Commentary

miR-21 alters circulating Treg function in vascular disease—hope for restoring immunoregulatory responses in atherosclerosis?

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Provenance: This is a Guest Commentary commissioned by Section Editor Zhijun Han, MD (Department of Laboratory Medicine, Wuxi Second Hospital, Nanjing Medical University, Wuxi, China).

Comment on: Li S, Fan Q, He S, *et al.* MicroRNA-21 negatively regulates Treg cells through a TGF-β1/Smad-independent pathway in patients with coronary heart disease. Cell Physiol Biochem 2015;37:866-78.

Submitted Nov 21, 2016. Accepted for publication Nov 25, 2016.

doi: 10.21037/atm.2016.12.72

View this article at: http://dx.doi.org/10.21037/atm.2016.12.72

The small molecule regulators of gene expression, microRNAs, have emerged as important mediators in a variety of cellular processes linked to disease. Much emphasis has been placed on measuring the expression of these molecules as novel biomarkers of disease. In particular, miRNA profiles of cancer have revealed both tumor specific signatures as well as highlighting common miRNAs with central roles in malignancy. Chief amongst these is microRNA-21, miR-21, the expression of which is enhanced in multiple solid tumors and lymphomas (1) where it regulates transformation by limiting the expression of various tumor suppressor genes (2). Outside of tumors, miR-21 is also highly expressed in cells of the immune system and its expression can be modulated by inflammatory stimuli during immune cell activation (3), which traffic through the circulation. miRNAs can also be found in circulation in secreted vesicles including exosomes released from various cell types associated with disease (4). This has led to an explosion in interest in measuring serum miRNA profiles during disease pathogenesis. A recently published article in *Cellular Physiology and Biochemistry* by Li and colleagues (5) measured miR-21 in serum from patients with vascular disease has revealed novel ways miR-21 can control circulating immune cell diversity and function, which impacts upon disease.

Although other groups measured circulating miR-21 in patients with atherosclerosis previously and found an increased level of miR-21 associated with increased risk of stroke (6), the study by Li and colleagues examines miR-21 in serum in various cohorts of patients with increasing severity. In particular, miR-21 expression is increased over 5-fold in peripheral blood mononuclear cells (PBMCs—a fraction of white blood cells including B, T-cells, monocytes, dendritic cells and neutrophils), in patients with severe vascular disease and a history of myocardial infarction relative to control patients with chest pains yet no vascular disease. Interestingly, in other intermediate patients groups with progressing from stable to unstable angina and increasing incidence of vascular disease there is also an increase in PBMC miR-21 expression implicating the induction of miR-21 in progression of atherosclerosis.

While atherosclerosis is associated with the recruitment of immune cells to areas of lipid deposition along blood vessels, particularly pro-inflammatory monocytes which foster and promote plaque inflammation (7), it is emerging that regulatory immune cells including FoxP3+ T-cells (Treg) infiltrate atherosclerotic plaques (8). However, the number and function of these cells decreases both in plaques and in circulation as disease progresses, consistent with a breakdown in tolerance and the appearance of pro-inflammatory T cells (9-12). The Li study confirmed this decline in circulating Treg numbers alongside the expression levels of TGF-β and FoxP3 mRNA in PBMC from atherosclerotic patients, reflected in the serum by decreased TGF-β protein levels when measured by ELISA. These decreases, like miR-21, are associated with severity of vascular disease and are more pronounced in cohorts with history of unstable angina and myocardial infarction. These

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intriguing findings suggest that an increase in PBMC miR-21 as disease progresses alters T-cell function to promote an immune-regulatory environment, specifically by reducing Treg numbers and activity.

