], Toru Suzuki [c], Chan-quan Nie [a], Yong hui Li [a], Yong Zhang [d], Li-li Song [a], Hui-jing Shi [a], Yan Liu [a], Cui-ying Zheng [a], Jin-kun Wen [a,**]

[a] Department of Biochemistry and Molecular Biology, Hebei Medical University, Zhongshan East Road, Shijiazhuang 050017, China
[b] Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224, USA
[c] Department of Cardiovascular Medicine, The University of Tokyo, Tokyo, Japan
[d] Department of Urinary Surgery, Second Hospital of Hebei Medical University, Pingan Road, Shijiazhuang 050000, China

ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 27 February 2015
Accepted 10 March 2015
Available online xxxx

Keywords:
miR-200c
Ubc9
SUMOylation
KLF4
VSMC
Proliferation
Chromatin remodeling

ABSTRACT

The regulation of vascular smooth muscle cell (VSMC) proliferation is an important issue because it has major implications for the prevention of pathological vascular conditions. Using microRNA array screen, we found the expression levels of 200 unique miRNAs in hyperplasic tissues. Among them, miR-200c expression substantially was down-regulated. The objective of this work was to assess the function of miR-200c and SUMOylated Krüppel-like transcription factor 4 (KLF4) in the regulation of VSMC proliferation in both cultured cells and animal models of balloon injury. Under basal conditions, we found that miR-200c inhibited the expression of KLF4 and the SUMO-conjugating enzyme Ubc9. Upon PDGF-BB treatment, Ubc9 interacted with and promoted the SUMOylation of KLF4, which allowed the recruitment of transcriptional corepressors (e.g., nuclear receptor corepressor (NCoR) and HDAC2) to the *miR-200c* promoter. The reduction in *miR-200c* levels led to increased target gene expression (e.g., Ubc9 and KLF4), which further repressed *miR-200c* levels and accelerated VSMC proliferation. These results demonstrate that induction of a miR-200c-SUMOylated KLF4 feedback loop is a significant aspect of the PDGF-BB proliferative response in VSMCs and that targeting Ubc9 represents a novel approach for the prevention of restenosis.

1. Introduction

Vascular smooth muscle cells (VSMCs) play pivotal roles in a variety of diseases, including atherosclerosis [1], hypertension [2], cancer [3], asthma, and vascular aneurysms [4]. Major challenges for the field of vascular medicine have been identifying environmental cues, signaling pathways, and molecular mechanisms that normally control VSMC proliferation and determining how these are disrupted in disease states. A key to understanding the basis of VSMC proliferation and differentiation

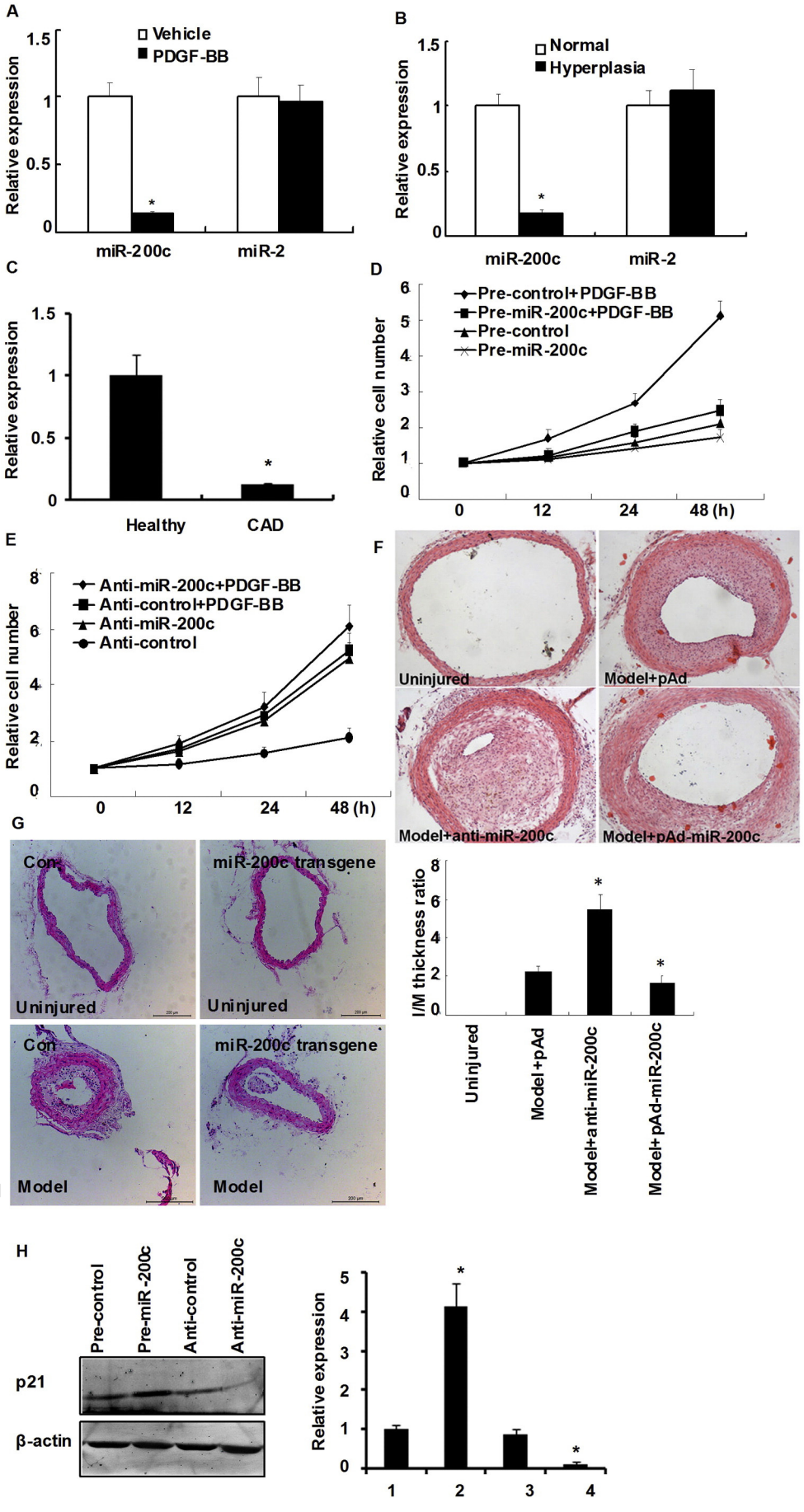
Table 1
Comparison of array analyses of kidney vascular tissues.

