Editorial

Dedifferentiation of atrial cardiomyocytes: from in vivo to in vitro

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Received 9 April 2002; accepted 9 April 2002

See article by Rücker-Martin et al. [3] (pages 38–52) in this issue.

1. Introduction

Atrial fibrillation (AF) is characterized by extensive electrical and structural remodeling [1,2]. The latter aspects have been associated with cardiomyocyte dedifferentiation because of the phenotypic similarities with fetal cardiomyocytes. The paper of Rücker-Martin et al. [3] in this issue describes the dedifferentiation of atrial myocytes both in vivo and in vitro and emphasizes the role of fibroblasts in the maintenance of the dedifferentiated state of the cardiomyocytes. The authors report that in biopsies from patients with AF, concomitantly suffering from valvular disease, the majority of atrial myocytes undergo changes which are reminiscent of dedifferentiation, akin to those seen in ventricular biopsies of patients with hibernating myocardium [1]. The atrial cardiomyocytes are myolytic, accumulate glycogen and myocyte bundles are separated by thick layers of fibrotic tissue. Reexpression and redistribution of structural proteins such as α-smooth muscle actin and titin show that the structurally altered myocytes adopt a dedifferentiated (fetal) phenotype. When cardiomyocytes of these patients were cultured a very similar dedifferentiated phenotype appears. Moreover, after prolonged time in culture these myocytes redifferentiate and regain the adult phenotype. Intriguingly, the dedifferentiation process is inhibited in the presence of abundant fibroblasts. The paper of Rücker-Martin et al. [3] points out the prominent role of matrix fibroblasts in the perpetuation of the dedifferentiated state of cardiomyocytes.

2. Structural remodeling of atrial myocytes

Apart from clear degenerative changes in fibrillating atria of patients which have been reported earlier by the same group and others [4,5], the present study shows that in such patients remodeling of the myocyte structure (dedifferentiation) and the surrounding connective tissue occurs. Structural hallmarks of dedifferentiation are myolysis, glycogen accumulation, dispersion of nuclear chromatin, changes in mitochondrial shape and size and loss of sarcoplasmic reticulum and T-tubules. All changes are accompanied by important alterations in the expression and organization of structural proteins such as α-smooth muscle actin, titin, myosin heavy chain, troponin, desmin [6–8].

The possible common trigger for these events might be Ca2+-overload. At the onset of AF, Ca2+-overload occurs, which will lead to induction of the proteolytic activity by the Ca2+-dependent calpain I, thereby inducing desmin degradation and sarcomere dissolution [3,9]. Moreover, down-regulation of the L-type Ca2+-channel, as shown within the first days after the onset of AF [10], will lead to decreased atrial contractility [11]. The decreased atrial contractility promotes passive stretch on the atrial wall which in turn initiates Ca2+-overload and proteolysis, and in such a way providing a basis of structural remodeling [12]. After long-term AF the intracellular Ca2+-concentration tends to normalize [13].

3. Are the phenotypic adaptations part of a cell survival program?

The subcellular adaptation in myocytes might be interpreted as a response to elevated stress whereby the myocytes switch from an adult (functional) to a fetal (survival) phenotype, a process akin to dedifferentiation. In all studies dealing with phenotypic adaptations of cardiomyocytes the dedifferentiation was incomplete, i.e.
4. The role of extracellular matrix and fibroblasts

Another important question relates to the role of the extracellular matrix and fibroblasts in the described atrial pathologies. Since in most of the patients an increased amount of connective tissue is observed and, moreover, as Rücker-Martin et al. [3] state in this paper ‘some degree of extracellular fibrosis was also seen in control patients above 62 years old’, the extracellular remodeling is an important determinant in the progression of the atrial fibrillation. Fibroblasts are the main producers of extracellular matrix components and enhanced proliferation of fibroblast in atrial tissue will likely result in increased synthesis of matrix material such as collagens, which could underly the delayed recovery of contractile function after cardioversion of long-term AF. Evidence for this was also found in patients with chronic ventricular hibernating myocardium, where the increased amount of connective tissue has been considered a possible candidate for the slow recovery of contractile function after restoration of blood flow [17]. Besides the delayed recovery of contractile function it is likely that an increased amount of connective tissue as a result of fibroblast proliferation, either in between individual myocytes or myocyte bundles, will lead to anisotropic conduction which promotes reentry and in turn stabilizes AF [18].

5. In vitro simulation of structural remodeling

The in vitro dedifferentiation and redifferentiation of atrial myocytes was previously reported for atrial and ventricular myocytes of different species [19–23]. The in vitro culture can serve as a model system to study the behavior of myocytes under different circumstances. In this way, mechanisms underlying the phenotypic changes can be unraveled. Hitherto, the isolation of cardiomyocytes from human heart has been very cumbersome. Rücker-Martin et al. [3] have a surprisingly high yield of surviving (Ca$^{++}$-tolerant) myocytes after the applied isolation procedure. This might be due to the sophisticated method the authors used for cell dissociation or to the fact that they used isolated cells from atrial instead of ventricular tissue.

