Keep calm and carry on: miR-1298 prevents up-regulation of Cx43 and secures a quiescent vascular smooth muscle cell

Kjestine Schmidt¹,² and Cor de Wit¹,²*

¹Institut für Physiologie, Universität zu Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany; and ²Deutsches Zentrum für Herz-Kreislauft-Forschung (DZHK) e.V. (German Center for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Lübeck, Germany

Online publish-ahead-of-print 21 July 2015

This editorial refers to ‘MicroRNA-1298 is regulated by DNA methylation and affects vascular smooth muscle cell function by targeting connexin 43’ by W. Hu et al., pp. 534–545.

Atherosclerosis constitutes the leading cause of cardiovascular disease resulting in ischaemia of dependent organs due to arterial occlusion. It affects not only heart and brain, but also limbs and other organs with fatal consequences for the individuum. The process invokes profound remodelling of the entire vascular wall driven by inflammation, which affects endothelial cells and promotes the activation of vascular smooth muscle cells (VSMCs). In healthy vessels, VSMCs retain a quiescent, contractile state but in response to environmental cues (chemical and mechanical) they adopt an activated, synthetic state characterized also by proliferation and migration. This remarkable plasticity is achieved through a substantial change in gene expression profile, which is likely governed by epigenetic and/or transcriptional control mechanisms.

In addition to transcriptional regulation, non-coding, single-stranded, small RNAs (typically ~22 nucleotides) contribute substantially to the phenotypic modulation of VSMCs through post-transcriptional regulation of gene expression (mRNA degradation or translational repression).¹² These so-called microRNAs (miRNAs/miRs) promote or inhibit the switch of VSMCs to the synthetic state. For example, the miR-143/145 cluster supports a contractile state and was down-regulated by vascular injury, implicating that its lack fosters the synthetic state.³⁴ Conversely, other miRNAs (miR-21, miR-221/222) were found to promote the synthetic state, proliferation of VSMCs, and to be enhanced in vascular injury.⁵ Most of these effects were attributable to the regulation of the expression levels of transcription factors, thereby modifying a multitude of genes and modulating or achieving the phenotypic switch of VSMCs.⁶ Due to the tremendous interest in this field of cellular gene regulation, new members are continuously added to this list of miRNAs (miR-663,⁷ miR-34c,⁸ and miR-195⁹) holding the promise of modulating vascular remodelling during atherosclerosis and neointima formation.¹⁰

Hy et al.¹¹ added miR-1298 to this ever expanding list. However, this miRNA did not act via regulation of transcription factors, but directly by the post-transcriptional modulation of the expression of the gap junction forming protein connexin 43 (Cx43). The authors started from the observation that miR-1298 is expressed in healthy human vessels in VSMCs, but strongly down-regulated in atherosclerotic arteries. Interestingly, this down-regulation occurred at the transcriptional level as a consequence of methylation-mediated epigenetic silencing. They demonstrated a hypermethylation (by 100%) of CpG-rich fragments in the region upstream of the miR-1298 core sequence in diseased arteries, an effective miR-1298 up-regulation by the inhibition of DNA methyltransferase in vitro, and an inhibition of the miR-1298 promoter due to methylation using a reporter gene assay. The functional consequences were evaluated vigorously in cultured VSMCs: overexpression of miR-1298 inhibited PDGF-induced cell cycle progression into the S-phase and attenuated VSMC proliferation and migration, suggesting that miR-1298 prevents the switch to the synthetic phenotype. Conversely, blocking miR-1298 specifically revealed the opposite, i.e. an increased number of VSMCs in the S-phase, enhanced proliferation and migration (Figure 1).

