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Commentary

Antiproliferative miR-212-5p: Promising RNA therapy for pulmonary hypertension

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In a recent paper published in *Molecular Therapy – Nucleic Acids*, Chen et al.[1] report that miR-212-5p was consistently and selectively upregulated in pulmonary arterial smooth muscle cells (PASMCs) of animal models and patients with pulmonary arterial hypertension (PAH). Interestingly, this microRNA (miRNA) inhibited PASMC proliferation *in vitro* and *in vivo*. The authors showed that exogenous microRNA-212-5p attenuated the induced PAH in two different rodent models. These findings suggest that exogenous administration of miR-212-5p may emerge as a potential therapy for PAH.

PAH is a malignant, progressive, and incurable disorder characterized by narrowing of the pulmonary arteries (PAs) leading to a rise in the pulmonary vascular resistance, right ventricle (RV) hypertrophy, RV failure, and eventually death.[2] It is widely recognized that a key piece in the PAH pathogenesis is the switch of PASMCs and endothelial cells (PAECs) to a proliferative phenotype, an epigenetic reprogramming leading to vascular obliteration. Residual vasoconstriction and thrombosis may also play an additional role. Because the current drugs addressing these latter components are only partially effective in reducing the symptoms and the progression of the disease, antiproliferative therapies are much needed.

miRNAs are epigenetic modulators that downregulate specific target genes and can interfere with multiple physiologic and pathologic cell functions, including cell survival, proliferation, and differentiation. miRNA-based therapies are very attractive due to the reduce nucleic acid size, cell-specific targetability, and role in pathophysiology.[3] Several strategies have been developed either to mimic or to inhibit miRNAs. Notably, such therapies have succeeded in preclinical studies, and some have even advanced into clinical phases, but there is still a need to progress our understanding as to reach effective licensed drugs. Recently, several studies have explored miRNAs in PAH, focusing both on their possible role as circulating biomarkers as well as their pathophysiological role in the disease. Thus, upregulated expression of deleterious miRNAs or downregulated expression of protective miRNAs has been reported to play an important role in PAH pathogenesis and progression. This also has therapeutic implications. As with other diseases, upregulated harmful miRNAs can be targeted with miRNA inhibitors, and, conversely, downregulated beneficial miRNAs can be rescued with miRNA mimics. In contrast, the study of Chen et al. shows that despite the fact that miR-212-5p was upregulated in PAH, it exerted a beneficial effect on disease progression. The authors interpreted their results as miR-212-5p being a compensatory protective response of the organism. This was confirmed using strain smooth muscle-specific miR-212-5p knockout mice. Thus, the removal of this protective mechanism significantly exacerbated the hypoxia-induced elevation of RV systolic pressure (a surrogate measure of PA pressure), RV hypertrophy, and PA wall thickening. Moreover, while the endogenous induction of miR-212-5p is insufficient to completely reverse PAH, supplementary exogenous miRNA can further reduce the hypertensive response in a rat model of severe PAH induced by hypoxia plus SU5416, an inhibitor of the VEGF receptor. In this model, the miRNA was given once PAH had developed, in a curative rather than a preventive approach, as it is desirable to be used in the clinical arena because patients are often diagnosed at advanced stages of the disease.

It is interesting to note that the expression of miR-212-5p determines the proliferative status of PASMC in culture, i.e., its inhibition induced proliferation, and, conversely, exogenous supplementation suppressed it, both in normoxic and hypoxic conditions. Under hypoxic conditions *in vivo,* inhibition and stimulation of the miRNA also produced stimulatory and inhibitory effects, respectively, on proliferation. It is tempting to speculate that a reduced expression of miR-212-5p may predispose individuals to develop PAH when exposed to hypertensive stimuli. In fact, none of the known factors involved in the development of PAH, including genetic mutations, drugs, connective tissue diseases, portal hypertension, HIV, or *Schistosoma* infections, is able, on its own, to induce PAH. An additional unknown stimulus, widely known as "second hit," is required. Whether basal expression of miR-212-5p may constitute a second hit deserves further study. Similarly, the different ability to overexpress the miRNA among patients with PAH might determine the severity and rate of progression of the disease.

The main weakness of Chen's study is that the molecular mechanism of the antiproliferative effect was not addressed. Future studies must identify the key mRNAs targeted by miR-212-5p and the affected signaling pathways.

One of the main limitations of the nucleic acid-based therapies is the inadequate organ

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distribution of the compounds, with incomplete delivery to the target tissue and/or access to off-target tissues. Chen et al. have explored different modes of administration of the microRNA, including intratracheal administration of adenoviral particles expressing miR-212-5p, intravenous administration of a miR-212-5p commercial mimic, and intratracheal endothelial-derived extracellular vesicles with an enriched miR-212-5p load. Although direct comparisons in the same animal model were not carried, the latter strategy seems to be the most promising. Extracellular vesicles were collected from the conditioned medium of ECs infected with lentiviral particles expressing the miR-212-5p sequence. In the mice model, the distribution of the vesicles was monitored after intratracheal administration, and the authors found them within the pulmonary vessel wall and in the airways 1 and 3 h after delivery, but they could not be detected at 24 h. In the rat model after 3 weeks of weekly administration of the vesicles, miR-212-5p expression was selectively increased in the lungs avoiding off-target delivery in other organs, and this was accompanied by reduced RV systolic pressure, RV hypertrophy, and PA wall thickness. The increase in expression remaining after 1 week was modest (∼1.3-fold) and it remains to be determined whether an optimized dosage regimen can improve the results.

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AUTHOR CONTRIBUTIONS

F.P.-V. and G.M.-P. have jointly conceived and written this article.

DECLARATION OF INTERESTS

The authors declare no competing interest.

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