Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo: XXXV

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Introduction: Cardiomyopathies are a heterogeneous group of myocardial diseases associated with mechanical and / or electrical dysfunction, which usually have inappropriate ventricular hypertrophy or dilation and are due to a variety of genetic causes. Cardiomyopathies are divided into two main groups: primary cardiomyopathies, which are mainly limited to the heart muscle, and secondary cardiomyopathies, which are related to generalized systemic disorders. Two important cardiomyopathies are being studied in our laboratory: the Dilated Cardiomyopathy (DCM), developed in subjects with Duchenne Muscular Dystrophy (DMD), and the Hypertrophic Cardiomyopathy (HCM), which is associated with various mutations, most of which reside in the genes that code for sarcomere proteins. During the first year of PHD, I focused on the study of a gene mutation present in the MYBPC3 gene, encoding the cardiac myosin binding protein C (cMyBP-C). This mutation is associated with the development of HCM, which is characterized by left ventricular hypertrophy and diastolic dysfunction. The genetic mutation is called E258K-cMyBP-C and it is a highly penetrant missense mutation with founder effect in Tuscany. We have recently investigated the mechanics and kinetics of contraction using the left ventricular (LV) tissue from three patients affected by HCM, with E258K mutation, and compared to those from donor hearts. The results suggested that the mutation may impair the energy of the sarcomere. To better explore the direct and indirect impact of the mutation, we generated induced pluripotent stem cells (hiPSCs), which were obtained by reprogramming monocytes isolated from HCM patients, with the E258K mutation, and from healthy subjects. hIPSCs have been differentiated into cardiomyocytes (hIPS -CMs) to provide information on the early stage of disease mechanisms.

Methods: Three different hIPSC lines were used: a control line, obtained from healthy subjects, a mutated cell line, obtained from patients with the E258K mutation, and an isogenic cell line, generated by the CRISPR-Cas9 gene correction of the E258K mutation. Through a differentiation protocol, cardiomyocytes were generated from all three hIPSC lines with visible spontaneous contraction after 8 days of culture. On day 20 of differentiation, individual cardiomyocytes were transferred to nanopatterned surfaces, which mimic the extracellular matrix, to improve cell maturation. Despite this process, such cardiomyocytes have showed more immature characteristics than native cardiomyocytes and therefore could help identify the molecular mechanisms that precede the hypertrophic phase of patients.

Results: At different time points of differentiation, protein C expression was observed; the data showed a reduced protein expression in the early stages of maturation in the mutated cardiomyocytes compared to controls. On day 60 after differentiation, cardiomyocytes were

used to perform optical action potential and calcium transient measurements. The results showed similar calcium transient decay in mutants and controls cardiomyocytes and similar cell shortening, suggesting a similar E-C coupling in earlier stages. To better understand the effects of the mutation, studies on the mechanics of myofibrils will be carried out and engineered cardiac tissues (EHTs) will be created with hIPS-CMs to perform contractile force measurements and to test some drugs on cardiomyocyte contraction.

- Partecipation in training courses:
  - *"Utilizzo e cura degli animali da laboratorio",* Università degli studi di Firenze, 11-12/11/19
  - *"Formazione per la sicurezza sul lavoro",* Università degli studi di Firenze, 07/09/20

- Attendance to lecture at Centro studi Fondazione Meyer:
  - *"Verso i pomeriggi con la scienza*", G. Cossu, 11/12/20

- Attendance to lecture at European Laboratory for Non-Linear Spectroscopy (LENS):
  - *"Channelopathies: zooming out from ion channels to gaze at the landscape",* A. Barbuti, 30/01/20

- Attendance in Thermofisher's scientific webinars:
  - *"Rilevazione di RNA e proteine in flow cytometry",* A. Gandelli, 21/04/20
  - *"Western Blot: realizza il tuo esperimento in 4 ore",* A. Gandelli, 22/04/20
  - *"Clonaggio molecolare: seleziona la tecnica più adatta alle tue esigenze",* A. Gandelli, 23/04/20
  - *"Nuovi immunoassay ad alta sensibilità",* A. Gandelli, 28/04/20
  - *"Western blot: total protein normalization e tecnologia No-Stain"*, A. Gandelli, 29/04/20
  - *"Attune NxT vs Covid-19: cosa dobbiamo capire",* A. Gandelli, 30/04/20
  - *"Gene synthesis: risparmia tempo ottimizzando il tuo Progetto di clonaggio"*, A. Gandelli, 06/05/20
  - *"Attune NxT: la citofluorimetria resa semplice, innovativa e flessibile"*, A. Gandelli, 07/05/20

- Attendance to lecture at University of Milan:
  - *"La percezione pubblica della scienza: sperimentazione animale, vaccini, scienza e Covid-19"*, E. Cattaneo, 27-28/04/20
  - *"Stem cells: from molecular physiology to human disease modelling"*, A. Barbuti, 27-28-29/05/20

- Attendance in Stemcell Technologies scientific webinars:
  - *"Improving reproducibility of your hPSC research by generating a high-quality cell bank"*, M. Hildebrandt, 06/05/20
  - *"Considerations for high-efficiency genome editing of hPSC"*, A. Watson, 13/05/20
  - *"Quality control guidelines for clinical-grade hIPSC lines"*, A. Gaffney, 20/05/20

- Attendance in International Society for Heart Research webinars:
  - *"Mitochondrial redox control in hereditary cardiomyopathies"*, C. Maack, 12/05/20
  - "*Top-down proteomics for deciphering hypertrophic cardiomyopathy*", T. Tucholski, 18/05/20
  - "*Context-specific network identifies new interactions in beta-adrenergic cardiac hypertrophy",* A. Khalilimeybodi, 21/05/20
  - *"Interplay between the ubiquitin-proteasome system and autophagy",* Xuejun XJ Wang, 22/05/20
  - *"Hypertrophic cardiomyopathy: sarcomeric mutations and disease modifiers*", D. Mosqueira, 05/06/20

- Attendance to Complementary skills at University of Siena:
  - "*Diritto dei brevetti",* V. Santoro, 11/05/20
  - "*Comunicare in ricerca*", E. Meli, 03/06/20
  - *"Spin off e start up della ricerca: concetti introduttivi e presupposti per la nascita di un'impresa"*, L. Zanni, 08/06/20
  - *"Nuovi strumenti per l'analisi della risposta immunitaria alla vaccinazione e all'infezione tramite un approccio si "Systems Biology",* A. Ciabattini, 11/06/20
  - *"I rapporti tra scienza e società tra persistenze",* A. Allansdottir, 17/06/20
  - *"Scientific writing and presentation",* J. Telford, 08/09/20

- 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