# Pericardial Fluid Exosomes: A New Material to Treat Cardiovascular Disease

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Recent studies using exosomes as a therapeutic platform for the treatment of vascular diseases have led to very encouraging responses.[1–3] To date, exosomes secreted by stem and progenitor cells in the myocardium have shown promising therapeutic benefits, although their precise cellular origin and mechanism(s) of action remain poorly characterized. In this issue of *Molecular Therapy*, Beltrami et al.[4] report that exosomes present in the pericardial fluid (PF) of patients undergoing aortic valve replacement (AVR) are enriched with microRNAs (miRNAs) co-expressed in patients' myocardium and vasculature. The authors further report that PF exosomes enhanced angiogenesis in cultured endothelial cells, improved blood flow recovery and capillary density, and reduced necrosis in mouse models of unilateral limb ischemia. The Beltrami et al.[4] study presents molecular evidence by studying one of the well-known let-7 family of miRNAs, miR-let-7b-5p, which is present at high levels within PF exosomes. The authors demonstrate that miR-let-7b-5p partially mediates the angiogenic potential of PF exosomes, which supports the conclusion that PF exosomes may have therapeutic potential for the treatment of vascular diseases.

The heart is the first functional organ to develop in the vertebrate embryo. During early developmental stages, it is partitioned into four distinct chambers forming two atria and two ventricles. A tough "double-walled sac" called pericardium encompasses the entire heart and the roots of the great vessels (ascending aorta, superior and inferior vena cava, pulmonary arteries, and pulmonary veins).[5] Pericardium protects the heart by preventing aberrant motion, excessive dilation due to acute volume overload, and the spread of infections from other organs. The pericardial sac comprises inner and outer continuous layers, between which is found a viscous fluid called pericardial fluid. Pericardial fluid prevents friction between the two layers of the pericardial sac and also serves as a shock absorbent to the heart.

Heart development and function is governed by coordinated expression of miRNAs, which are ~22 nt-long short RNAs that regulate gene expression by post-transcriptional silencing of miRNAs.[6] Differentiation and development of cardiac lineages are marked by specific expression of cardiomiRs, including miR-1, miR-133, and miR-208. The importance of miRNAs in normal cardiac function and under pathological conditions was elegantly demonstrated by cardiac-specific deletion of the miRNA-processing enzyme, Dicer, which resulted in rapid progression of dilated cardiomyopathy, heart failure (HF), and postnatal lethality in Dicer mutant mice.[7] In HF, aberrant expression of cardiac miRNAs is marked by maladaptive changes to gene expression programs, ultimately affecting calcium and sarcomeric function.[8–10] Recent studies have uncovered exosomes, which are 30–150 nm-sized extracellular vesicles, as carriers of functional miRNAs and proteins for exchange between cells. Specifically, cardiac progenitor or stem-cell-derived exosomes are gaining significant attention for the clinical treatment of heart disease due to their remarkable ability to induce ischemic tissue repair and regeneration. While the underlying mechanisms are not completely understood, the beneficial effects of certain stem-cell-derived exosomes are, at least in part, mediated by promoting angiogenesis.[11–13] One of the current challenges is to identify the precise roles of exosomes based on their cellular origin within the heart and to delineate whether exosomes with different cellular origin have distinct angiogenic potential.

Beltrami et al[4]. addressed one such challenge by investigating PF obtained from patients undergoing AVR surgery. They demonstrated that PF is enriched with miRNAs expressed in the heart and thoracic vasculature. Specifically, by subjecting PF exosomes to proteinase K and RNase A digestion, validated at least 16 bona fide cardiovascular miRNAs that are present at high levels within PF exosomes. To date, very little evidence has been presented showing exosomes from PF as regulators of endothelial biology and vasculature. This study is one of the very few that highlighted PF as a stimulator of therapeutic angiogenesis via direct exchange of exosomal miRNAs. Using mouse models of unilateral limb ischemia, the authors further demonstrated that PF exosomes significantly restored limb blood-flow recovery and toe survival. To gain molecular insights, they examined whether one of the highly enriched let-7 family of miRNAs, let-7b-5p, could mediate the beneficial effects of PF exosomes. By using a miRNA mimic and inhibitor assays, the authors show that let-7b-5p, at least in part, mediates the beneficial angiogenic activity of PF exosomes. Interestingly, let-7b-5p promoted angiogenesis by post-transcriptionally silencing a miRNA that encodes TGFBR1, which is an anti-angiogenic protein.[14] This study adds significant knowledge to the existing literature pertaining to cardioprotective roles of exosomes and exosomal miRNAs, with particular attention paid to PF as a novel therapeutic material.

In addition, this study has significant relevance to the clinical setting because of the utilization of human PF material. It presents one of the earliest insights into the miRNA

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composition of PF exosomes. Pericardium is well known for its fortified content, which is typically enriched with proangiogenic paracrine effectors secreted by cardiac cells.[15] The consensus is that pericardium reflects immediate changes occurring in the molecular environment surrounding the post-ischemic myocardium. Moreover, PF isolated from HF patients is enriched with growth factors and other factors that may not be detected in the blood plasma of HF patients. The present study underscores that several cardiovascular miRNAs are present at high levels in PF and are often not detected in patients' plasma. It has been shown in earlier studies that the PF total microRNA profile (exosomal plus non-exosomal) represents an active secretory process, including several known cardiac exosomal and cardiac fibroblast-derived exosomal miRNAs.[16] However, these miRNAs did not show significant variation across early and advanced stages of HF. This observation is particularly unexpected, because several known proangiogenic proteins were found to be increased in the PF of patients with myocardial ischemia.[15] This may suggest that changes in PF may be an early event in the pathology of HF and that HF changes are reflected early in the PF long before a clinical diagnosis. A thorough analysis of PF exosome-derived miRNAs between control and disease populations is needed to understand disease progression and to exploit the PF exosomes in regenerative medicine. In patients, PF is accessible through invasive techniques and, as such, can be a conduit for exosomes in a variety of cardiovascular diseases.

The study by Beltrami et al.[4] presents evidence for the complexity of the cargo carried by patient-derived PF exosomes. Nevertheless, the authors do not completely address the supplemental role of exosome-free PF in angiogenesis. To associate the functional data of exosome-free PF with the molecular composition and to address whether the beneficial proangiogenic (miRNA and protein) components of PF are predominantly present in the exosomes and not in the non-vesicular component will have particular importance for clinical translation. Moreover, while there is increasing evidence for a strong role for exosomal miRNAs in promoting angiogenesis, any significant contributions made by exosomal proteins are often overlooked. Beltrami et al.[4] investigated in detail the miRNA content of PF exosomes, but determining the protein content and its role in promoting angiogenesis should strengthen the current understanding of PF-exosomes particularly toward clinical translation. This should be interesting, because the authors report that proangiogenic let-7b-5p is only partially responsible for mediating the observed angiogenic potential of PF exosomes. Nevertheless, this study has taken the initiative to determine the composition as well as the critical function of patient-derived PF exosomes. Future studies will be required to test the feasibility of PF exosomes (particularly cardiovascular patient-derived exosomes) in providing a therapeutic platform to treat vascular diseases.

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