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EDITORIAL

Arrhythmogenic cardiomyopathy: the ongoing search for mechanism-driven therapies meets extracellular vesicles

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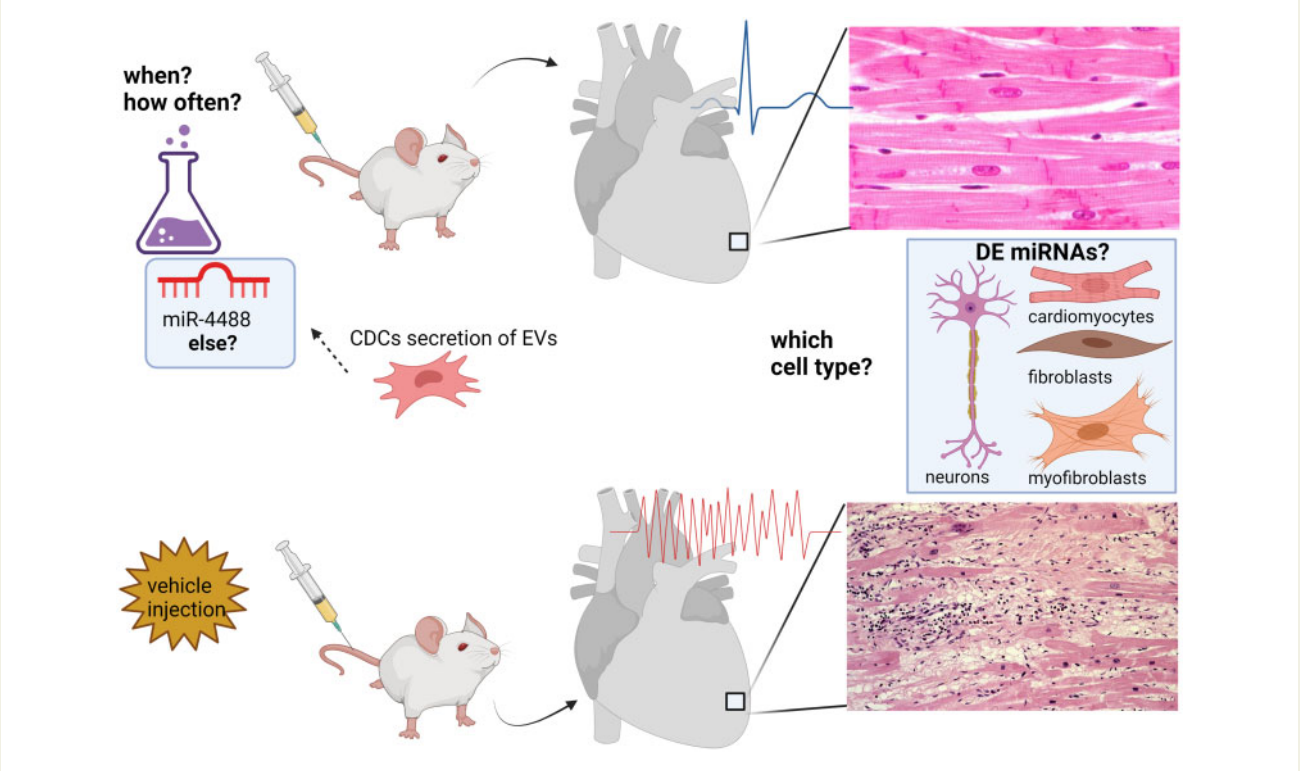
This editorial refers to 'Extracellular vesicles from immortalized cardiosphere-derived cells attenuate arrhythmogenic cardiomyopathy in desmoglein-2 mutant mice', by Y.N. Lin *et al.*, doi:10.1093/eurheartj/ehab419.



Graphical Abstract Curative therapy in AC: outstanding questions. Upper panel: delivery of CDC-derived EVs seems to attenuate AC in desmoglein-2 mutant mice, thus being proposed as a novel therapeutic option. However, there are many outstanding questions: what is the content of the 'magic potion' besides mir-4488? Is a single dose sufficient to achieve persistent beneficial responses and what is the duration of cardio-protective effects after completion of the EV treatment cycle? Which cell types are affected and what is their role in disease development? Bottom panel: delivery of placebo (vehicle injection) does not attenuate AC in desmoglein-2 mutant mice with onset of ventricular arrhythmias, massive necrosis, inflammatory response, and replacement-type fibrosis. CDCs, cardiosphere-derived cells; EVs, extracellular vesicles; DE, differential expression; AC, arrhythmogenic cardiomyopathy.