However, one point that hampers the interpretation of their data is related to the starting material, i.e. it is not clear in what state of differentiation the isolated cells are at the beginning of the cell culture. The time course of dedifferentiation cannot be established when the culture consists of a mixture of differentiated and dedifferentiated myocytes. Moreover, it is not known if a selection is made between differentiated versus dedifferentiated myocytes during the isolation procedure, based on their different resistance to stress factors applied during the isolation procedure.

Similar to the in vivo situation, the cell culturing did not give rise to a far advanced dedifferentiation state with reoccurrence of cell division. Co-culturing of cardiomyocytes with fibroblasts was previously described by Dispersyn et al. [19]. In contrast to human atrial cells as reported in this paper, the dedifferentiation in rabbit myocytes occurred only in the presence of fibroblasts. The most intriguing observation in Rücker-Martin’s paper is that redifferentiation of human atrial myocytes was prevented in the presence of abundant fibroblasts. From their and previously published papers by Decker et al. and Dispersyn et al. [19,20] one could conclude that fibroblasts stimulate cardiomyocyte dedifferentiation and inhibit re-differentiation. However, it is not clear how fibroblasts influence the differentiation state of the myocytes. That firm contacts between fibroblasts and myocytes exist in the culture systems is evidenced by time-lapse microcinematographic experiments where stretching of myocytes by fibroblasts is commonly seen, and by the formation of gap-junctions between the two cell types [3,19]. Whether locally produced or activated paracrine factors in combination with close cell–cell interactions between fibroblasts and myocytes are involved remains to be established.

6. Role of stretch in initiation and maintenance of the dedifferentiated phenotype

Myocardial stretch may play a predominant role in the trigger of dedifferentiation of cardiomyocytes. Dedifferentiation of atrial myocytes occurs in atria from patients with volume overload due to valvular disease, although to a lesser extent than when accompanied by AF [24,25]. The absence of atrial contractility due to AF, induces a much higher degree of passive stretching of the atria which in turn sustains the dedifferentiated stage of the cells. Ven-
tricular volume-overload in dogs with chronic AV block was also shown to induce dedifferentiation in a small percentage of the myocytes [15]. In patients with severely volume overloaded ventricles dedifferentiated cardiomyocytes are present in high numbers [26]. In pigs with chronic hibernating myocardium dedifferentiated cardiomyocytes also appear in the areas remote from the ischemic zone, i.e. in areas were only volume-overload occurs [27].

In addition, when myocytes are embedded in an enlarged matrix of connective tissue, contractility becomes further depressed thereby promoting passive stretch. The point of no return might be reached whereby contractility is irreversibly lost and constitutes the underlying cause of permanent AF. An important issue evolving from these observations is that in order to achieve successful re-differentiation it might be crucial that elevated stretch is removed.

7. Unsolved issues and future perspectives

The electrophysiological characterization of atrial myocardium, in which dedifferentiated myocytes predominates has not been assessed so far. Such studies might provide new clues to explain the maintenance of AF and its reoccurrence after cardioversion. This is based on the assumption that dedifferentiated myocytes have different electrophysiologic characteristics, given the structural resemblance between dedifferentiated cardiomyocytes and other less differentiated myocytes such as P-cells of which it is known that they have a different action potential duration.

One point that is rightfully emphasized by Rücker-Martin et al. [3] is the possible implication of the role of fibroblasts in the extracellular matrix volume. An increased amount of connective tissue promotes anisotropic conduction which thereby can stabilize AF. However, the exact role of the extracellular matrix in stabilizing AF is not known and needs to be further explored. The author’s statement ‘given the marked accumulation of extracellular matrix in diseased atria and also with age, it is likely that proliferation of fibroblasts occurs also in situ and thus could represent a factor of irreversibility and aggravation of cellular remodeling and contribute to the increase in incidence of atrial fibrillation with aging’ is of cardinal importance. Prevention of structural remodeling during AF might be an important new issue in the treatment of AF [28]. Finally, the interaction between cardiomyocytes and fibroblasts needs to be dealt with in greater detail. An unsolved issue remains how fibroblasts influence the differentiation stage of the myocytes. This includes the cell–cell contacts and ways of transport of components from one cell to another. Also a possible role for other cell types present in the intact heart, as e.g. endothelial and smooth muscle cells, needs further study. The role of cell–cell interactions between myocytes and non-myocyte cells such as fibroblasts has to be investigated. The role of locally produced or activated paracrine factors needs to be thoroughly assessed. Moreover, it is not known whether cell–cell contacts between myocytes and non-myocytes do occur in vivo under normal or certain stress conditions such as ischemia, volume/pressure overload, or atrial fibrillation.

References


