Messages from the heart

Chantal Boulanger, Xavier Loyer, Pierre-Michael Coly, Nicolas Amabile

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EDITORIAL on:
Myocardial Hypoxic stress mediates functional extracellular vesicle release
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Chantal M. Boulanger[1], Xavier Loyer[1], Pierre-Michael Coly[1], Nicolas Amabile[1,2]
1 : Université de Paris, PARCC, INSERM UMR970, Paris, France
2 : Institut Mutualiste Montsouris, Paris, France

Corresponding address:
Chantal M Boulanger, PharmD PhD,
Paris Cardiovascular Research Center,
Inserm UMR 970,
56 rue Leblanc, 75737 Paris

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For more than two decades, extracellular vesicles (EVs) have increasingly stimulated the interest of cardiovascular scientists as biological effectors and biomarkers of disease progression. Circulating levels of human procoagulant, endothelial or platelet-derived vesicles increase within hours after the onset of myocardial ischemia and monitoring their levels improves predictions of major adverse cardiovascular events (1–3). Ischemia-reperfusion injury in mice also rapidly augments both circulating EV numbers and their content in cardiac and muscle microRNAs (4,5). However, all efforts to identify specific cardiac EV protein markers in the plasma of patients with heart disease have remained in vain, despite evidence for the presence of EVs in infarcted heart tissue itself (6,7).

In this issue of European Heart Journal, Anselmo and his colleagues (8) reveal that the human myocardium continuously releases EVs in the circulating blood and that several cardiac diseases, involving a degree of tissular ischemia, further amplify the phenomenon. For this, they designed an elegant flow cytometry approach to identify the presence of SIRPα (CD172), a surface marker of cardiomyocyte differentiated from human pluripotent stem cells (iPSC)(9), while excluding from the analysis plasma EVs from other cellular origin that might also express this marker. The cardiomyocyte origin of isolated CD172+EVs was further confirmed by the presence of Troponin-T and their specific enrichment in cardiac microRNA. A gradient in CD172+EVs between the coronary sinus and the systemic circulation also supported this conclusion.

Anselmo *et al.* report that plasma CD172+EV levels increase in a large group of patients with aortic stenosis, but also in other patients with cardiovascular diseases, and that surgical aortic valve replacement significantly reduced the elevated CD172+EVs. Cellular hypoxia, a common condition involved in cardiac stress, may trigger cardiomyocyte EV release in patients, as it does from iPSC-derived cardiomyocyte in culture (Figure 1). However, we cannot exclude the possibility that impaired clearance, for example by the spleen or the liver (10), could result in high plasma levels of these vesicles in patients.

The lower levels of CD172+EVs found in some patients with aortic stenosis remain unexplained. These low concentrations could reflect an impaired vesiculation capacity, suggesting a decreased adaptative process related to the loss of cardiomyocytes that is observed in advanced cardiac diseases. Alternatively, they could result from an increased clearance or from the molecular interaction of CD172 with its CD47 receptor on circulating cells (11)(Figure 1).

Unexpectedly, Anselmo et al. observed that patients with the highest circulating levels of CD172+EVs at inclusion had a more favorable prognosis after trans-catheter aortic valve replacement; in this regard, this finding contrasts with the prognostic values of Troponin T and N-terminal prohormone of brain natriuretic peptide. However, the multiple Cox analysis demonstrated that increased cardiac CD172+EV levels were independently associated with greater survival after one year. These results certainly open new avenues for the development of prognostic biomarker application in patients with cardiac diseases, but the complex and time-consuming flow cytometry approach developed by Anselmo et al. may not be compatible with routine analysis in clinical labs, nor the study of very large cohorts of patients. Further analytical progress will no doubt be required.

In order to better understand the paradoxical prognostic value of high levels of CD172+EVs, the authors attempted to decipher the vesicles' possible functional effects on isolated adult murine cardiomyocytes (Figure 1). They demonstrated that CD172+EVs from hypoxic iPSC-derived cardiomyocytes increased both intracellular calcium levels and cell shortening in a concentration-dependent manner, whereas EVs from normoxic cardiomyocytes, or EVs of other cellular origin, were without effect. CD172+EVs isolated from the plasma of patients with aortic stenosis also increased the contraction of isolated cardiomyocytes. As the sphingolipid signature of circulating EVs is affected by myocardial ischemia (12), Anselmo *et al* identified ceramide enrichment in circulating CD172+EVs isolated from patients with aortic stenosis and demonstrated that both ceramides and CD172 were instrumental in mediating their positive inotropic effect *in vitro*. Whether or not this effect contributes to the better prognosis of patients remains unclear. Future studies evaluating the prognostic value of EV expressing both CD172 and ceramide would be helpful.

