MicroRNA-21 and the Vulnerability of Atherosclerotic Plaques

Temo Barwari,[1] Marieke Rienks,[1] and Manuel Mayr[1]

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.[1] suggest the local delivery of microRNA-21 (miR-21) mimics 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.[2]

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 (SMCs), 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 (DESs) are designed to inhibit vascular SMC proliferation to reduce neo-intima formation within the stent lumen. While 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 DESs 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.[3–6] For example, pharmacological and genetic miR-21 inhibition markedly decreased neo-intima formation in vein grafts[5,6] and balloon-injured carotid arteries.[3] 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.[2]

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.[7] The results presented by Jin et al.[1] in this issue propose the use of miR-21 mimics to enhance plaque stability. Unstable human plaques and atherosclerotic lesions in apolipoprotein E (apoE)-deficient mice ($Apoe^{-/-}$) 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 ($Apoe^{-/-}miR-21^{-/-}$) 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 while 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.[8] 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).[9] miR-21 also directly targets phosphatase and tensin homolog (PTEN), steering macrophages toward a reparative phenotype that promotes resolution of inflammation and tissue recovery.[10] 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.[11] also focused on miR-21 in the context of atherosclerosis. 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 $miR-21^{-/-}$ bone marrow into $Ldlr^{-/-}$ 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.[1] also highlight the impact of miR-21 on the influx of macrophages. $Apoe^{-/-}miR-21^{-/-}$ 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.,[11] miR-21 *null* peritoneal macrophages displayed an increase in oxidized LDL uptake and foam cell formation through enhanced nuclear factor κB (NF-κB) signaling. Notably, secreted factors from $Apoe^{-/-}miR-21^{-/-}$ macrophages could suppress SMC proliferation *in vitro*.

[1]King's British Heart Foundation Centre, King's College London, London, UK

Correspondence: Manuel Mayr, King's British Heart Foundation Centre, King's College London, 125 Coldharbour Lane, London SE5 9NU, UK.
E-mail: manuel.mayr@kcl.ac.uk
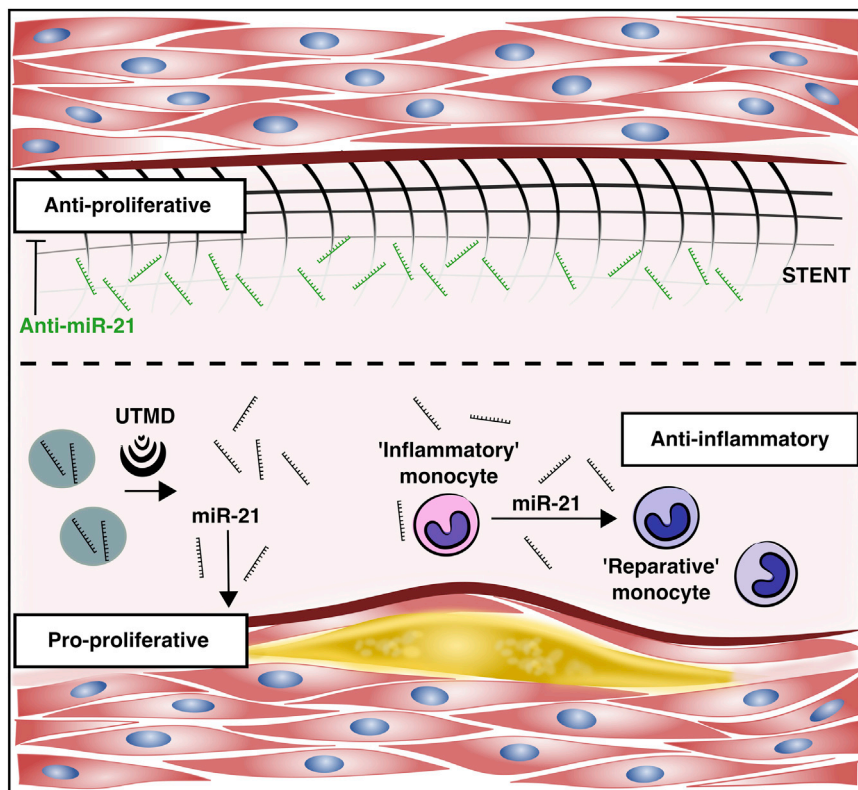
Figure 1. MicroRNA-21-Based Therapy for Plaque Vulnerability and In-Stent Restenosis
Delivery of microRNA-21 (miR-21) mimic to atherosclerotic plaques by ultrasound-targeted microbubble destruction (UTMD) enhances smooth muscle cell proliferation while also steering monocytes toward a reparative, anti-inflammatory phenotype. Conversely, stents coated with miR-21 inhibitor (anti-miR-21) prevent in-stent restenosis through their anti-proliferative effect on smooth muscle cells.

