SCHEDA RELAZIONE DOTTORANDI

Al collegio docenti del Dottorato in Medicina Molecolare

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Introduction

Optical imaging is considerably limited by light scattering, a physical phenomenon that prevents light to penetrate deep into the tissue. In the last few years a number of optical clearing methodologies have been developed to overcome this limitation. This year I focused my work on the optimization of an optical clearing and staining protocol to successfully image cardiac tissue features in a murine model of arterial hypertension and in controls. Indeed, one important feature of this condition is remodelling of intramural small coronary arteries and arterioles. We investigated the implications of this remodelling in the downstream vascular organization, in particular at the capillary level. We used Spontaneously Hypertensive Rats (SHR) exhibiting many features of the human hypertensive cardiomyopathy and the normotensive Wistar Kyoto Rats (WKY) at different time-points of age (4, 8, 18 and 24 weeks). We investigated if microvascular remodelling precedes or follows the onset of systemic hypertension and left ventricle hypertrophy and how it correlates with other elements of myocardial remodelling (e.g. myocyte disarray). Now we are trying to extend the analysis of the tissue features in the whole-heart in mice with different cardiac pathologies to compare them to controls.

Methods

We generated 3D high-resolution mesoscopic reconstructions of the entire network of SHR hearts combining gel-based fluorescent labelling of coronaries with a CLARITY-based tissue clearing protocol (Fig 1). We also co-stained the tissue with a Wheat Germ Agglutinin (WGA) conjugated to Alexa Fluor 594 to detect cellular membrane. Collagen was detected through second harmonic generation. We imaged the tissue using a custom-made Two-Photon Fluorescence Microscope. We performed morphometric quantification of the capillary network over time to assess capillary diameter and linear density using software Amira 5.3 (Thermo Fischer) and a custom-made Labview interface (National Instruments). To quantify myocardial disarray and vascular angular dispersion in the tissue, we developed an automatic software tool to estimate the 3D orientation of vessels and cardiomyocytes. The software virtually dissects the volume in portions and applies a Structure Tensor analysis to extract the main direction of the cells or vessels, estimating multiple local disarrays. We are optimizing a different tissue transformation protocol, the SHIELD protocol, which offers some advantages compared to CLARITY: hearts are able to become completely transparent in about 18-20 days compared to the 3-4 months needed using the CLARITY methodology. We are also trying to develop a different protocol to label the vascular network without using the gel, which remains stuck into the cardiac cameras, proving fluorescence from the inside.

Results

In SHRs, we found significant remodelling of the capillary network density and dispersion. SHR capillary density is increased in both ventricles and at all ages, including before the onset of systemic hypertension (Fig 2). This result suggests that remodelling occurs independently from the onset of systemic hypertension and left ventricular hypertrophy. On the contrary, capillary angular dispersion increases with time in SHR. Consistently, our multicolor imaging underlined a strong correlation between vascular dispersion and cellular disarray. Together the data show that 3D high-resolution

reconstruction of the capillary network can unveil anatomic signatures in both physiological and pathological cardiac conditions, thus offering a reliable method for integrated quantitative analyses.

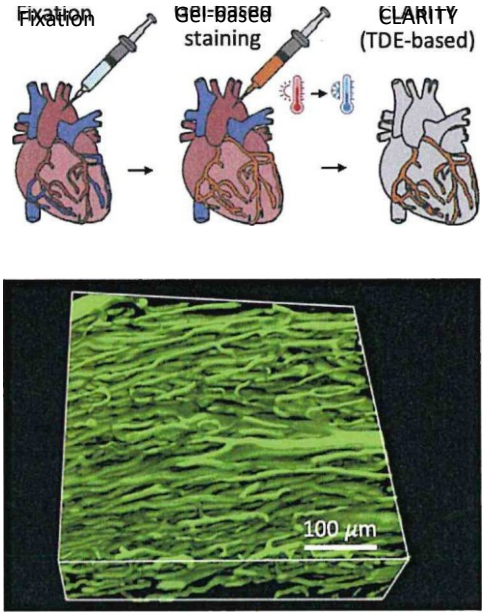
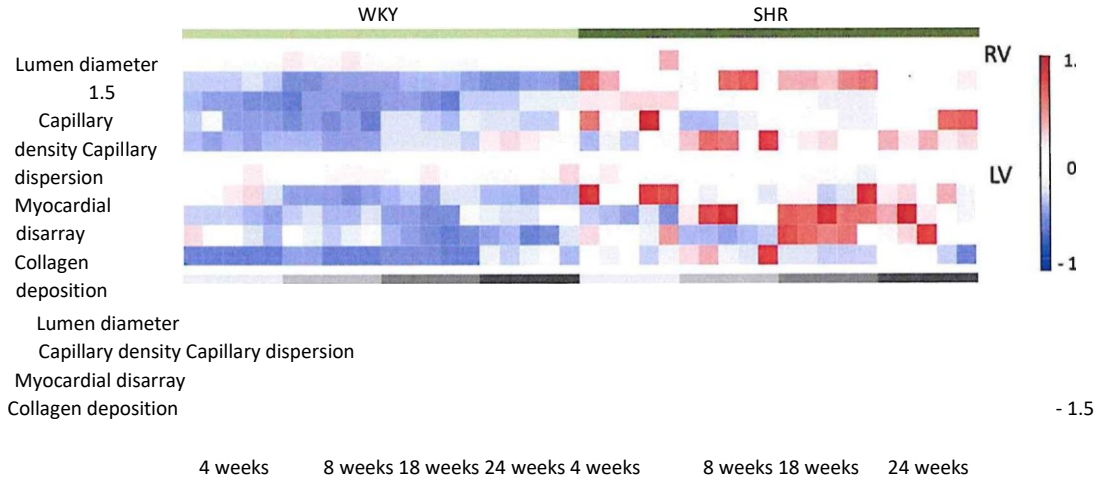


Figure l: On the top: Diagram showing the main steps of the BSA-FITC/CLARITY protocol. Heart is rapidly isolated, cannulated through the proximal aorta and perfused with fixative solution. Coronaries are stained with BSA-FITC gel and the organ is subjected to modified CLARITY protocol. On the bottom: Representative 3D reconstruction of a stack of 450 x 450 x 300 pm^3 acquired with a custom- made Two Photon Fluorescence Microscope. 3D reconstruction was created using



ImageJ 2.0.0.

Figure 2: Heat Map summarizing vascular, cellular and collagen parameters in all the experimental classes. Five different ventricular regions are shown for each experimental class. All the parameters have been normalized on the same scale. RV is right ventricle (above) and LV is left ventricle (below). Heat Maps were created using OriginPro 9.0,

Publications

- 2020 — Olianti C, Costantini I, Giardini F, Lazzeri E, Crocini C, Ferrantini C, Pavone FS, Camici PG, Sacconi L; 3D imaging and morphometry of the heart capillary system in spontaneously hypertensive rats and normotensive controls. Scientific Reports; doi.org/l O. 1038641598-020-711749

Abstracts

2020 - 3D imaging and morphometry of the heart capillary system in spontaneously hypertensive rats and normotensive controls, Biophysical Society, San Diego, Poster performed by Dott. Sacconi.

2020 - 3D imaging and morphometry of the heart capillary system in spontaneously hypertensive rats and normotensive controls, Frontiers in CardioVascular Biomedicine, Budapest (conference postponed).

2020 - 3D imaging and morphometry of the heart capillary system in spontaneously hypertensive rats and normotensive controls — Working Group, Amsterdam (conference postponed).