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# **MicroRNA-21 and the Vulnerability of Atherosclerotic Plaques**

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Stabilizing atherosclerotic lesions and preventing plaque ruptures can be seen as the holy grail in vascular medicine. In this issue of *Molecular Therapy*, Jin *et al* suggest local delivery of a microRNA-21 (miR-21) mimic as a potential therapeutic strategy to reduce plaque vulnerability. Interestingly, inhibition of this microRNA has also been proposed as a therapeutic approach for in-stent restenosis, where the application of anti-miR-21 oligonucleotides on stents improved vessel patency<sup>1</sup>.

Atherosclerosis is a chronic inflammatory disease resulting from the build-up of lipid deposits in the innermost layer of an artery. Apart from lipids, atherosclerotic plaques comprise mainly smooth muscle cells, macrophages, calcium and fibrous connective tissue. Upon rupture or erosion of vulnerable atherosclerotic plaques, platelet aggregation results in thrombus formation that can occlude the vessel lumen. Stents are metallic scaffolds placed into the occluded segment of a diseased artery to hold it open. Current drug-eluting stents (DES) are designed to inhibit vascular smooth muscle cell (SMC) proliferation to reduce neo-intima formation within the stent lumen. Whilst greatly enhancing stent patency in coronary arteries, these anti-proliferative agents also inhibit the proliferation of endothelial cells, thus delaying re-endothelialization and arterial healing. After DES implantation, dual antiplatelet therapy is therefore required to prevent the inherent risk of thrombosis and stent occlusion. Alternatives to current DES that would inhibit SMC proliferation without delaying re-endothelialization should result in less thrombogenicity.

In the vasculature, miR-21 has previously been implicated as a determinant of SMC proliferation<sup>2-5</sup>. For example, pharmacological and genetic miR-21 inhibition

markedly decreased neo-intima formation in vein grafts<sup>4,5</sup> and balloon-injured carotid arteries<sup>2</sup>. These findings led to the exploration of local therapeutic strategies by coating stents with miR-21 inhibitors. When deployed in denuded arteries in rats, this approach prevented SMC-mediated in-stent restenosis without delaying re-endothelialization<sup>1</sup>.

Conversely, SMC proliferation can also be beneficial in stabilizing the vessel wall and atherosclerotic plaques. For example, the proliferative effects of miR-21 mimics in SMCs reduced the expansion of abdominal aortic aneurysms<sup>6</sup>. The results presented by Jin *et al* in this issue of the journal propose the use of miR-21 mimics to enhance plaque stability. Unstable human plaques and atherosclerotic lesions in apolipoprotein E (apoE) deficient mice (*ApoE*<sup>-/-</sup>) exhibit lower miR-21 levels. Based on *in situ* hybridization and immunohistochemistry, this expression pattern was ascribed to SMCs. The authors further show that plaques in *ApoE/miR-21* double deficient mice (*ApoE*<sup>-/-</sup>*miR21*<sup>-/-</sup>) were more prone to rupture. Additionally, the unstable plaques were shown to harbor lower levels of the RE1-silencing transcription factor (REST). REST has been identified as a regulator of miR-21 whilst also being a direct target of miR-21. Subsequent *in vitro* analysis of SMCs revealed an anti-proliferative effect of REST, confirming the proliferative role of miR-21. Finally, local delivery of miR-21 mimics to carotid plaques using ultrasound-targeted microbubble destruction enhanced plaque stability. These findings suggest that by increasing SMC proliferation, miR-21 mimics could stabilize the SMC-rich fibrous cap that shields the lipid-filled core of atherosclerotic plaques.

As is the case for most miRNAs, miR-21 is ubiquitously expressed<sup>7</sup>. It is particularly abundant in circulating hematopoietic cells, where it appears to act as an 'emergency brake' on inflammation. In macrophages, miR-21 directly targets the pro-inflammatory programmed cell death protein 4 (PDCD4), thereby increasing the secretion of anti-inflammatory interleukin-10 (IL-10)<sup>8</sup>. MiR-21 also directly targets phosphatase and tensin homolog (PTEN), steering macrophages towards a reparative phenotype that promotes resolution of inflammation and tissue recovery<sup>9</sup>. Thus, miR-21 may also modulate tissue inflammation, in part through monocyte differentiation toward an anti-inflammatory macrophage phenotype.

A recent study by Canfrán-Duque *et al* also focused on miR-21 in the context of atherosclerosis<sup>10</sup>. MiR-21 accumulated in murine atherosclerotic plaques along with CD68, a macrophage marker. Deficiency of miR-21 in bone marrow cells promoted vascular inflammation and plaque necrosis in low-density lipoprotein receptor (LDLr) *null* mice. Compared to wild-type bone marrow, transplantation of *miR-21*<sup>-/-</sup> bone marrow into *LDLr*<sup>-/-</sup> mice resulted in larger and less stable atherosclerotic plaques due to increased inflammatory cell infiltration. Thus, miR-21 expression in murine hematopoietic cells attenuates vascular inflammation. The findings of Jin *et al* also highlight the impact of miR-21 on the influx of macrophages. *ApoE*<sup>-/-</sup>*miR21*<sup>-/-</sup> mice displayed more advanced plaque formation at an early age, with a concomitant increase of macrophage infiltration and foam cell formation. In line with the recent findings of Canfrán-Duque *et al*, miR-21 *null* peritoneal macrophages displayed an increase in oxidized LDL uptake and foam cell formation through enhanced NF-κB signaling. Notably, secreted factors from *ApoE*<sup>-/-</sup>*miR21*<sup>-/-</sup> macrophages could suppress SMC proliferation *in vitro*.

