MICRORNA-25 UPREGULATION PROTECTS SPINAL CORDS, YET IS BAD FOR THE HEART: THE DARK SIDE OF NONCODING RNAS

To the Editor:

We read with great interest the article published by Zhao and colleagues,[1] which reported that neuroprotection of ischemic spinal cords can be enhanced by exosomes overexpressing microRNA (miRNA) 25 (*miR-25*). Although a growing number of novel therapies have been developed for the spinal cord damage, a trend toward the discovery of noncoding components of the genome including miRNA can significantly contribute to the clinical medicine and this study served as a prime example. Despite their promise however, the fact remains that miRNA-based therapeutics have yet to enter the clinic. With growing numbers of studies in this category we anticipate that this critical milestone will be reached in the near future.

Insights into the roles of miRNAs in development of neurologic and cardiovascular diseases have made miRNAs attractive tools and targets for novel therapeutic approaches. MiRNA mechanisms of action involve the regulation of gene expression by inhibiting protein translation or by triggering cleavage of the target messenger RNA involved in disease processes.[2] *MiR-25*, a member of the *miR-106b-25* cluster, has been reported to be involved in essential cellular processes and located within intron 13 of the minichromosome maintenance protein 7 (*MCM7*) gene on chromosome 7q22.1. Because *miR-106b-25* is usually expressed in terminally differentiated cells, they tend to accumulate in less differentiated and more specifically proliferating cells. Therefore, miRNAs may represent ideal targets for gene and cell therapy addressing critical disease processes involving the regulation of proliferation.

In our previous studies in the heart, we found that *miR-25* delayed calcium uptake in cardiomyocytes and up-regulated in heart failure, both in mice and humans. Adeno-associated virus serotype 9–mediated overexpression of *miR-25* resulted in a significant loss of the heart's contractile function. Injection of an antisense oligonucleotide against *miR-25*, however, dramatically halted established heart failure, improving cardiac function and overall survival in various animal models.[3,4]

It is important to note that the manipulation of miRNAs too aggressively in either direction can cause controversial results. Expressional change of *miR-25* seems to act as a double-edged sword specific to different target messenger RNAs. Accumulating evidence has shown that *miR-25* regulates different biologic processes and could result in various diseases sharing the same pathologic mechanisms. An imbalance of *miR-25* could trigger off-target side effects in remote healthy tissues. For example, Zhao and colleagues[1] demonstrated as a positive effect that *miR-25*–enriched exosomes inhibited expression of the gene for reduced nicotinamide adenine dinucleotide phosphate oxidase 4, *Nox4*. However, *Nox4*-null animals had development of exaggerated contractile dysfunction, hypertrophy, and cardiac dilatation, whereas *Nox4*-transgenic mice were protected.[5] In another example, Li and associates[6] recently reported that overexpression of *miR-25* in normal mice causes heart hypertrophy, cardiomyocyte fibrosis, and cell apoptosis.[6] Moreover, many research studies have demonstrated that the upregulation of *miR-25* potentiates cellular transformation. These deleterious effects are associated with the progression of several malignant solid tumors in human and animal models.

Therefore, although miRNA-targeting biology has advanced rapidly in recent times, caution in therapeutic strategy specific to the disease processes must be considered in the context of application. Preclinical efficacy studies with *miR-25* have proved it to be effective in targeting different pathologic processes; however some hurdles, including safety, evaluation of expression level in the different tissues, and also limiting off-target side-effects, remain to be overcome for successful clinical application.

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References

1. Zhao L, Jiang X, Shi J, Gao S, Zhu Y, Gu T, et al. Exosomes derived from bone marrow mesenchymal stem cells overexpressing microRNA-25 protect spinal cords against transient ischemia. *J Thorac Cardiovasc Surg*. 2019;157:508-17.
2. Katz MG, Fargnoli AS, Kendle AP, Hajjar RJ, Bridges CR. The role of microRNAs in cardiac development and regenerative capacity. *Am J Physiol Heart Circ Physiol*. 2016;310:H528-41.

