Cardiomyocyte - stromal cells interplay as a novel platform for mechanistic insights in arrhythmogenic cardiomyopathy

A.S. Maione1, L. Iengo1, L. Sala2, F. Lodola3, M. Lippi1, A. Zaza3, C. Tondo1, G. Pompilio1, E. Sommariva1

1Monzino Cardiology Centre, Milan, Italy
2Istituto Auxologico Italiano IRCCS, Milan, Italy
3University of Milano-Bicocca, Milan, Italy

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Background: Arrhythmogenic Cardiomyopathy (ACM) is an inherited heart disorder characterized by a high incidence of sudden death at young age. Mutations linked to ACM occur primarily in desmosomal genes (e.g. PKP2) [1]. Different cell types individually contribute to set the ACM phenotype with either functional abnormalities (cardiomyocytes; CM) [2]; or fibro-fatty substitution (cardiac mesenchymal stromal cells; CMSC) [3]; these two cell types interact and show a reciprocal influence [4]. The relative contribution of the different cell type derangements to determine disease evolution and electrical instability is still unclear.

Purpose: Based on this evidence, we hypothesize that the use of a multicellular cardiac system, modelling the functional interaction between CM and CMSC, will allow the understanding of the relative contribution of the different cell types to the ACM phenotypes.

Methods: Primary stromal cells (carrier of the PKP2 mutation c.2013delC) have been collected from a right ventricle biopsy sample; control stromal cells obtained from a biobank. iPSC reprogrammed from the same ACM patient and the isogenic line in which the PKP2 mutation c.2013delC was corrected [5] were used. Cardiac cocultures (cc) were assembled by combining 85% iPSC-CM and 15% primary CMSC, either mutated or control, in different combination (as in Table 1). Movies of contracting clusters have been collected and analysed by the MUSCLEMOTION tool [6]. Fibro-fatty accumulation have been evaluated by immunofluorescence analysis by confocal microscopy.

Results: Immunofluorescence analysis highlighted that ACM cc (cc 4) accumulate more lipids and collagen than healthy control (HC) ones (cc 3). Since intermediate fibro-fatty levels were observed in ‘mixed cc’ (cc 1 and 4), we conclude that ACM iPSC-CM are able to influence fibro-adipo-commitment of CMSC. Contraction studies showed a high propensity for cc monolayers which include ACM iPSC-CM (cc1 and cc2) to display arrhythmic events. An increase in the percentage of arrhythmic events was triggered when the ACM CMSC were replacing HC CMSC, indicating that ACM CMSC can affect iPSC-CM rhythm.

Conclusion: Overall, our data confirm the importance of the cardiomyocytes and stromal cells interplay during ACM pathogenesis. Furthermore, our novel advanced ACM cell model can recapitulate different phenotypes of ACM and offers a unique opportunity for validating pharmacological therapies to modulate ACM-related characteristics (e.g. fibro-adipose replacement, contractile defects and proarrhythmic events).