MicroRNA Passenger Strand
Orchestral Symphony of Paracrine Signaling

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Study Hypothesis
During the past few years, increasing studies have identified microRNAs to be present in the circulation, either encapsulated in microvesicles or exosomes or associated with RNA-binding proteins or lipoproteins. Recent studies have suggested that circulating microRNAs may function in cell–cell communication, being transported from one cell type to another and regulating target gene expression in recipient cells. Although it has been generally thought that the passenger strand of the microRNA duplex is degraded during microRNA biogenesis and only the guide strand of the microRNA duplex is selected to become the mature functional microRNA, there is mounting evidence that passenger strand microRNAs can also target miRNAs and have biological functions in pathologies such as cancer. In the current study, Bang et al. present evidence that exosomes produced by cardiac fibroblasts contain passenger strand microRNAs, which are transferred to cardiomyocytes and play a role in the development of fibroblast-derived cardiomyocyte hypertrophy, revealing a novel method of paracrine communication between cardiac fibroblasts and cardiomyocytes.

How Was the Hypothesis Tested?
The authors used electron microscopy to demonstrate the ability of neonatal rat cardiac fibroblasts to produce and secrete exosomes (fibroblast-derived exosomes). They further confirmed the identity of the exosomes by performing Western blotting and fluorescence-activated cell sorting analyses for the presence of an exosomal marker protein. To assess the microRNA content of fibroblast-derived exosomes, the authors used a microRNA profiling assay and, in particular, detected the presence of the passenger strand of microRNA-21 (miR-21*). Expression of miR-21* in fibroblast-derived exosomes was confirmed by quantitative real time-polymerase chain reaction and RNA sequencing. Using confocal microscopy and coculture assays, the authors investigated the ability of fibroblast-derived exosomes and microRNAs to be transported to and taken up into cardiomyocytes. Moreover, they tested whether exosome-derived miR-21* modulated cardiomyocyte cell size by incubating cardiomyocytes with exosomes isolated from the conditioned medium of fibroblasts that were transfected with a precursor of miR-21* (pre–miR-21*). To further study the role of miR-21* in cardiomyocytes, the authors performed proteome profiling in cardiomyocytes transfected with pre–miR-21* or a control microRNA to identify potential targets that are regulated by miR-21*. Proteome profiling analysis revealed that sorbin and SH3 domain-containing protein 2 (SORBS2) was strongly downregulated and PDZ and LIM domain 5 (PDLIM5), which had been previously implicated in cardiomyopathy, was also among the downregulated targets. The authors examined whether SORBS2 and PDLIM5 play a role in cardiomyocyte hypertrophy by using small interfering RNAs to knockdown either SORBS2 or PDLIM5.

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in cardiomyocytes. Finally, the authors induced a prohypertrophic condition in C57BL/6N mice by implanting osmotic angiotensin II–containing minipumps and then injected the mice with a cholesterol-modified miR-21* antagonist (miR-21* antagomir) or a scrambled control antagomir to determine the importance of miR-21* for cardiac hypertrophy in vivo.

Principal Findings
Electron microscopic analysis revealed the presence of multivesicular bodies, structures which are formed during exosome biogenesis, in the cytoplasm of neonatal rat cardiac fibroblasts. The multivesicular bodies were found to fuse with the plasma membranes of cardiac fibroblasts, resulting in the release of exosomes into the extracellular fluid. The authors confirmed the identity of the exosomes by performing Western blotting and fluorescence-activated cell sorting analyses to detect the presence of the exosomal marker protein CD63. Using a microRNA profiling assay, the authors compared the expression of microRNAs from fibroblast-derived exosomes and fibroblast cells and identified 50 microRNAs to be enriched in fibroblast-derived exosomes. Of these 50 microRNAs, 26% were passenger strand microRNAs. Previous studies have reported that miR-21 is an important player in fibroblast biology. Interestingly, the authors found that miR-21* was among the passenger strand microRNAs that were enriched in fibroblast-derived exosomes. Both quantitative real time-polymerase chain reaction and RNA sequencing confirmed that miR-21* was enriched in fibroblast-derived exosomes, whereas miR-21 is enriched in cardiac fibroblasts.

