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PATHOPHYSIOLOGICAL AND THERAPEUTIC IN VITRO ASSESSMENT OF PATIENT-SPECIFIC IPS CELL-DERIVED CARDIOMYOCYTES

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1. Project summary

The main objective of this project was to evaluate the usefulness of cardiomyocytes derived from induced pluripotent cells (iPS-CM) as a model for the study of hereditary arrhythmogenic diseases (ICA).

In addition, we proposed to investigate the possible impact of the genetic background and cardiac cellular components on the effects of mutations located in the cardiac sodium channel ($Na_V1.5$) on the activity of the sodium current. This is based on the premise that the relationship between the genotype and the phenotype of ICAs is complex and is dictated in principle by specific tissue factors and the patient's genetic background. In this project we focused on a family that carries a mutation in the SCN5A gene (which encodes the cardiac sodium channel) associated with Brugada syndrome (BrS). This channel is responsible for the depolarization phase of the cardiac action potential (CAP) It is known that mutations in this channel cause abnormalities in the CAP that result in a typical BrS electrocardiogram (ECG) with an elevation in the ST segment in leads V1, V2 and V3, which is an arrhythmogenic substrate and may lead to sudden cardiac death. Our objective was to study the sodium current in patient-specific iPS-CM derived from skin biopsies from four members of the family under study, three of whom are carriers of the same mutation (c. 4573 G> a; NaV1. 5_p.V1525M) and one who is a non-carrier. We aimed to detect possible differences in the characteristics of the sodium current between family members. In addition, we compared the characteristics of the sodium current from these patient-specific iPS-CM with the current resulting from heterologous transfection of the mutant and non-mutant sodium channel. Our experiments showed that the $Na_V1.5$-V1525M mutation produces a drastic reduction in sodium current in two of the patient-specific lines of iPS-CM with respect to the non-carrier patient, while the third patient-specific line does not present changes in the current. On the other hand, although the mutation caused a loss of sodium channel function in transfected tSA201 cells, this reduction in current was not as large as that observed in the patient-specific iPS-CM. This finding supports our hypothesis that the phenotypic expression of mutations in the sodium channel, associated with Brugada syndrome, is determined or modulated by the cell type and the specific genetic heritage of each individual.

This finding is important to understand the incomplete penetrance and variable expressivity of the ICA, since two individuals with the same mutation could have different cell phenotypes depending on the presence of other variations in genes that affect the activity of the heart. In addition, it suggests that the effect of a mutation depends on the interaction of the channel with regulatory proteins, typical of cardiomyocytes.

We also studied a mutation in the *SCN1B* gene that encodes two isoforms of the cardiac sodium channel regulatory proteins, the β1 and β1b subunits. This mutation was found in a child who presented neurodevelopmental abnormalities in combination with cardiac dysfunction. The mutation in *SNC1B*, 308 A> T causes an amino acid change, p.D103V, in both the β1 and β1b subunits. Therefore, and because these subunits regulate both cardiac and neuronal sodium channels and the patient has neurological and cardiac symptoms, we studied the effects of mutated β1 and β1b subunits on the sodium current from tSA201 cells expressing the $Na_V1.1$ or $Na_V1.5$ channels. In this case we could not obtain skin samples from the patient, but we observed in our heterologous transfection model, that the mutation causes a loss of function in both types of sodium channels. The child's mutation in the *SCN1B* gene was inherited from his father. In addition, the child is a carrier of two mutations in the *POLR1C* gene inherited one from the father and one from the mother. Neither the father nor the mother of the child has any type of neurological or cardiac symptom. On the other hand a sister of the child, also a carrier like him of the three variants (two in the *POLR1C* and the *SCN1B* variant), had a fetal diagnosis of bradycardia, postnatal AV block and early postnatal death due to multi-organ failure. Our results suggest that the phenotypic expressiveness of the mutation in *SCN1B* depends on the combination of other variants present in the genome of the child and his sister, but not in the parents.

