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RESEARCH NEWS

Rethinking replating

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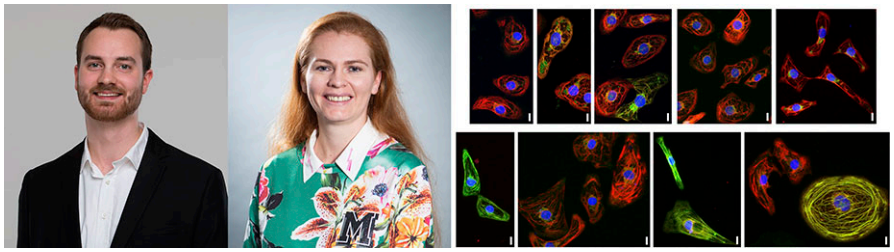
***JGP* study (In this issue, Osten et al. https://doi.org/10.1085/jgp.202313377) suggests that, by altering mechanosensitive signaling pathways, replating stem cell-derived cardiomyocytes changes myosin expression and contractile function.**

Human pluripotent stem cell-derived cardiomyocytes have many potential uses, from regenerative medicine to pharmacological assays and studies of cardiac function and development. Many of these applications require the cardiomyocytes to be replated on fresh surfaces shortly before they are analyzed. In this issue of *JGP*, however, Osten et al. reveal that replating induces a temporary change in myosin heavy chain (MyHC) expression and contractile function that researchers likely need to consider when interpreting their results (1).

MyHC expression is the major determinant of a cardiomyocyte's contractile properties. Human adult atrial cardiomyocytes predominantly express α-MyHC, whereas ventricular cardiomyocytes mainly express β-MyHC (2). Stem cell-derived cardiomyocytes differ from adult cardiomyocytes in several important respects, but their maturation can be improved by culturing them for long time periods on stiff matrices (3). In 2016, Natalie Weber and colleagues at the Institute of Molecular and Cell Physiology, Hannover Medical School, discovered that these long-term cultures adopt a ventricular phenotype, with over 80% of cells exclusively expressing β-MyHC (4).

However, when one of Weber's colleagues, Bogdan Iorga, analyzed the contractile function of these long-term cultured stem cell-derived cardiomyocytes—having first replated them onto glass coverslips in a micromechanical setup—he noticed that they didn't behave like cells expressing β-MyHC (1).

"He saw that the kinetic parameters of crossbridge cycling were accelerated compared to non-replated cells," explains Felix Osten, a graduate student in Joachim Meissner's laboratory and co-first author with Weber on the new *JGP* paper.



Felix Osten (left), Natalie Weber (center), and colleagues including Bogdan Iorga and Joachim Meissner report that replating long-term cultures of mature stem cell-derived cardiomyocytes induces temporary changes in myosin expression and contractile function. The vast majority of long-term cultured stem cell-derived cardiomyocytes (top row) exclusively express β-MyHC (red). But many replated cells (bottom row) express α-MyHC (green) as well as, or instead of, β-MyHC.

Faster crossbridge kinetics are associated with α-MyHC expression, because this isoform has a higher ATPase activity than β-MyHC (5). Accordingly, the researchers found that replating caused a significant increase in the number of stem cell-derived cardiomyocytes expressing α-MyHC, either alone or in combination with β-MyHC. RNA-sequencing confirmed that the expression of mRNA encoding α-MyHC was dramatically upregulated 2 d after replating, while the levels of mRNA encoding β-MyHC were slightly reduced, resulting in nearly equal levels of the two MyHC isoforms.

1–2 wk after replating, however, the majority of cells reverted to expressing β-MyHC exclusively, and their crossbridge cycle kinetics slowed to the levels expected for mature ventricular cardiomyocytes.

To find out what regulates these changes in MyHC expression, Osten et al. compared RNA-seq data from replated and non-replated stem cell-derived cardiomyocytes. The researchers found that replating causes the upregulation of many genes involved in cell adhesion and mechanotransduction, particularly genes associated with integrin-based signaling pathways.

Integrins are mechanosensitive components of the costamere structures that link sarcomeres to the extracellular matrix, and might therefore be able to sense the changes in matrix stiffness associated with cell replating and initiate signaling pathways that alter MyHC expression. Osten et al. found that focal adhesion kinase (FAK), a signaling protein downstream of integrins, is active when stem cell-derived cardiomyocytes are cultured for long periods on stiff matrices. Inhibiting FAK reduced the number of β-MyHC-expressing cells, confirming that mechanosensitive signaling pathways control MyHC expression.

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Osten et al. note that, although MyHC is the main determinant of contractile properties, replating cardiomyocytes may also alter the expression of other components of the sarcomere. Moreover, replating could have other effects on cellular function; Osten et al. found that Ca^{2+} transients are slowed in replated cells, and the increased ATP consumption by α-MyHC could have important implications for cellular energetics.

"People might not be aware that detaching and replating can have such an impact on stem cell-derived cardiomyocytes and their downstream applications," Osten says.

"For example, in our institute we study hypertrophic cardiomyopathies involving mutations in β-MyHC," Weber adds. "So, for us, it's very important to be aware of this upregulation of α-MyHC due to replating and to develop different protocols."

References

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