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Arrhythmogenic cardiomyopathy (AC) is nowadays a well-recognized inherited heart muscle disease, mostly due to mutations in genes encoding desmosomal proteins, predisposing to stress-related life-threatening ventricular arrhythmias, particularly in the young and in athletes.[1,2] The overt AC phenotype is characterized by loss of cardiac myocytes and fibro-fatty replacement of the ventricular myocardium. It is noteworthy that such a histological hallmark was initially thought to be the result of a congenital maldevelopment of the myocardium ('dysplasia').[2] Increasing awareness of the familial background, together with the discovery, in the last 20 years, of the causative genetic mutations, led to a change in understanding of the disease as progressive myocardial dystrophy ('cardiomyopathy'). The morphological changes of the ventricular myocardium are in fact dynamic, age-related, and typically start from the epicardium, with a wave-front extension toward the endocardium often becoming transmural, with wall thinning and aneurysm formation. Although the disease was originally described as predominantly affecting the right ventricle, variants with early or even dominant left ventricular involvement have been increasingly reported.[1]

Despite major achievements in the understanding of the disease aetiology (i.e. identification of novel disease-causing mutations), the mechanisms underlying AC pathogenesis remain incompletely understood. Several studies have implicated the canonical Wnt/β-catenin, as well as Hippo and transforming growth factor (TGF)-β pathways in fibro-fatty myocardial degeneration.[3,4] However, these mechanisms do not comprehensively explain the high phenotypic variability of AC. Inevitably, the knowledge gap on disease pathogenesis reflects the absence of efficient mechanism-driven therapies. In fact, current therapeutic strategies are mainly based on restriction from physical exercise, antiarrhythmic drugs, catheter ablation, and implantable cardioverter defibrillators, all of which are aimed at preventing arrhythmic sudden cardiac death, but are not targeted to counteract AC myocardial remodelling. Thus, AC is in urgent need of mechanistic insight, guiding the development of specific therapeutic approaches to strike at the sign of a disease that is a major killer in the young and in athletes. The lag in the identification of novel therapies suggests that looking at AC from a novel perspective might provide translational knowledge. Current research has treated AC as a disease of desmosomes and, as such, a cardiomyocyte-restricted disorder. However, we and others showed that desmosomal proteins are also expressed by cardiac and extra-cardiac non-myocyte cells, which thus harbour AC variants. Consistent with this, we recently demonstrated that cardiac- and bone marrow-derived mesenchymal stromal cells are additional cell types affected in the disease, indicating that AC should be thought of as a multicellular and multiorgan disorder.[5]

Since the early descriptions of the disease, inflammation has been recognized as a prominent feature of AC, thus attracting the interest of several groups as a potential target in the cascade of events culminating in AC structural and functional remodelling. Inflammatory infiltrates are detectable in the heart of most AC patients, especially in those who died suddenly.[2] Moreover, in experimental animal models, we first demonstrated that in *Dsg2* transgenic mice, myocyte necrosis is the key initiator of myocardial injury, triggering progressive myocardial damage, including an inflammatory response and massive calcification within the myocardium, injury repair with fibrous tissue replacement, and myocardial atrophy.[6] More recently, we added a new molecular player, galectin-3, which switches on and attenuates the inflammatory response based on desmosomal instability and modulation of Wnt signalling.[7]

It is noteworthy that, particularly in paediatric patients, AC may manifest with the so-called 'hot phase', characterized by chest pain and release of myocardial enzymes in the setting of normal coronary arteries.[8] Endomyocardial biopsy, performed during such an acute phase, can reveal histological evidence of myocarditis, which may reflect an active disease phase, associated with its onset or accelerated progression (evolutive *poussée*). In addition, elevated circulating levels of proinflammatory cytokines, which may be released by cardiomyocytes, have been found in AC patients.[9] Autoimmune responses have recently been identified in AC patients and mutation carriers, and correlated with disease severity, thus supporting the use of anti-inflammatory drugs as a therapeutic option.[10] Multiple murine AC models have been employed to design possible treatment strategies to rescue the disease phenotype, supporting the therapeutic benefit of blunting inflammatory signalling, through complement modulation, inhibiting the inflammasome, or blocking the glycogen synthase kinase-3β(GSK3β)/nuclear factor (NF)-κB pathway.[11–13]

