BRAND X

SMALL RNAS

Protecting a healthy circulation

> This work … provides an example of vesicle-mediated miRNA transfer

The transcription factor Krüppel-like factor 2 (KLF2), which is expressed in endothelial cells under shear stress, has a protective function against atherosclerosis. Dimmeler and colleagues now show that KLF2 induces the expression of the microRNAs (miRNAs) miR-143 and miR-145 in endothelial cells, and that these are transported to adjacent smooth muscle cells to carry out a vasculoprotective function.

First, the authors observed that KLF2 regulated several miRNAs in human vascular endothelial cells under conditions of shear stress. Of these miRNAs, the cluster containing miR-143 and miR-145, which are known to be expressed by smooth muscle cells, was the most profoundly upregulated by KLF2 and shear stress. Further experiments confirmed that these miRNAs are expressed by endothelial cells *in vivo* and are directly activated by KLF2.

So how does KLF2-dependent miR-143 and miR-145 expression in endothelial cells affect the vasculature? It had been observed previously that mice lacking KLF2 have a normal endothelium but dysfunctional and disorganized smooth muscle cells. Furthermore, miR-143 and miR-145 are known to be crucial for proper smooth muscle cell function, which suggests a physiological link, mediated by the miRNAs, between endothelial KLF2 expression and the underlying smooth muscle cells.

To test this hypothesis, Dimmeler and colleagues analysed the RNA content of extracellular vesicles isolated from the supernatant of endothelial cells overexpressing KLF2 or exposed to shear stress. They found that these vesicles were enriched in miR-143 and miR-145. Furthermore, electron microscopy observations and treatment with phospholipid membrane disruptors showed that the vesicles carrying miR-143 and miR-145 are mostly exosomes, which are small vesicles surrounded by a lipid bilayer.

Next, the authors co-cultured endothelial cells and smooth muscle cells, separated by a 0.4 μm pore-size membrane that prevents direct contact or transfer of large vesicles, to show that miRNAs can be actively transferred from endothelial to smooth muscle cells through small vesicles, such as exosomes. Furthermore, when smooth muscle cells were co-cultured with endothelial cells overexpressing KLF2, their levels of miR-143 and miR-145 increased and expression of miR-143 and miR-145 target genes was reduced. This indicates that transfer of miRNAs from endothelial to smooth muscle cells is induced by KLF2 and that this leads to miRNA target gene repression.

Blood vessels exposed to laminar blood flow have high shear stress, express KLF2 and are protected from atherosclerosis. To examine whether miRNA-mediated gene repression induces this atheroprotective effect, the authors isolated vesicles from KLF2-expressing mouse endothelial cells and subsequently injected them into *Apoe*$^{-/-}$ mice kept on a high fat diet. This significantly reduced the number of fatty lesions areas in the aorta and, importantly, the atheroprotective effect was abolished if miRNA expression was inhibited.

This work shows that KLF2-expressing endothelial cells protect against atherosclerotic lesions in a miR-143- and miR-145-dependent manner and provides an example of vesicle-mediated miRNA transfer as a means for cell-to-cell signalling.

Kim Baumann

ORIGINAL RESEARCH PAPER Hergenreider, E. *et al.* Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nature Cell Biol.* 12 Feb 2012 (doi:10.1038/ncb2441)