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 (Figure 1). 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.[12] Also, the plasticity of SMCs is currently under debate, with lineage tracing experiments suggesting a potential SMC origin of macrophage-like cell types.[13,14] Finally, apoE mediates the reverse cholesterol transport,[15] and atherosclerosis is known to differ between man and apoE *null* mice.[16]

Despite these notes of caution, the study by Jin et al.[1] 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.[1] 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.

ACKNOWLEDGMENTS

REFERENCES

1. Jin, H., Li, D.Y., Chernogubova, E., Sun, C., Busch, A., Eken, S.M., Saliba-Gustafsson, P., Winter, H., Winski, G., Raaz, U., et al. (2018). Local Delivery of miR-21 Stabilizes Fibrous Caps in Vulnerable Atherosclerotic Lesions. Mol Ther. *26*, this issue, 1040–1055.

2. Wang, D., Deuse, T., Stubbendorff, M., Chernogubova, E., Erben, R.G., Eken, S.M., Jin, H., Li, Y., Busch, A., Heeger, C.-H., et al. (2015). Local microRNA modulation using a novel anti-miR-21-eluting stent effectively prevents experimental in-stent restenosis. Arterioscler. Thromb. Vasc. Biol. *35*, 1945–1953.

3. Ji, R., Cheng, Y., Yue, J., Yang, J., Liu, X., Chen, H., Dean, D.B., and Zhang, C. (2007). MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ. Res. *100*, 1579–1588.

4. Wang, M., Li, W., Chang, G.-Q., Ye, C.-S., Ou, J.-S., Li, X.-X., Liu, Y., Cheang, T.-Y., Huang, X.-L., and Wang, S.-M. (2011). MicroRNA-21 regulates vascular smooth muscle cell function via targeting tropomyosin 1 in arteriosclerosis obliterans of lower extremities. Arterioscler. Thromb. Vasc. Biol. *31*, 2044–2053.

5. McDonald, R.A., Halliday, C.A., Miller, A.M., Diver, L.A., Dakin, R.S., Montgomery, J., McBride, M.W., Kennedy, S., McClure, J.D., Robertson, K.E., et al. (2015). Reducing in-stent restenosis: Therapeutic manipulation of miRNA in vascular remodeling and inflammation. J. Am. Coll. Cardiol. *65*, 2314–2327.

6. McDonald, R.A., White, K.M., Wu, J., Cooley, B.C., Robertson, K.E., Halliday, C.A., McClure, J.D., Francis, S., Lu, R., Kennedy, S., et al. (2013). miRNA-21 is dysregulated in response to vein grafting in multiple models and genetic ablation in mice attenuates neointima formation. Eur. Heart J. *34*, 1636–1643.

7. Maegdefessel, L., Azuma, J., Toh, R., Deng, A., Merk, D.R., Raiesdana, A., Leeper, N.J., Raaz, U., Schoelmerich, A.M., McConnell, M.V., et al. (2012).

MicroRNA-21 blocks abdominal aortic aneurysm development and nicotine-augmented expansion. Sci. Transl. Med. *4*, 122ra22.

8. Barwari, T., Joshi, A., and Mayr, M. (2016). MicroRNAs in cardiovascular disease. J. Am. Coll. Cardiol. *68*, 2577–2584.

9. Sheedy, F.J., Palsson-McDermott, E., Hennessy, E.J., Martin, C., O'Leary, J.J., Ruan, Q., Johnson, D.S., Chen, Y., and O'Neill, L.A. (2010). Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat. Immunol. *11*, 141–147.

10. Das, A., Ganesh, K., Khanna, S., Sen, C.K., and Roy, S. (2014). Engulfment of apoptotic cells by macrophages: a role of microRNA-21 in the resolution of wound inflammation. J. Immunol. *192*, 1120–1129.

11. Canfrán-Duque, A., Rotllan, N., Zhang, X., Fernández-Fuertes, M., Ramírez-Hidalgo, C., Araldi, E., Daimiel, L., Busto, R., Fernández-Hernando, C., and Suárez, Y. (2017). Macrophage deficiency of miR-21 promotes apoptosis, plaque necrosis, and vascular inflammation during atherogenesis. EMBO Mol. Med. *9*, 1244–1262.