Name	Fold	Expression (hyperplasia vs control)
hsa-miR-548a-5p	10.75	Upregulated
hsa-miRPlus-F1050	54.13	Upregulated
hsa-miR-10a*	10.38	Upregulated
hsa-miR-744	9.08	Upregulated
hsa-miR-302c*	15.25	Upregulated
hsa-miR-30c-1*	13.70	Upregulated
hsa-miR-27a	2.40	Upregulated
hsa-miR-342-5p	0.07	Downregulated
hsa-miR-221	0.28	Downregulated
hsa-miR-212	0.09	Downregulated
hsa-miR-200c	0.09	Downregulated
hsa-miR-1915*	0.10	Downregulated
hsa-miR-222	0.26	Downregulated
hsa-miR-564	0.02	Downregulated
hsa-miR-143	0.44	Downregulated
hsa-miR-130b*	0.04	Downregulated
hsa-miR-363*	0.09	Downregulated
hsa-miR-340*	0.14	Downregulated
hsa-miR-668	0.09	Downregulated
hsa-miR-215	0.14	Downregulated
hsa-miR-589	0.13	Downregulated
hsa-miR-122*	0.11	Downregulated
hsa-miR-2113	0.10	Downregulated

Abbreviations: ATRA, all-trans retinoic acid; cdk2, cyclin-dependent kinase 2; HASMC, human aortic smooth muscle cell; HDAC2, histone deacetylase 2; KLF, Krüppel-like factor; LSD1, lysine-specific demethylase 1; miR, microRNA; NCoR, nuclear receptor corepressor; p21, $p21^{WAF1/Cip1}$; PDGF, platelet-derived growth factor; qRT-PCR, quantitative real-time polymerase chain reaction; SRF, serum response factor; SUMO, small ubiquitin-like modifier; Ubc9, E2-conjugating enzyme Ubc9; UTR, untranslated regions; VSMCs, vascular smooth muscle cells

* Correspondence to: B. Zheng, Department of Biochemistry and Molecular Biology, Hebei Medical University, No. 361, Zhongshan East Road, Shijiazhuang 050017, China. Tel.: +86 311 86265563.
** Correspondence to: J.-K. Wen, Department of Biochemistry and Molecular Biology, Hebei Medical University, No. 361, Zhongshan East Road, Shijiazhuang 050017, China. Tel.: +86 311 86266180.
E-mail addresses: doublezb@sina.com (B. Zheng), wjk@hebmu.edu.cn (J. Wen).

http://dx.doi.org/10.1016/j.yjmcc.2015.03.011
0022-2828/



Please cite this article as: Zheng B, et al, miR-200c-SUMOylated KLF4 feedback loop acts as a switch in transcriptional programs that control VSMC proliferation, J Mol Cell Cardiol (2015), http://dx.doi.org/10.1016/j.yjmcc.2015.03.011