Along this line, looking at immunomodulation as a possible strategy to treat AC, Lin and colleagues[14] tested the potential anti-inflammatory effects of extracellular vesicles (EVs), obtained from cardiosphere-derived cells (CDCs), in a pre-clinical model of AC; their results are reported in this issue of the *European Heart Journal*. EVs are membrane-enclosed vesicles, carrying cell-specific cargoes of proteins, lipids, metabolites, and nucleic acids, which may impinge on the activity and phenotype of different cell types. Delivery of cell-selective EVs has been looked upon as a novel therapeutic option in the treatment of cardiac diseases, including myocardial ischaemia and heart failure, as well as for immune-modulatory and regenerative therapies. The therapeutic effect of EVs in AC has not, to the best of our knowledge, been tested previously. The results presented above, together with the promising results achieved in Duchenne muscular dystrophy, prompted Lin and colleagues[14] to undertake a pioneering study testing whether the anti-inflammatory and antifibrotic effects of EVs, secreted by immortalized CDCs, could be beneficial in AC.

Firstly, the authors chose the most suitable animal model, which is here represented by desmoglein-2 mutant mice (*Dsg2* $^{mut/mut}$), previously shown to replicate well both human genetics and clinical phenotype, including myocardial inflammation.[3,12] These mice were treated with EVs from 1 month of age, when myocardial damage is undetectable, and delivered for 4 or 8 consecutive weeks. EV treatment had amazing effects at both the structural and functional level. In fact, Lin and colleagues demonstrate that intravenously injected EVs reduced the immune response and the rate of cell death, mitigated intercalated disc remodelling and fibrous tissue deposition, while improving cardiac function and attenuating spontaneous and induced arrhythmia incidence. The functional effects have been attributed to accelerated cardiac conduction and improved repolarization, resulting from rescue of Cx43 down-regulation, fibrosis, and inflammation. The clear-cut effect on QTc interval prolongation, an ECG sign which is not part of the human AC clinical picture, could influence the reduced arrhythmogenicity observed in mice treated with EVs. To define the mechanism underlying reduced fibrotic remodelling, the authors looked at the state of cell death in hearts from vehicle- vs. EV-treated AC mice and demonstrated a significant decrease in the number of apoptotic nuclei, as well as necrotic cardiomyocytes, in hearts from mice undergoing EV

delivery. Notably 'serum troponin I levels were low regardless of genotype or therapy at both baseline and endpoint, suggesting the necrotic process was insidious with periodic acute bursts'. Furthermore, in line with the authors' guiding hypothesis, EV treatment resulted in the reduction of cardiac content of proinflammatory cytokines, NF-κB phosphorylation, and inflammasome activation. By combining *in vitro* and *ex vivo* analyses with RNA sequencing, the authors demonstrated that the observed salutary effects can be ascribed to the EV-mediated transfer to cardiomyocytes of hsa-miR-4488, which 'mitigates NF-κB activation'. It is noteworthy that while the role of microRNAs as disease biomarkers has been demonstrated in several studies, none of them has yet provided compelling evidence of a pathogenetic significance.[15]

Overall, EVs seem to be the 'magic potion' that might attenuate the AC phenotype. However, there are several outstanding points that still need to be resolved before clinical translation might be contemplated. Firstly, the duration of the cardio-protective effects after completion of the EV treatment cycle has to be verified. In previously proposed experimental therapies, acting via inhibition of inflammatory responses, e.g. inhibition of GSK3β with SB216763, efficacious treatment required chronic drug administration. In fact, a single dose or a frequently repeated multiple drug regimen might not suffice to achieve persistent beneficial responses, particularly in conditions such as AC, which usually starts in adolescents/young adults and develops, with repeated bouts, with an age-related penetrance. Additionally, the long-term effects following discontinuation of EV administration, as well as the possible adverse consequences of long-term EV therapy, should be evaluated. In the AC context, progress in understanding these factors is essential to develop pharmacological therapies, in the absence of a definitive eradicating gene therapy. Moreover, it is well known that high-intensity/competitive physical exercise is associated with earlier disease onset, accelerated disease progression, and increased arrhythmic risk in AC patients and mutation carriers.[1] Studies specifically addressing the effects of EV-based therapy on exercise-related structural changes and arrhythmias are warranted. Finally, putting together the results of Lin and colleagues with our novel concept of AC as a multicellular and multiorgan disorder, it would be interesting to evaluate whether the EVs target other cardiac (i.e. stromal and vascular cells, or neurons) and extracardiac (i.e. bone marrow and spleen) cell types, which may be involved in the disease (*Graphical Abstract*). Thus, many questions need to be addressed before moving EV therapy from bench to bedside.

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