12. Langley, S.R., Willeit, K., Didangelos, A., Matic, L.P., Skroblin, P., Barallobre-Barreiro, J., Lengquist, M., Rungger, G., Kapustin, A., Kedenko, L., et al. (2017). Extracellular matrix proteomics identifies molecular signature of symptomatic carotid plaques. J. Clin. Invest. *127*, 1546–1560.

13. Shankman, L.S., Gomez, D., Cherepanova, O.A., Salmon, M., Alencar, G.F., Haskins, R.M., Swiatlowska, P., Newman, A.A.C., Greene, E.S., Straub, A.C., et al. (2015). KLF4-dependent pheno- typic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. Nat. Med. *21*, 628–637.

14. Bennett, M.R., Sinha, S., and Owens, G.K. (2016). Vascular smooth muscle cells in atherosclerosis. Circ. Res. *118*, 692–702.

15. Zanotti, I., Pedrelli, M., Potì, F., Stomeo, G., Gomaraschi, M., Calabresi, L., and Bernini, F. (2011). Macrophage, but not systemic, apolipoprotein E is necessary for macrophage reverse cholesterol transport in vivo. Arterioscler. Thromb. Vasc. Biol. *31*, 74–80.

16. Allahverdian, S., Pannu, P.S., and Francis, G.A. (2012). Contribution of monocyte-derived macrophages and smooth muscle cells to arterial foam cell formation. Cardiovasc. Res. *95*, 165–172.

Keep Quiet and Stay in Line! Smart Polymers to Keep an Eye on Pancreatic Tumors

Pierre Cordelier[1]

Pancreatic cancer is among the deadliest epithelial malignancies and one of the few solid neoplasms with a rising incidence worldwide. Pancreatic cancer is the 12th most common cancer in the US[1] and, paradoxically, is predicted to become the second most common cause of cancer-related deaths within the next decade or so.[2] Pancreatic cancer prognosis is largely dependent on the stage of diagnosis. Unfortunately, pancreatic tumors are rarely detectable at early stage, and the vast majority of patients (80%–90%) are diagnosed with local and/or distant metastasis, with only 3% of patients surviving to 5 years.[3] Improving survival will definitely require better therapeutics for late-stage disease. In this issue of *Molecular Therapy*, Naqvi et al.[4] describe a new strategy to impair the metastatic spread of experimental pancreatic cancer tumors using nucleic acid binding poly- mers (NABPs). They show that a third-generation polyamidoamine dendrimer called PAMAM-G3 was efficient in treating a well-calibrated aggressive experimental mouse model of pancreatic cancer dissemination.

The authors took advantage of the fact that PAMM-G3 binds pro-inflammatory extra-cellular nucleic acids and nucleic acid-protein complexes to skew toll-like receptor (TLR) activation.[5] These compounds, originally developed as a tool for gene delivery, proved to be safe and effective in treating experimental models of lupus, acute liver failure, and influenza infection.[5,6] Interestingly, circulating nucleic acids such as cell-free DNA (cfDNA), pathogen associated molecular patterns (PAMPs), and damage associated molecular patterns (DAMPs) are hallmarks of various carcinomas and usual suspects in TLR-dependent metastatic dissemination, notably of pancreatic cancer cells.[7] Blunting the dialog between cancer cells and their immediate microenvironment may not only directly impair primary tumor metastatic spread, but also jeopardize the pre-conditioning of distant pre-metastatic sites for remote cancer cell implantation.

With this in mind, the authors of the new study found that PAMAM-G3 behaves at least as a two-edged sword to abrogate TLR activation and nuclear factor κB (NF-κB) nuclear translocation induced by cell-free DNA (cfDNA) in cancer cell lines from pancreatic origin. The preferred mechanism of action, which still needs to be clarified in these particular cells, is that PAMAM-G3 decreases the cellular uptake and the subcellular localization of TLR9 agonists from the endosome to the nucleus to prevent TLR9 activation.[5] Interestingly, as this NABP exerts its function by depleting DAMPs and PAMPs upstream of TLR9,

[1]Université Fédérale Toulouse Midi-Pyrénées, Université Toulouse III Paul Sabatier, INSERM, CRCT, Toulouse, France

Correspondence: Pierre Cordelier, PhD, INSERM U1037 Cancer Research Centre of Toulouse, Team 102, Avenue Hubert Curien, 31037 Toulouse Cedex 1, France.
E-mail: pierre.cordelier@inserm.fr