GW23-e0919  INSULIN PROMOTES VASCULAR SMOOTH MUSCLE CELL PROLIFERATION VIA MICRORNA-208-MEDIATED DOWNREGULATION OF P21

doi:10.1136/heartjnl-2012-302920a.37

Y Zhang, C Zeng. Department of Cardiology, Daping Hospital, The Third Military Medical University, Chongqing. Chongqing Institute of Cardiology, Chongqing

Objectives Abnormal vascular smooth muscle cell (VSMC) proliferation is considered a key risk factor in many cardiovascular diseases, including hypertension. There is now convincing evidence...
that humoral factors, including insulin, regulate both normal vessel homeostasis and abnormal arterial growth that occurs in vascular disease. Although the growth-promoting effect of insulin on several types of cultured VSMCs has been demonstrated, the underlying mechanism remains vague.

MicroRNAs (miRNAs) are key regulators of gene expression, which are involved in many physiological cellular pathways, including cell growth, differentiation, and apoptosis. MiRNAs are initially transcribed by RNA polymerase II (Pol II) in the nucleus to form large pri-miRNA transcripts. The pri-miRNAs are processed by the RNase III enzymes, Drosha and Dicer, to generate 18- to 24-nucleotide mature miRNAs. The mature miRNAs regulate gene expression in one of two ways that depend on the degree of complementarity between the miRNA and its target. MiRNAs that bind to 3'UTR of mRNA with imperfect complementarity block protein translation. In contrast, miRNAs that bind to mRNA with perfect complementarity induce targeted mRNA cleavage. Currently, more than 400 miRNAs have been cloned and sequenced in human, and the estimated number of miRNA genes is more than 1000 in the human genome. As a group, miRNAs are estimated to regulate 30% of the genes of the human genome.

The role of miRNAs is well-established in the genesis of malignancy, given that cell dedifferentiation, growth, and apoptosis are important cellular events in the development of cancer. There is increasing evidence that miRNAs are expressed in the cardiovascular system and participate in many important biological functions. Because abnormal VSMC proliferation shares similar cellular events and molecular mechanisms with cancer, we hypothesised that endogenous miRNAs may be involved in insulin-induced VSMC proliferation and further contribute to the pathology of hypertension.

**Methods** VSMC proliferation was determined by [3H]-thymidine incorporation; specific miRNA changes in insulin-stimulated VSMCs were detected by miRNA chips and real-time PCR. Target of the specific miRNA was predicted by bioinformatics analysis (TargetScan prediction programme, release 5.1). The relationship of the specific miRNA and the target was further demonstrated by luciferase reporter construct and luciferase assay. Cell cycle was analysed by Fluorescence-activated cell sorting (FACS) analysis and experimental approaches. P21 expression was determined by immunoblotting.

**Results** In this study, we found that insulin increased VSMC proliferation and miR-208 expression. Overexpression of miR-208 increased basal and insulin-mediated VSMC proliferation. Although miR-208 inhibitor, by itself, had no effect on VSMC proliferation, it reduced the insulin-mediated cell proliferation. Moreover, miR-208 increased the transformation of cell cycle from G1 phase to the S phase. Bioinformatics analysis found that p21, a member of the cyclin-dependent kinase (CDK)-inhibitory proteins family, may be the target of miR-208. Insulin decreased p21 expression in VSMCs; transfection of miR-208 also decreased p21 protein expression. In the presence of miR-208 inhibitor, the inhibitory effect of insulin on p21 expression in VSMCs was partially blocked. The interaction between miR-208 and p21 was direct. Using a luciferase reporter with entire wild-type p21 3’UTR or a mutant p21 3’UTR in HEK293 cells, we found that miR-208 decreased but neither miR-208 mimic nor the mutant p21 3’UTR had any significant effect on the luciferase activity.

**Conclusions** This study indicates that miRNAs, miR-208, in particular, are involved in the insulin-induced VSMC proliferation via down-regulation of its potential target, p21, a key member of CDK-inhibitory protein family and maybe further contribute to the pathology of hypertension.
DOWNREGULATION OF P21 MICRORNA-208-MEDIATED MUSCLE CELL PROLIFERATION VIA INSULIN PROMOTES VASCULAR SMOOTH Y Zhang and C Zeng

Heart 2012 98: E17-E18
doi: 10.1136/heartjnl-2012-302920a.37

Updated information and services can be found at:
http://heart.bmj.com/content/98/Suppl_2/E17.3

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Hypertension (3006)
Drugs: cardiovascular system (8842)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/