The paper by Anselmo *et al* is an important step forward in the field of cardiovascular extracellular vesicles and we are just beginning to elucidate the complex mechanisms of EV contribution to cardiovascular disease development. Future studies will be necessary to better understand how cardiac CD172+EVs are taken up or endocytosed by cardiomyocytes (13). The mechanism by which ceramide causes shortening of cardiomyocyte will also require additional investigations, in particular in view of the potential deleterious effects of this sphingolipid in cardiac cells (14). There is currently no evidence in Anselmo's study that plasma EVs cross the capillary barrier to increase the contractility of healthy cardiomyocytes; a direct paracrine effect of EVs generated in cardiac muscle is also a tempting hypothesis (Figure 1). Interestingly, cardiac EVs accumulate in myomectomy fragments of interventricular septum in patients with aortic stenosis (7) and could contribute to the positive

inotropic effect, in addition to regulating the local inflammatory responses. Cardiomyocytes may also release bioactive EVs that do not harbor CD172, as rightfully acknowledged by Anselmo *et al* (8). Furthermore, circulating EVs have other cardioprotective effects through the Heat shock protein/Toll like receptor-4 axis (5). Whether or not circulating EVs, in particular those originating from cardiomyocytes, actively take part in the crosstalk between organs remains unclear.

Future preclinical animal models allowing EV tracking in vivo are much needed to track cardiac EVs and finally demonstrate how the diseased part of the myocardium sends specific messages to the healthy part of the muscle in order to boost its contractile function. Certainly, the study by Anselmo *et al.* highlights extracellular vesicles released from the myocardium as previously unsuspected “spurs for a tired horse” (8).

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References:

1. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation. 2000 Feb 29;101(8):841–3.
2. Nozaki T, Sugiyama S, Koga H, Sugamura K, Ohba K, Matsuzawa Y, et al. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. J Am Coll Cardiol. 2009 Aug 11;54(7):601–8.
3. Sinning J-M, Losch J, Walenta K, Böhm M, Nickenig G, Werner N. Circulating CD31+/Annexin V+ microparticles correlate with cardiovascular outcomes. Eur Heart J. 2011 Aug;32(16):2034–41.
4. Deddens JC, Vrijsen KR, Colijn JM, Oerlemans MI, Metz CHG, van der Vlist EJ, et al. Circulating Extracellular Vesicles Contain miRNAs and are Released as Early Biomarkers for Cardiac Injury. J Cardiovasc Transl Res. 2016 Aug;9(4):291–301.
5. Vicencio JM, Yellon DM, Sivaraman V, Das D, Boi-Doku C, Arjun S, et al. Plasma exosomes protect the myocardium from ischemia-reperfusion injury. J Am Coll Cardiol. 2015 Apr 21;65(15):1525–36.
6. Sahoo S, Losordo DW. Exosomes and cardiac repair after myocardial infarction. Circ Res. 2014 Jan 17;114(2):333–44.
7. Loyer X, Zlatanova I, Devue C, Yin M, Howangyin K-Y, Klaihmon P, et al. Intra-Cardiac Release of Extracellular Vesicles Shapes Inflammation Following Myocardial Infarction. Circ Res. 2018 Jun 22;123(1):100–6.
8. Anselmo A, Frank D, Papa L, Anselmi CV, Di Pasquale E, Mazzola M, et al. Myocardial hypoxic stress mediates functional cardiac extracellular vesicle release. Eur Heart J. 2021 in press;
9. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, et al. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nat Biotechnol. 2011 Oct 23;29(11):1011–8.

10. Matsumoto A, Takahashi Y, Chang H, Wu Y, Yamamoto A, Ishihama Y, et al. Blood concentrations of small extracellular vesicles are determined by a balance between abundant secretion and rapid clearance. J Extracell Vesicles. 2020 Sep;9(1):1696517.
11. Seiffert M, Brossart P, Cant C, Cella M, Colonna M, Brugger W, et al. Signal-regulatory protein alpha (SIRPalpha) but not SIRPbeta is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34(+)CD38(-) hematopoietic cells. Blood. 2001 May 1;97(9):2741–9.
12. Burrello J, Biemmi V, Dei Cas M, Amongero M, Bolis S, Lazzarini E, et al. Sphingolipid composition of circulating extracellular vesicles after myocardial ischemia. Sci Rep. 2020 Sep 30;10(1):16182.
13. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles. 2014;3.
14. Russo SB, Baicu CF, Van Laer A, Geng T, Kasiganesan H, Zile MR, et al. Ceramide synthase 5 mediates lipid-induced autophagy and hypertrophy in cardiomyocytes. J Clin Invest. 2012 Nov;122(11):3919–30.