2. Results obtained

Our results demonstrate that the effects of mutations in *SCN5A* on sodium current activity in iPS-CM have characteristics that are not evident in studies conducted in heterologous expression models. This implies that the cellular phenotype of a mutation depends on both specific components of the cell type and the specific genetic variants (pathogenic or not) of each individual. These findings had not been demonstrated

before in relation to Brugada syndrome and it was possible to obtain them thanks to the help of the La Marató TV3 Foundation.

3. Relevance with possible future implications

This result can be very useful in the genetic diagnosis of hereditary arrhythmias related to mutations in the sodium channel. In this work the cellular components that modify the cellular phenotypic expression of a mutation have not been studied. However, in the future, the identification of these components may serve to predict the degree of individual pathogenicity of a mutation. Our work demonstrates the need and usefulness of looking for these modifying factors.

4. Scientific Bibliography generated

Publications in indexed scientific journals

Elisabet Selga, Franziska Sendfeld, Rebecca Martinez-Moreno, Claire N Medine, Olga Tura-Ceide, Ian Wilmut, Guillermo J Pérez, Fabiana S Scornik Ramon Brugada and Nicholas L Mills. Sodium Channel Current Loss of Function in Induced Pluripotent Stem Cell-derived Cardiomyocytes from a Brugada Syndrome Patient. J Mol Cell Cardiol. 2018 114:10-19. doi: 10.1016/j.yjmcc.2017.10.002. Epub 2017 Oct 9.

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National and International meetings presentations

2019-2020

Rebecca Martinez-Moreno, Elisabet Selga, Georgia Sarquella-Brugada, Ramon Brugada, Guillermo J. Perez, Fabiana S. Scornik. Comparative study of the effects of an *SCN5A* mutation within a family diagnosed with brugada syndrome using iPS-CM. Oral presentation at the 64th Annual Meeting of the Biophysical Society on February 19th, 2020.

Rebecca Martinez-Moreno, Elisabet Selga, Georgia Sarquella-Brugada, Ramon Brugada, Guillermo Perez, Fabiana Scornik. Cardiac Sodium Current is Severely Impaired in Induced Pluripotent Stem Cell-Derived Cardiomyocytes from Brugada Syndrome Patients. Biophysical Journal 116(3):390a-391a, 2019. DOI: 10.1016/j.bpj.2018.11.2114.

2018-2019

Rebecca Martinez-Moreno, Elisabet Selga, David Carreras Gorgals, Georgia Sarquella Brugada, Ramon Brugada, Guillermo Pérez, Fabiana Scornik. Cardiac sodium current is severely impaired in induced pluripotent stem cell-derived cardiomyocytes from Brugada Syndrome patients. VII Congreso Red Española de Canales Iónicos. Cáceres 15-17 May 2019.

Rebecca Martinez-Moreno, Helena Riuró, Elisabeth Selga, Michael F. Wangler, Ramon Brugada, Guillermo J. Pérez, Fabiana S. Scornik. An SCN1B Variant Found in a Child Diagnosed with Epilepsy and Brugada Syndrome Modifies Brain-Type (NaV1.1) and Cardiac-Type (NaV1.5) Sodium Currents. Biophysical Journal, 114: 490ª, 2018.

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2017-2018

Rebecca Martínez-Moreno, Helena Riuró, Elisabet Selga, Michael Wangler, Ramon Brugada, Guillermo J. Pérez and Fabiana Scornik. An Scn1b Variant Found in a Child Diagnosed with Epilepsy and Brugada Syndrome Modifies Both Brain-Type (Nav1.1) and Cardiac-Type (Nav1.5) Sodium Currents. VI Congreso de la Red Española de Canales Iónicos, 6-8 September, 2017 Santiago de Compostela, Spain.

Elisabet Selga, Ramon Brugada, Guillermo Pérez and Fabiana Scornik. Introduction of single point mutations using a CRISPR-Cas9 double nicking strategy and ssODNs. Cell Symposia, CRISPR: from Biology to Technology and Novel Therapeutics. October 22-24, 2017, Sitges, Spain.

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