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3. Jeong D, Yoo J, Lee P, Kepreotis SV, Lee A, Wahlquist C, et al. MiR-25 tough decoy enhances cardiac function in heart failure. *Mol Ther*. 2018;26:718-29.
4. Wahlquist C, Jeong D, Rojas-Muñoz A, Kho C, Lee A, Mitsuyama S, et al. Inhibition of miR-25 improves cardiac contractility in the failing heart. *Nature*. 2014;508:531-5.
5. Zhang M, Brewer AC, Schröder K, Santos CX, Grieve DJ, Wang M, et al. NADPH oxidase-4 mediates protection against chronic load induced stress in mouse hearts by enhancing angiogenesis. *Proc Natl Acad Sci U S A*. 2010;107:18121-6.
6. Li H, Xie Y, Liu Y, Qi Y, Tang C, Li X, et al. Alteration in microRNA-25 expression regulate cardiac function via renin secretion. *Exp Cell Res*. 2018; 365:119-28.

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REPLY: A "SIMPLE" WAY FOR ENHANCED EXOSOMES TO ALTER GENE EXPRESSION: SIMPLE BUT WRONG–COMPLEX BUT RIGHT

Reply to the Editor:

Exosomes are a subtype of transport vesicles that are released by almost all cell types and are present in most if not all biologic fluids. Exosomes carry biologically active cargo, especially microRNA (miRNA), and can alter the phenotype of recipient cells. After the Nobel Prize in Physiology or Medicine in 2013 was awarded to 3 groups of investigators for their work on defining how cells communicate with exosomes, there was understandable enthusiasm regarding the possibility of manipulating cellular function with synthetically enhanced exosomes. The combination of work done by these investigators identified 3 biological principles about how cells communicate through the cellular machinery of exosomes. These Nobel laureates were recognized for (1) identifying a set of genes that are required for microvesicle (ie, exosome) creation and transport, (2) defining how exosomes delivered their cargoes to the right places, and (3) discovering the cellular and extracellular machinery that controls exosome release of chemical messengers.

This exceptional work spurred an enormous interest in how cells communicate. Perhaps the most exciting aspect of potential importance is the use of enhanced exosomes. Enhanced exosomes are man-made exosomes that carry a specific miRNA that has a particular site of action and a specific cellular response of interest. The use of enhance exosomes to alter cellular function seemed like a simple means of fixing or altering cellular function; however, as so often seems to be the case in complex cellular systems, simple is wrong and complex is right.

Previous articles in the *Journal* have highlighted some potential areas of interest in which enhanced exosomes might prove useful in modifying cellular function in spinal cord ischemia. These preliminary studies identified a specific miRNA, identified as miR-25, that showed some exciting potential limitations of spinal cord ischemia with enhanced exosomes containing miR-25.[1] This very preliminary report suggested some exciting possibilities, but accompanying editorials and previous *Journal* articles added appropriate caution about how unintended consequences associated with enhanced exosomes might modify cellular function to provide adverse outcomes.[2,3]

In the current issue of the *Journal*, yet another cautionary note surfaces in the world of intracellular communication surrounding exosomes. A letter to the Editor by Katz and colleagues[4] raises the issue of unintended consequences of enhanced exosome-guided cellular interventions. In their letter, Katz and colleagues[4] identify potential adverse effects of miR-25–enhanced exosomes that might alter cardiac function. Katz and colleagues[4] suggest that miR-25–enhanced exosomes might cause cardiomyocyte fibrosis and cell apoptosis. They point out that other research suggests that miR-25–enhanced exosomes can trigger cellular transformations that could lead to progression of several human and animal model malignant solid tumors.

There is little doubt that the exciting possibilities attributed to enhanced exosomes must be tempered by some as yet not fully understood harsh realities. There are multiple uncertainties associated with exosomes, not the least of which is exactly how best to create, stabilize, and harvest exosomes for analysis and ultimately for intervention in experimental models.[5,6] Perhaps the most important concern revolves around unintended consequences of enhanced miRNAs carried by exosomes. Exosomes can generate a protumor environment that is essential for tumorigenesis.[6] It is uncertain that modification of exosomes to overexpress an miRNA of interest will have only a very specific influence; rather, they may in fact promote undesirable metabolic pathways with adverse outcomes. Much more work needs to be done before a clear-cut, reproducible enhanced exosomes containing therapeutic miRNAs can be used in human disease, but that does not mean that we should stop doing research in this very intriguing area. More work needs to be done and we should not be surprised when simple systems prove to be wrong and complex systems are the correct path to follow.