Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo: XXXV

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Attività scientifica svolta nel 2° anno di Dottorato, Anno Accademico 2020-2021

Introduction: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy, characterized by asymmetric left ventricular hypertrophy and diastolic dysfunction. Most genotyped HCM patients harbor a mutation in one of the genes coding for cardiac sarcomeric proteins, the cardiac contractile building blocks. Mutations in *MYBPC3*, the gene coding for cardiac myosin-binding protein-C (cMyBP-C), are the most common cause of hypertrophic cardiomyopathy (HCM). During the second year of PHD, I focused on the study of the *MYBPC3-c772G>A* mutation, which is a highly prevalent mutation in the Tuscany. This mutation leads to a reduction of cMyBP-C expression and impairs sarcomere energetics and cross bridge cycling. Specific drug treatments to prevent HCM are not yet available and for this reason the goal of my project is to use patient-specific cells to study disease-related mechanisms in vitro and to identify molecular targets to validate a pharmacological approach. For in vitro disease modeling and for the study of personalized therapies, I used human Engineered Heart Tissues (EHTs) generated by hIPSC-CMs. The hIPSC lines are obtained from HCM patients, with *c772G>A* mutation, and from healthy individuals and are differentiated in CMs for EHTs formation. This patient-specific cell-based approach allows to recapitulate the cardiac phenotype of patients in vitro, giving the opportunity to test drugs, that can be used for specific treatments to reduce the risk of arrhythmias, such as the Mavacamten, an allosteric inhibitor of sarcomeric myosin, which could reduce the impairment of sarcomere energetics.

Methods: HiPSC lines are obtained from patients with the *c772G>A* mutation and are compared with hiPSC lines obtained from healthy people, with the addition of a CRISPR/Cas9 genome edited isogenic control line. The stem cells are differentiated into cardiomyocytes in monolayers and at day 15 of differentiation, single cardiomyocytes are used for EHTs formation. EHTs are made using shaped molds to cast a mixture of immature hiPSC-CMs and a semisolid medium, containing fibrin. After fibrin polymerization between PDMS posts, the resulting tissues are kept in culture until day 50 p.d.

Results: During the maturation, the EHTs are used for optical analysis in auxotonic conditions, by detecting the deflection of the flexible calibrated posts. Optical analysis showed that all EHTs developed greater tension generating capacity and reduced their spontaneous beating frequency at Ca^{2+} [0.4mM]. At day 50 p.d. the EHTs are detached from pillars and are used for mechanical investigations in isometric conditions under pacing stimuli and higher calcium concentrations (4mM). Under these conditions, the effect of Mavacamten 1μM is tested. A reduction of the tension generated by patient EHTs is observed in the presence of the drug. In the future, this approach will be applied to evaluate

a long-lasting treatment of patient-EHTs with Mavacamten to verify potential prevention of disease mechanisms.

- Attendance in ISHR-JMCC webinars:
  - *"Amino terminus of cardiac myosin binding protein-C regulates cardiac contractility*", S. Sadayappan, 09/08/21
  - *"Nano-scale morphology of cardiomyocyte t-tubule/sarcoplasmic reticulum junctions revealed by ultra-rapid high-pressure freezing and electron tomography",* M. Cannell, 24/05/21

- Attendance to lecture at University of Florence:
  - *"Atrial fibrillation: studying specific triggering mechanisms in search of a preventive approach",* A. Barbuti, 19/05/21

- Attendance to meeting of cardiac arrhythmias at Accademic Medical Center of Amsterdam with the lecture "Electrophysiology of Life-threatening Arrhythmias", 09/09/21

- Attendance in Stemcell Technologies scientific webinars:
  - *"Scaling Up hPSCs for future cell therapies",* N. Leite, 22/04/21
  - *"Innovation Showcase: Quality by Design: Reagents and Support for hPSC-Derived Cell and Gene Therapies"*, L. Csontos, 25/06/21
  - *"Plenary VI: Cellular Therapy and Tissue Engineering",* S. Louis, 26/06/21
- Attendance to Complementary skills at University of Siena:
  - "*Creating value from large archive and big data*", L. Neri, 25/05/21
  - *"I rapporti tra scienza e società", A. Allansdottir, 31/05/21*
  - "Soft Skills per fare ricerca nelle organizzazioni", A. Romano, 07/06/21
  - *"Possibili percorsi formativi post PhD", G. Prota, 02/07/21*

- Attendance to the virtual European Muscle Conference, Warsaw, Poland, September 20-22, 2021 with the poster "*ENGINEERED HEART TISSUES for testing personalized therapy in hypertrophic cardiomyopathy: the impact on a MYBPC3 mutation with founder effect in Tuscany*"

- From September 2021 to date, visiting PhD student at the Department of Physiology of the University of Maastricht, under supervision of Prof. Ulrich Schotten, Professor of cardiac electrophysiology.

- Pubblication: *"Optical investigation of action potential and calcium handling maturation of hIPSC-Cardiomyocytes on biomimetic substrate"*

  Josè Manuel Pioner, Lorenzo Santini, Chiara Palandri, Daniele Martella, Flavia Lupi, **Marianna Langione**, Silvia Querceto, Bruno Grandinetti, Valentina Balducci, Patrizia Benzoni, Sara Landi, Andrea Barbuti, Federico Ferrarese Lupi,Luca Boarino, Laura Sartiani, Chiara Tesi, David L. Mack, Michael Regnier,Elisabetta Cerbai, Camilla Parmeggiani, Corrado Poggesi, Cecilia Ferrantini and Raffaele Coppini; International Journal of Molecular Sciences 2019 Aug 3;20(15):3799. doi: 10.3390/ijms20153799