The authors showed that incubating cardiomyocytes with fluorescently labeled exosomes derived from cardiac fibroblasts resulted in the internalization of the labeled exosomes into the cytoplasm of the cardiomyocytes, as analyzed by confocal microscopy. In addition, confocal microscopic analysis of cardiomyocytes cocultured with cardiac fibroblasts transfected with a fluorescently labeled precursor microRNA revealed the presence of the labeled precursor microRNA in the cardiomyocytes. Next, the authors found an enrichment of miR-21* expression in cardiomyocytes cocultured with cardiac fibroblasts that were transfected with pre–miR-21*. Moreover, the authors observed that cardiomyocytes incubated with exosomes isolated from the conditioned medium of fibroblasts transfected with pre–miR-21* exhibited an increase in cell size. Transfection of cardiomyocytes with pre–miR-21* resulted in an increase in cardiomyocyte cell size, whereas the opposite was seen when cardiomyocytes were transfected with an inhibitor of miR-21*. Taken together, these findings suggest that fibroblast-derived exosomes containing miR-21* can be secreted from cardiac fibroblasts and taken up into cardiomyocytes, leading to the induction of cardiomyocyte hypertrophy.

Proteome profiling analysis of cardiomyocytes transfected with pre–miR-21* or a control microRNA revealed that SORBS2 and PDLIM5 are downregulated after transfection of miR-21* and thus are candidate targets of miR-21* in cardiomyocytes. Transfection of cardiomyocytes with pre–miR-21* reduced the protein levels of SORBS2 and PDLIM5. Incubation of cardiomyocytes with miR-21*-transfected fibroblast exosomes also resulted in decreased mRNA levels of SORBS2 and PDLIM5 in cardiomyocytes. Of note, SORBS2 has been reported to be downregulated during cardiac pathologies and mice with a cardiomyocyte-specific deficiency of PDLIM5 develop cardiomyopathy, suggesting a potential role for these proteins in mediating the hypertrophic effects of miR-21* in cardiomyocytes. The authors showed that cardiomyocytes transfected with small interfering RNAs directed against SORBS2 or PDLIM5 exhibited an increase in cardiomyocyte cell size, supporting a role for SORBS2 and PDLIM5 in cardiomyocyte hypertrophy. Interestingly, the authors found that the levels of miR-21* were increased in pericardial fluid of C57BL/6N mice with transverse aortic constriction–induced cardiac hypertrophy compared with that of sham-operated mice. Furthermore, administration of miR-21* antagomir to mice with angiotensin II–induced cardiac hypertrophy resulted in reduced heart/body weight ratio and decreased cardiomyocyte diameter compared with mice with angiotensin II–induced cardiac hypertrophy treated with a scrambled control antagomir. Taken together, these results provide support for a model in which miR-21*-enriched exosomes are secreted from cardiac fibroblasts and transported to cardiomyocytes, where exosome-derived miR-21* negatively regulates the expression levels of SORBS2 and PDLIM5 to induce a cardiac hypertrophic response.

Implications
This study uncovers a novel exosome-mediated paracrine mechanism by which cardiac fibroblasts communicate with cardiomyocytes to induce cardiac hypertrophy. The finding that passenger strand microRNAs, specifically miR-21*, are present in fibroblast-derived exosomes and can modulate target gene expression in recipient cardiomyocytes further demonstrates that passenger strand microRNAs are not passive bystanders and do have biological function, particularly in the circulation acting as key regulators in paracrine signaling networks involved in cardiac hypertrophy. Strategies designed to inhibit the function of miR-21* may be an innovative microRNA-based therapeutic approach in the treatment of cardiac hypertrophy and heart failure.

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Disclosures
None.

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