

ESC Council on Basic Cardiovascular Science

First Contact Initiative Grant 2016 Report

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Current Institution: University of Bristol (UK). Bristol Heart Institute, Lab of Experimental Cardiovascular Medicine (supervisor: Professor Paolo Madeddu)

Host institution: Tel Aviv University (Israel). Neufeld Cardiac Research Institute directed by Professor Jonathan Leor. Heart Center, Sheba Medical Center, Tel-Hashomer.

Duration of the visit: 26th March to 7th April 2017

Project: Contribution of pericytes to cardiac arteriogenesis in the healthy and infarcted heart

First of all I wish to express my immense gratitude to the ESC Council on Basic Cardiovascular Science for awarding me with one of the First Contact Initiative Grants 2016. This bursary has supported me over a 2-week visit to Tel-Aviv University. I had the great fortune to meet with Professor Leor and work at his lab based at the Neufeld Cardiac Research Institute, which is part of one of the most prestigious centres for cardiovascular research. I wish also to express my gratitude to Prof Leor for welcoming me in his team, offering me the opportunity to perform some important preliminary experiments for my project.

Background to the project. The human neonatal heart is a plastic tissue in which the vascular network grows to support the increasing perfusion needs of the growing organ. Moreover, the new-born human heart is capable of significant functional recovery after an acute ischemic event. At the opposite, the revascularisation of the human adult heart after an acute injury such a myocardial infarction (MI) does not occur at levels able to support the functional recovery of the organ. A better understanding of the molecular and cellular mechanisms implicated in the age-related decline of arteriogenesis might provide novel therapeutic targets for the treatment of myocardial ischemia.

Cardiac pericytes (CPs) are perivascular cells particularly abundant around the cardiac vasculature, where they have several functions, among which supportive and trophic functions for endothelial cells. A published study demonstrated epicardial CPs contribute to the formation of coronary arteries during the mouse heart development. We hypothesize CPs still retain this arteriogenic ability in the post-natal heart. In order to investigate this theory, we decided to use a transgenic mouse model in which platelet derived growth factor receptor beta (PDGFR β)-positive cells (that identify pericytes with a high specificity) are recognised by the red fluorescent marker tdTomato, constitutively expressed under the PDGFRB gene promoter. This mouse model, available in Professor Leor's lab, allows the tracing of the CP fate *in vivo*.

Aim of the visit to the host institution. The aim of my visit to the host lab was to perform some preliminary histological evaluations in the transgenic adult mouse hearts to determine:

1. If arterial vascular smooth muscle cells (VSMCs) express the tdTomato-PDGFR β marker;
2. If MI is a stimulus for recruitment of CPs to the site of injury;
3. If tdTomato-PDGFR β -positive CPs contribute to the generation of new arteries in the infarcted myocardium.

Moreover, given the macrophages contribution to the angiogenic process has been previously recognised, I am planning to study if the interaction between CPs and macrophages further supports the arteriogenic process in the heart. During my stay at the Tel Aviv lab, I have learnt the protocol for isolation of cardiac macrophages from murine hearts.

Experimental work.

During these preliminary experiments, I have analysed heart sections of adult *tdTomato-PDGFR β* mice, which underwent either the permanent ligation of the left anterior descending coronary artery for induction of MI (N=1) or sham operation (N=1). Animals have been sacrificed 7 days post-surgery. PDGFR β -positive CPs have been recognised by the red fluorescent marker tdTomato, while mature contractile VSMCs have been identified by immunofluorescent staining for the highly specific marker smooth muscle-myosin heavy chain (SM-MHC).

Sections have been analysed with a confocal-fluorescence microscope (Zeiss).

Results.

VSMCs forming arteries are positive for SM-MHC but negative for PDGFR β (**A**). This means SMCs in this mouse model do not express PDGFR β that is a marker proper of pericytes and other cells. This was a first encouraging observation, because it means the transgenic mouse model is good for tracing the transition from the CP to the VSMC, and possible cells expressing both SM-MHC and PDGFR β might represent a transitional form between the CP and the VSMC, showing a contribution of CP to the arteriogenic process.

We could not appreciate many PDGFR β -positive cells in the sham animal (**B**) and in the remote zone of infarcted mouse hearts (**C**). At the opposite, we observed a massive recruitment of PDGFR β -positive cells to the infarcted area (**D and respective magnifications**). This evidence is of great interest because it suggests CPs play a primary role in the healing of the infarcted myocardium. However, in these first experiments we were not able to identify arterial vessels containing PDGFR β -positive cells, necessary to demonstrate the direct contribution of CPs to the post-MI remodelling arteriogenesis. Moving from these promising results, further experiments will be conducted in the future with a larger number of animals to better investigate the phenomenon object of our question. Moreover, additional VSMC markers will be evaluated, as well as markers of macrophages and inflammatory cells will be assessed in order to discriminate other blood-derived PDGFR β -positive cells from pericytes.