Previous work in the area of T-cell biology has implicated miR-21 in multiple levels of control of T-cell fate, function and diversity and this new data showing modulation of miR-21 negatively regulating T-reg function in atherosclerosis, while increasing our knowledge also increases the complexity whereby miR-21 can control immune function. miR-21 is known to be specifically expressed in the Treg subset (13) and promotes Treg differentiation by positively regulating expression of FoxP3 itself, the Treg-specific transcription factor. miR-21 also has the potential to positively regulate Treg activity by directly targeting a proposed negative regulator of TGF-β signaling, SMAD7 (14,15). In contrast, other studies have suggested that miR-21 in fact restrains Treg activity through intrinsic T-reg-specific mechanisms and limiting FOXP3 activity (16) or indirectly by promoting the activity of Th17 cells, whose pro-inflammatory nature counters Treg function (14). With this in mind, Li and colleagues found that the decrease in TGF-β and FoxP3 expression in PBMC from diseased patients was consistent with a decrease in mRNA expression of the miR-21 target *SMAD7*. While this suggests the increase in PBMC miR-21 has functional consequences by reducing the expression of a known miR-21 target, it also suggests the net anti-inflammatory effects of this on T-reg numbers and activity may proceed through an alternative mechanism.

Although it is likely that miR-21 controls Treg activity through repression of alternative target mRNAs, it is also possible that during atherosclerosis progression, the increase in PBMC miR-21 which alters Treg activity, may occur in other cell types which drive suppression of Tregs. In particular, the role of inflammatory monocytes, known to increase during hyperlipidemia (17), or Th17 cells, known to negatively regulate Treg activity through miR-21 in other contexts (14), could mediate the effects here. Indeed, the cells expressing induced-miR-21 in disease remain unidentified and although Treg numbers decrease with vascular disease, the possibility exists that the remaining cells enhance miR-21 to mediate suppressive effects. Although the current study did not examine miR-21 in Treg in atherosclerotic plaques, miR-21 has been reported to be up-regulated in human plaques (18) and differences in local and peripheral regulatory T-cell function may exist. The authors highlight a previous study which demonstrated that Tregs derived from umbilical cord blood (13), which represented a heterogenous population distinct from those of circulating PBMC, also express miR-21 to promote Treg activity through FoxP3.

Examining other autoimmune diseases reveals interesting differences relating to circulating miR-21 levels and immune cell function. For example, rheumatoid arthritis patients, characterized by chronic inflammation in synovial joints, display decreased serum miR-21 levels (15). This is associated with decreased Treg function and an increase in the number and activity of the Th17 subset, promoting chronic inflammation. In this particular situation, loss of miR-21 allowed expression of another predicted target, *STAT3*, which acts as a Th17-specific transcription factor. Again, the cells in which miR-21 expression is lost in during disease are not clear, although here it is likely that Th17 polarization is promoted by an increase in *STAT3* expression in T-cell precursors. This has the net effect of limiting Treg activity. In another model of autoimmune disease, the EAE model of multiple sclerosis, targeting miR-21 using antisense was shown to block Th17 mediated inflammation leading to reduced disease burden (14). In mouse models of SLE, both targeting of miR-21 by antisense and miR-21 deletion decreased Th17 cells with a consequent increase in Tregs, conferring protection against disease (19,20). These results confirm that the miR-21/Treg axis is active in disease and confirmation of the results in the Li *et al.* study using similar tools for vascular disease are forthcoming. In particular, identifying the mRNA targets specific to each disease through which miR-21 has its effects on immune cell function will add to the complexity behind control of T-cell fate by this important miRNA and will allow more specific targeting for improved therapies.

In summary, the study by Li *et al.* provides primary patient data highlighting an important role for miR-21 in the pathogenesis of vascular disease. Notably, as disease progresses there is a loss of an important immunoregulatory T-cell subset in circulation which is driven by miR-21 and identifying the cellular and molecular mechanisms controlling this will illuminate our knowledge of T-cell function, tolerance and inflammation in atherosclerosis. In particular, identifying the signals, such as lipids or lipid-induced inflammatory mediators, which drive miR-21 expression during disease progression as well as identifying the overexpressing subsets in PBMCs and correlating these observed differences with local immune cells in the plaque, will improve our understanding of miR-21 function in immunity.

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Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Hackett EE, Sheedy FJ. miR-21 alters circulating Treg function in vascular disease—hope for restoring immunoregulatory responses in atherosclerosis? Ann Transl Med 2017;5(1):21. doi: 10.21037/atm.2016.12.72

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