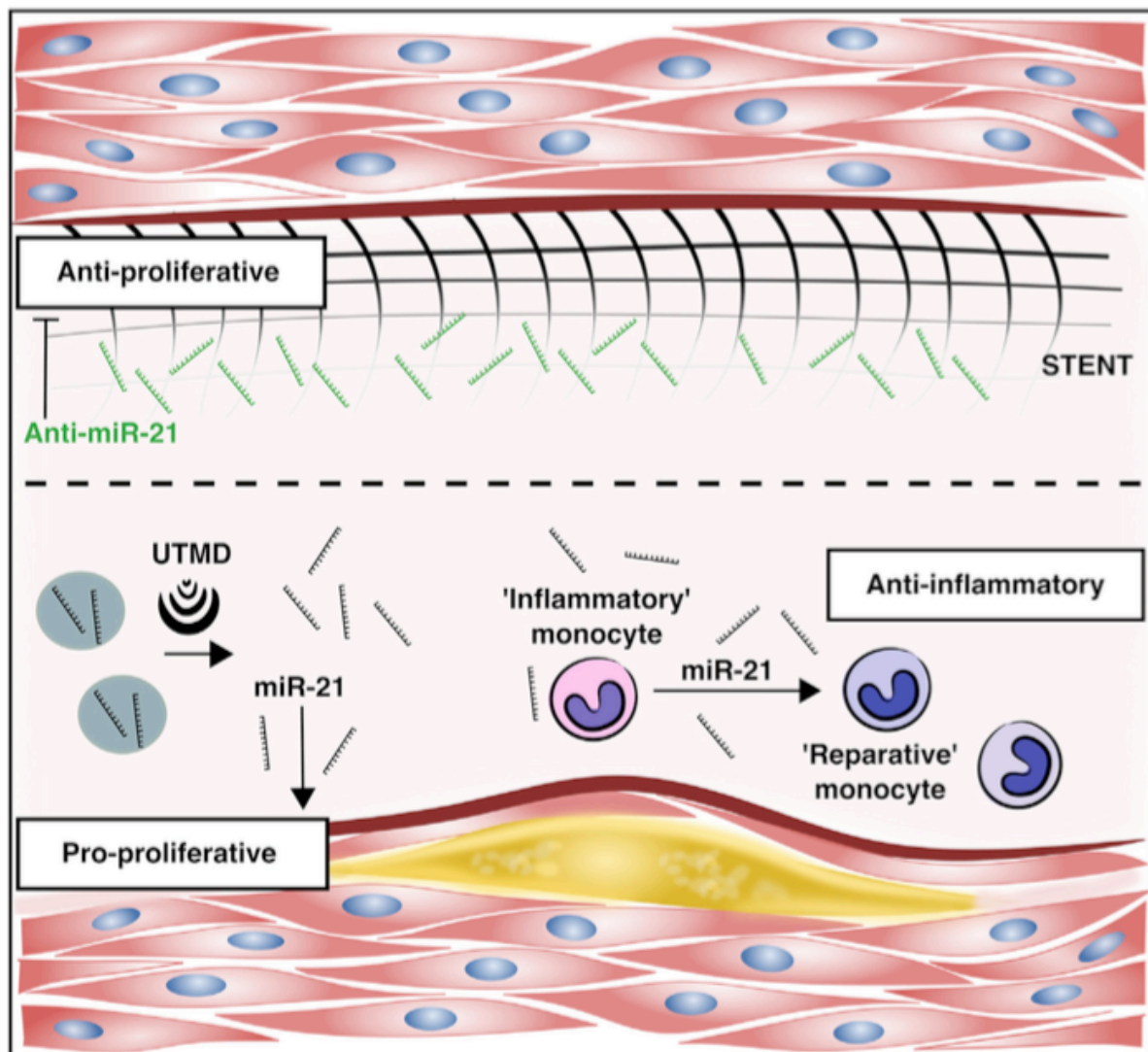
A common challenge in miRNA research is to identify the cell types that are responsible for the observed therapeutic benefits. Although the present study confirms a mechanistic link between miR-21 and SMC proliferation, it remains unclear whether the beneficial effect relies predominantly on SMCs, macrophages or other cell types. Using a multi-omics approach, we recently identified a protein signature of symptomatic atherosclerotic plaques that implied a shared involvement of immune cells and SMCs<sup>11</sup>. Also, the plasticity of SMCs is currently under debate, with lineage tracing experiments suggesting a potential SMC origin of macrophage-like cell types<sup>12,13</sup>. Finally, apoE mediates the reverse cholesterol transport<sup>14</sup> and atherosclerosis is known to differ between man and apoE *null* mice<sup>15</sup>.

Despite these notes of caution, the study by Jin *et al* advances our insight into the role of miR-21 in vascular biology. This is a timely contribution as miRNA-based therapies progress to clinical application. Clinical trials are currently underway in patients with Alport Syndrome, where systemic miR-21 inhibition is evaluated for the treatment of renal fibrosis. A better understanding of the effects of miR-21 in the context of other diseases is therefore highly relevant. The findings of Jin *et al* implicate that local therapy with miR-21 may indeed achieve higher drug concentrations at the target site and could minimize the risk of systemic side effects from miRNA therapeutics. Nevertheless, even local therapy for ubiquitously expressed miRNAs could have potential systemic effects, for example by affecting circulating cells in the blood stream.

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## Figure legends

**Figure 1. MicroRNA-21-based therapy for plaque vulnerability and in-stent restenosis.** Delivery of microRNA-21 (miR-21) mimic to atherosclerotic plaques by ultrasound-targeted microbubble destruction (UTMD) enhances smooth muscle cell proliferation, whilst also steering monocytes towards a reparative, anti-inflammatory phenotype. Conversely, stents coated with miR-21 inhibitor (anti-miR-21) prevent in-stent restenosis through their anti-proliferative effect on smooth muscle cells.





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