Mechanistically, the authors examined Cx43 as a target based on its complementary mRNA 3‘-UTR sequence allowing miR-1298 to prevent Cx43 translation. Indeed, Cx43 protein levels (shown by immunostaining and western blot) were enhanced in human atherosclerotic arteries, and concomitantly assessed miR-1298 levels were reduced suggesting a negative regulation. An inhibitory effect of miR-1298 on Cx43 was verified in cultured VSMCs at the protein and mRNA level, pointing towards Cx43 mRNA as a direct target of miR-1298 through accelerated degradation. In fact, they identified specific binding sites on the Cx43 mRNA for miR-1298 that are decisive for the regulation using reporter gene assays. However, effects on Cx43 may still be an inhibitory effect: miR-1298 on Cx34 was verified in cultured VSMCs at the protein and mRNA level, pointing towards Cx43 mRNA as a direct target of miR-1298 through accelerated degradation. In fact, the authors transfected VSMCs with Cx43 that is non-responsive to miR-1298 (lentiviral approach omitting the crucial miR-binding sites). This elegant approach would render other miR-1298 effects intact. As intended, miR-1298 was not able to inhibit Cx43 expression using the lentiviral approach. The overexpression of Cx43
enhanced proliferation and migration in otherwise non-stimulated VSMCs. The enhancing effect was insensitive to concomitant miR-1298 treatment, demonstrating that the down-regulation of Cx43 by miR-1298 is required to prevent the switch to the synthetic state.

A synthetic phenotype can likewise be induced by external stimulation (e.g. using PDGF) which up-regulated Cx43. Gap junctional coupling was verified by dye transfer and attenuated by miR-1298 (which also decreased Cx43 expression in this setting), indicating that additionally functional effects of Cx43 are affected. However, Cx43 may also invoke cellular signalling through kinases such as ERK. Although the authors did not examine this signalling pathway in detail, ERK was activated by Cx43 overexpression and basal ERK activation reduced by miR-1298 verifying effects on a distinct function of Cx43 in VSMCs.

Finally, the efficacy of miR-1298 was examined in the rat in vivo by provoking neointima formation in the carotid artery. Lentiviral transfection of miR-1298 reduced, whereas transfection of the miR-1298 inhibitor enhanced neointima formation. This was associated with concordant changes in the proliferation marker Ki67. The Cx43 expression was increased after injury and, conversely, overexpression of Cx43 enhanced neointima formation. While miR-1298 effectively blunted Cx43 expression in injured arteries, it did not reduce lentiviral-induced Cx43 expression because this approach circumvents the mRNA degradation by miR-1298 (due to the missing-binding sites).

Under these conditions (i.e. with unrestricted Cx43 expression), miR-1298 loses its strong protective effect against neointima formation substantiating that prevention of Cx43 up-regulation is required to abrogate neointima formation.

The data connect different avenues that regulate VSMC phenotype, namely miRNAs and connexins. Cx43 has been demonstrated before to induce a phenotypic switch and fosters the synthetic state, and now, the authors provided a pathway from methylation through miRNA expression directly targeting Cx43 expression. Interestingly, other miRNAs (e.g. miR-1, miR-206, and miR-130a) likewise target the expression of Cx43 in skeletal muscle and osteoblasts during physiologic differentiation, but also pathologically in cardiac muscle or cancer cells. This is the decisive distinction of miR-1298 compared with many miRNAs modulating the VSMC phenotypic: miR-1298 does not act through transcription factors, but the repression of Cx43 mRNA degradation that is also involved in physiological differentiation processes. Most interestingly, this process is initiated by epigenetic mechanisms (methylation, as shown for other miRs in cancer) representing a master regulator of cell behaviour in response to environmental cues. A myriad of stimuli that may lead to gene methylation come to mind (inflammation, haemodynamics, etc.) and have to be verified.

The targets at the end of the pathway are likewise not clearly identified: gap junctional communication between adjacent cells, activation...
of intracellular signalling pathways (e.g. ERK), or release of substances through hemichannels formed by Cx. 31

**Conflict of interest:** none declared.

**Funding**

Studies performed in the author’s own laboratory are supported by the Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK).

**References**