In addition, during my stay at the host lab, I had the pleasure to present my work during a seminar, and this has been an amazing opportunity to discuss the project with Professor Leor and his team, and receive important feedbacks and advices for the future.

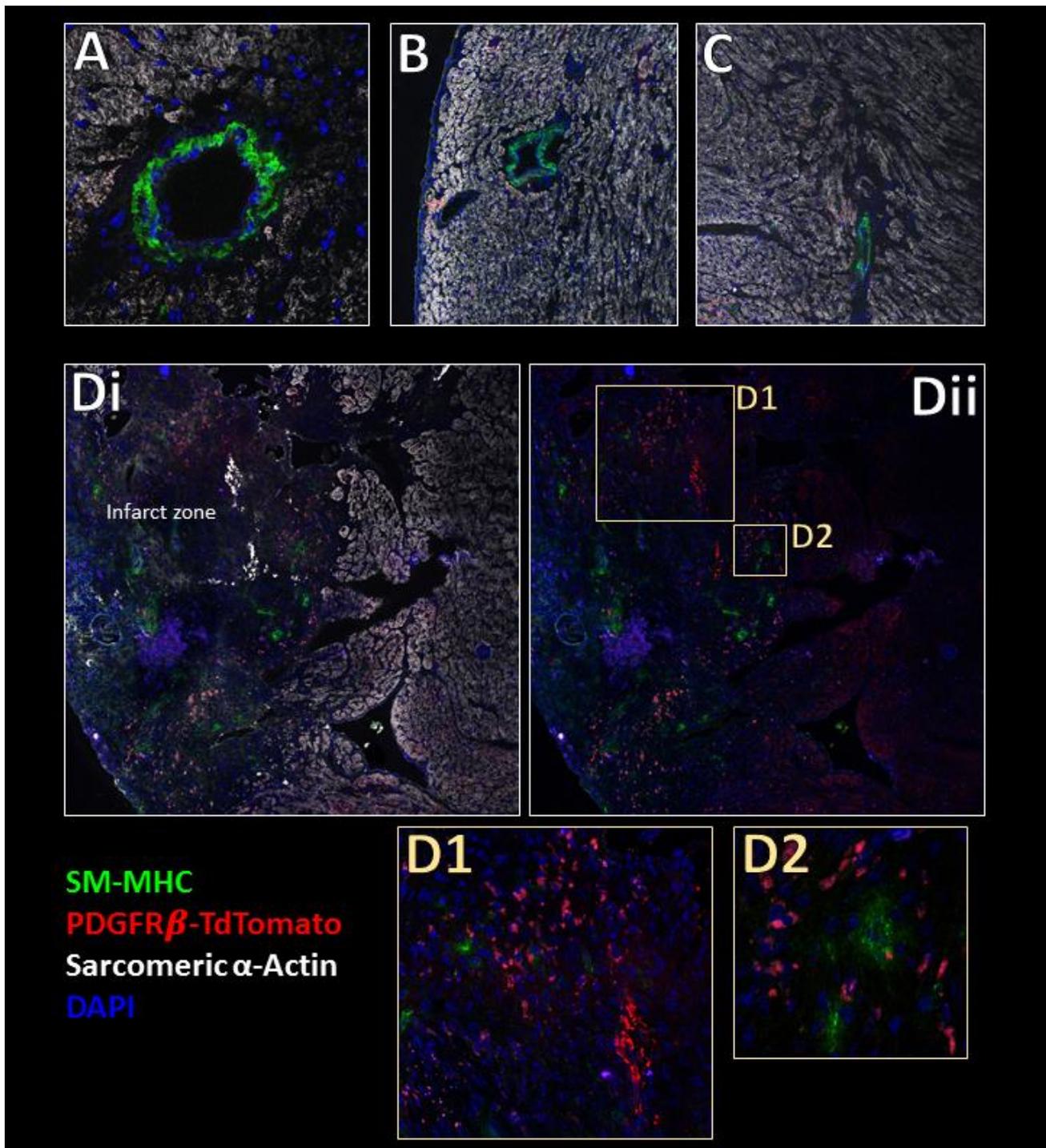


Figure legend. Confocal fluorescence microscope images. (A) Arterial SMCs are negative for PDGFR- β . Magnification 400X (B) Sham mouse heart section. Magnification 100X (C) Remote zone of a MI mouse heart section. Magnification 100X. (D) Infarct and peri-infarct zone of a MI mouse heart section. Magnification 100X (reconstruction of 9 images). In (D1) and (D2), two fields at higher magnification to appreciate better PDGFR- β -positive cells recruited to the infarcted area.

Future collaboration.

Despite the limited time spent in Tel Aviv did not allow me to perform more experiments, we agreed both teams are happy to continue this collaboration in the future. I wish to thank both Prof Jonathan Leor and my supervisor Prof Paolo Madeddu for being very supportive for this initiative.

Prof Leor will send me more histological samples in Bristol to continue the histological evaluation.

In conclusion, my visit to Professor Leor's lab has been an enriching experience, not only at the professional but also at the personal level.