iPSC-derived cardiomyocytes as a model to dissect mechanical dysfunctions of caveolinopathies

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Summary

Cardiac diseases remain one of the major causes of mortality and morbidity in our society with enormous costs for the health system. Arrhythmias and cardiomyopathy diseases are difficult to prevent/cure because the molecular mechanisms behind their onset are in most cases not fully clarified. Causes and effect are often confused, even when directly studying patients’ cardiomyocytes, because of the maladaptive remodelling imposed by electro-mechanical alterations. To overcome this limitation, studying the arrhythmogenic risk associated with genetic cardiac diseases using patient-derived iPS-CMs, provides a good model.

Caveolinopathies are a group of muscular diseases that arise from mutation in the caveolin-3 gene (CAV3). Several CAV3 variants have been found in patients with both skeletal and cardiac pathologies1. While electrophysiological alterations behind caveolinopathies have been partly elucidated using different models2, the impact of such mutations on cardiomyocyte contraction and thus on the risk of developing cardiomyopathy, although quite probable, has never been studied before. Caveolin-3 along with cholesterol, forms membrane caveolae and plays a key role in the maintenance of plasma membrane integrity and interacts with several signaling proteins and ion channels.

Here, CardioExcyte 96 and FLEXcyte 96 compared relative amplitude and kinetics of contraction and relaxation in patient/control hiPS-CMs in order to shed light on the relations between electrical and mechanical dysfunctions. This analysis offered various advantages, such as the possibility of electrical stimulation, recordings in an environment with an elastic surface area resembling that of the native cardiac tissue, as well as high throughput3,4.

Results

Here, the CAV3 variant T78K, associated with Rippling muscle disease and HyperCKemia (HCK), was investigated. Human cardiomyocytes (CM) were differentiated from induced pluripotent stem cells (iPSC) derived from a patient carrying this heterozygous mutation and from a healthy control. Among others, proteomic analysis revealed alteration in calcium handling and sarcomeric proteins which promoted the analysis of contraction properties using the CardioExcyte96 and FLEXcyte96 platforms.

Figure 1A shows the contraction profiles of control and T78K hiPSC-CMs monolayers as recorded on rigid (CardioExcyte 96) and flexible substrate (FLEXcyte 96).

Figure 1: A Contraction amplitude (top) and impedance (lower) recordings of mean beat traces generated for control and T78K hiPSC-CMs. B Comparison of contraction rate and amplitude of respective hiPSC-CMs as recorded on the CardioExcyte 96 and the FLEXcyte 96.
Further analysis of the contraction properties of control and T78K hiPSC-CMs revealed increased mean beat amplitude (at 1Hz), with faster rising and falling time in T78K hiPSC-CMs compared to control, as shown in Figure 2. Lastly, the cells were paced at various rates (1Hz, 1.5Hz and 2Hz) and the same parameters were analyzed. This revealed the inability of T78K-CM to adapt contraction to increasing rates, suggesting a possible excitation-contraction coupling dysfunction.

These results highlight the importance of use of hiPSC-CMs, together with contractile force recording systems, for understanding patient-specific pharmacological effects, aimed at preventing the life-threatening outcomes associated with cardiac diseases.

In summary, the CardioExcyte and FLEXcyte 96 were successfully used to record contractility of patient-derived human iPSC cardiomyocytes. Here, it was shown, for the first time, how the CAV3 T78K mutation determines an altered excitation-contraction coupling and a potential proarrhythmic profile. This highlights the need for cardiac monitoring in patients with CAV3 mutations. This analysis could allow to deepen the understanding of these pathologies unmasking the true causes and to lay the basis for a personalized-medicine approach aimed at preventing/curing the life-threatening outcomes of maladaptive remodelling.

References

Methods
Cells
Control and patient derived T78K mutation hiPSC-CM cell lines were developed and differentiated in the laboratory of Prof. Andrea Barbuti, Università degli Studi di Milano.

Figure 2: Contraction analysis under pacing conditions indicating mechanical dysfunction in T78K hiPSC-CMs compared to control cells. Scatter plots represent the effects of various pacing rates on A amplitude, B pulse width at 50%, C falling time and D rising time of mean beats as recorded on CardioExcyte 96.

Interestingly, spontaneous beating of T78K hiPSC-CMs was observed when the cells were recorded on flexible substrate, corresponding with increased spontaneous beating rate previously obtained through patch clamp analysis. These results were not observed when impedance was recorded on rigid substrate.