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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 colleagues⁴ raises the issue of unintended consequences of enhanced exosome-guided cellular interventions. In their letter, Katz and colleagues⁴ identify potential adverse effects of miR-25-enhanced exosomes that might alter cardiac function. Katz and colleagues⁴ 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.

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Victor A. Ferraris, MD, PhD
Tyler Gill Professor of Surgery
University of Kentucky
Lexington, Ky

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REPLY: MICRORNA-BASED SPINAL CORD PROTECTION: THE POSSIBLE SIDE EFFECT CANNOT BE NEGLECTED

Reply to the Editor:

MicroRNAs (miRNAs) are short, single-stranded, noncoding RNAs that modulate the expression of target proteins by annealing to complementary sequences in their target messenger RNAs (mRNAs). Because individual miRNAs often target numerous related mRNAs, the modulation of a single miRNA can have multiple biologic functions through regulation of different target proteins in different tissues or cells in different physiologic or pathophysiologic processes. Although miRNA-targeted modifications are becoming an attractive tool in preclinical study, they still have not entered the clinic.

In my group's recent report,¹ the miRNA *miR-25* was found to be upregulated in spinal cords by exosomes derived from mesenchymal stem cells genetically modified to overexpress *miR-25* and induced neuroprotective effects against transient ischemia, possibly by inhibition of reduced

nicotinamide adenine dinucleotide phosphate oxidase 4. Although the neuroprotection of *miR-25* was highlighted in our report, the side effects of *miR-25* in the central nervous system as well as other tissues were not fully investigated. Some effects are known; *miR-25* functions as an oncogenic miRNA, playing crucial roles in the development of many tumor types, including brain tumors.² The potential tumorigenic effects on the nervous system may be a side effect not to be ignored when upregulation of *miR-25* is used to induce neuroprotection. Furthermore, the function of *miR-25* in other organs or tissues besides the nervous system should also be considered. I thank Katz and colleagues for their insightful comments on the important role of *miR-25* in the myocardium. Inhibition of *miR-25* has been indicated to be a clinical candidate for therapy of heart failure through myocyte calcium ion handling.³ In this light, upregulation of *miR-25* in myocardium would result in cardiac risks. However, *miR-25* has also been reported to protect cardiomyocytes against oxidative damage by targeting the mitochondrial calcium uniporter.⁴ Expressional change of *miR-25* seems to act as a double-edged sword specific to different target mRNAs under different pathophysiologic processes, even in the same tissue. In fact, the application of miRNA-targeted therapy may be very complex because of the multiple functions of a single miRNA achieved by targeting different mRNAs. When one role of any given miRNA by targeting a special protein is underlined, the other functions of the miRNA by targeting other proteins would turn into side effects, which should be avoided. In our recent study,¹ the exosomes containing elevated *miR-25* were administered by intrathecal injection, not intravenous injection, which may concentrate the *miR-25* within spinal cords and to a certain extent avoid the distribution with the blood flow of *miR-25* to other organs, such as the heart. Because the exosome can penetrate the blood brain barrier, however, the *miR-25* expression in organs beyond the spinal cord cannot be avoided completely by the intrathecal injection, and this expression should be evaluated accurately in further investigations. Before moving forward with *miR-25* as a clinical candidate for spinal cord protection, it will be important to learn about the expression levels and functions of *miR-25* outside the spinal cord and its effects not related to alleviation of oxidative stress. Our recent study¹ is just a preliminary animal experiment, and there is still a long way to go before the possible clinical application of *miR-25* for spinal cord protection.

Enyi Shi, MD, PhD
Department of Cardiac Surgery
First Affiliated Hospital
China Medical University
Shenyang, China