PHYSIOLOGY

Fibroblast-myocyte coupling in vivo

Fibroblasts in scar tissue elicit cardiac excitation and promote arrhythmia in mouse hearts

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Traditionally, cardiac electrophysiology has focused on myocytes, the heart muscle cells that generate action potentials and are electrically excitable. Non-myocytes within the heart are often considered barriers to action potential propagation. Typically defined as extracellular matrix producing cells, cardiac fibroblasts are one of the largest non-myocyte cardiac populations. However, they have emerged as being important for maintaining normal cardiac function and mediating cardiac remodelling during pathology, when their number substantially increases (*1*). The presence of electrical coupling between fibroblasts and myocytes has been established in vitro and more recently demonstrated in situ (*2-4*). But definitive in vivo evidence has been lacking. On page xxx of this issue, Wang *et al.* (*5*) show that fibroblasts and myocytes are electrically coupled in living mice. This finding could transform the understanding of cardiac connectivity and arrhythmogenesis, with profound implications for the management of heart disease patients.

Wang *et al*. engineered an optogenetic transgenic mouse that expressed the light sensitive channelrhodopsin (CHR2) in fibroblasts, enabling real-time stimulation of membrane depolarization specifically in resident fibroblasts by illumination with blue light. In animals subjected to coronary occlusion to mimic myocardial infarction, blue light illumination of the resulting fibroblast rich scar drove organ-wide cardiac excitation in vivo and in excised perfused hearts. Increasing frequency of illumination increased heart rate with a 1:1 response, demonstrating functional electrical coupling between CHR2-expressing fibroblasts and myocytes. When optical pacing ceased, a subset of hearts did not return to normal sinus rhythm and displayed ectopic beats and atrioventricular conduction block, suggesting that depolarization of fibroblasts can initiate arrythmia.

Cardiac arrhythmia is an important complication in patients with myocardial infarction and can lead to sudden cardiac death. Fibrotic tissue promotes arrhythmia by acting as a physical barrier to action potential conduction between myocytes. Co-cultures of fibroblasts and myocytes has demonstrated that coupling between the two cell types can alter myocyte excitability, repolarisation, and conduction, promoting arrhythmogenic conditions (*6*). Wang *et al.* showed that fibroblast-myocyte coupling can promote arrhythmia in vivo. Although the exact mechanism leading to the observed arrhythmia remains elusive, the authors suggest that changes in myocyte excitability and conduction driven by the depolarisation of scar fibroblasts could induce arrhythmia after myocardial infarction.

It is important to note that Wang *et al.* used synchronous, global depolarization of scar fibroblasts to drive organ-wide cardiac excitation and promote arrhythmogenesis. Although local changes in fibroblast depolarization could occur in response to mechanical stretch or ischaemia in the scar (*7*), global depolarisation of fibroblasts in vivo is unlikely due to the heterogeneous nature of the infarcted myocardium. In vitro and computational studies indicate that a substantial number of fibroblasts functionally coupled to myocytes is required to promote arrhythmogenesis (*6*). Whether local depolarisation of a small number of fibroblasts in the scar or border zone would be sufficient to alter cardiac excitation and trigger arrhythmia remains to be determined. But focal activation using a micron-diameter light source in the model developed by Wang *et al*. could start to address this question.

Electrical coupling between fibroblasts and myocytes is generally thought to be mediated by gap junctions which directly connect the cytoplasm of neighboring cells (*1*). Using conditional knockout of gap junction protein connexin43 (CX43) in fibroblasts, Wang *et al.* show that this gap junctional protein is not essential for fibroblast-myocyte communication in vivo. It would be interesting to see whether loss of CX43 in fibroblasts would affect arrhythmogenicity. In vitro experiments performed in fibroblasts deficient in CX40, CX43, CX45 and pannexin-1 (PANX1) suggest that these membrane channel proteins are also unnecessary for hetero-cellular coupling. Follow-up computational studies indicated that ephaptic coupling, a non-gap junctional coupling mechanism which facilitates cell-to-cell transfer of electrical activation via the extracellular space (*8*), may be sufficient to couple cells. Proving the presence of ephaptic coupling in vivo and dissecting its contribution to arrhythmogenesis will be challenging but essential to develop therapies to block fibroblast-mediated cardiac arrhythmia.

The findings of Wang *et al*. have important implications for ablation procedures that are commonly used to treat arrhythmia. The frequent recurrence of ventricular tachycardia in these patients (*9*) could result from the coupling of fibroblasts with residual myocytes at the ablation site, leading to new arrhythmogenic substrates. Targeting fibroblast-myocyte coupling in this setting could be a promising strategy to prevent recurrence. Conversely, interventions that enhance fibroblast-myocyte coupling and conduction might be beneficial in bridging scar tissue and reducing conduction block, therefore preventing re-entrant ventricular tachycardia (*7*). The prevalence of diffuse and focal fibrosis in many other forms of heart disease further widens the relevance of these findings with potential roles of fibroblast-myocyte coupling in hypertrophic and arrhythmogenic right ventricular cardiomyopathies, which are common causes of sudden cardiac death in young people.

These results will also be important for the cardiac cell therapies which are being developed to replace myocardium lost in disease (*10*). Electrical coupling between resident scar fibroblasts and transplanted myocytes could alter donor myocyte excitability, repolarisation, and conduction, promoting substrates for focal or re-entrant arrhythmia (*11*). Alternatively, fibroblasts could assist donor myocyte integration by forming passive conduction connections to the host's myocardium, promoting electrophysiological synchronisation.

Advances in genetic tools (*12*), coupled with biomedical imaging (*13*), now make it possible to track and manipulate the function of individual cell types, allowing molecular processes and pathways to be investigated and multicellular contributions to complex organ systems disentangled in situ and even in

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vivo. The approach used by Wang *et al.* only highlights the beginning of what will be possible using these advanced genetic systems. The exact number and location of fibroblasts required to initiate arrhythmia, and the contribution of other non-myocytes and transplanted donor cells to cardiac electrophysiology can now be investigated and controlled in model organisms. This could initiate a step-change in our understanding of cardiac electrophysiology and uncover therapeutic targets for heart failure, arrythmia, and sudden cardiac death.

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