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ESC Council on Basic Cardiovascular Science

First Contact Initiative Grant 2017 Report
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Current Institution: Department of Electrophysiology, Helios Heart Center Leipzig, Germany, Prof. Bollmann, MD, PhD and Prof. Hindricks, MD

Host institution: Regenerative Medicine Center, UMC Utrecht, The Netherlands, Prof. Pieter Doevendans, MD, PhD and Prof. Joost Sluijter, PhD

Project: Human left atrial appendage resident progenitor cells in atrial fibrillation and fibrosis and potential implications in treatment of arrhythmia

First, I would like to thank the ESC Council on Basic Cardiovascular Science for awarding me with one of the First Contact Initiative Grants 2017. The grant gave me the opportunity to visit the labs at the Hubrecht Institute and UMC Utrecht (working group of Pieter Doevendans, MD, PhD and Joost Sluijter, PhD) to perform important preliminary steps for my project. Under supervision of Dr. Klaus Neef and Corina Metz I had the opportunity to gain insight in *in vitro* culture of cardiac progenitor cells. I started with the isolation of cardiac progenitor cells from fresh surgical tissue and continued the culture using selective growing conditions. This training provided a successful beginning of my own studies to propagation and differentiation of progenitor cells from left atrial appendages.

Background:
Cardiac progenitor cells (CPCs) provide a tremendous potential in disease-modeling and developing therapeutic approaches. Growing interest focused on resident CPCs. Currently, it is generally accepted that CPCs have the potential of stimulating regeneration, even if the detailed underlying mechanisms are still unknown and other CPC populations than the widely used c-kit-positive cells are studied scarcely. Both atria and atrial appendages constitute a promising reservoir for multiple types of CPCs which can be isolated. Human left atrial appendage progenitor cells content and distribution in human left atrial appendages, have not been described in detail to date.

Report:
In a first step I had the opportunity to get introduced to the CPC isolation protocol that has been published by Smits and colleagues in *Nature protocols* at the UMC Utrecht (1). Corina Metz, one of the coauthors and Klaus Neef, PhD, introduced me into the basic techniques in cell isolation using magnetic cell sorting with iron-labeled mouse anti-Sca-1 antibodies.
Following theoretical discussion and instruction about the protocol steps and variations, we prepared a CPC isolation test run. Subsequently, we performed a test isolation with fresh cardiac left atrial appendage tissue from cardiac surgery and further cell culture (Figure A, B). Later on, Tina Fischer, a technician at Heart Center Leipzig, joined me to get familiar with the new technique. Again we went through the protocol together with Klaus Neef and Corina Metz and optimized several preparation

details to allow a smooth transfer of the technique to our lab at Hear Center Leipzig. Following expanding cell numbers, cultured cells were cryo-conserved and are now available for further experiments on our site.
In the next step we are going to continue cell culture at Leipzig and characterize the isolated cells using FACS and immunofluorescence based microscopy at Heart Center Leipzig.
Additionally, we already discussed further project steps (immunohistological characterization of distribution patterns in left atrial appendages) and established a protocol for detection of c-kit positive and Isl-1 positive cells with histological immunofluorescence techniques.
To prepare further experimental approaches at Heart Center Leipzig, I already submitted an application for systematic tissue collection during cardiac surgery to our local ethics committee, and after its successful approval, I began to collect left atrial appendages from cardiac surgeries according to the ethical guidelines. So I have been able to build up a continually growing biobank. Following the initial basic histological workup, I applied the immunohistological protocols to detect and localize CPCs (Figure C, D). For several months I have been supervising the experimental work of a medical student who joined my group to prepare the medical doctor thesis in this field. At the moment we are working on the optimization of the immunofluorescence protocols (Figure E).

In conclusion, the bursary from ESC Council on Basic Cardiovascular Science enabled me a successful beginning of my studies in the field of *in vitro* investigations of heart CPCs during my visit at UMC. I also want to express my gratitude to Prof. Doevendans and Prof. Sluijter for welcoming me in their team and to Dr. Klaus Neef and Corina Metz for their help with hands-on training in CPC isolation. In addition to that, the research stay did not only extend and improve my methodical skills, but also inspired and helped me to consider and focus my further research plans. Subsequent to the ESC first initiative grant, I received a further local grant from the ProCordis Foundation, that supports to finance both our histological studies and a starter kit for CPC isolation. I will now be able to establish the newly learned techniques to our laboratory in Leipzig and I am looking forward to first results. It is one of my greatest ambitions to continue and expand this project besides my clinical training in cardiology and electrophysiology.

Yours Sincerely

Dr. Laura Ueberham

Reference

(1) Human cardiomyocyte progenitor cells differentiate into functional mature cardiomyocytes: an in vitro model for studying human cardiac physiology and pathophysiology.Smits AM[1], van Vliet P, Metz CH, Korfage T, Sluijter JP, Doevendans PA, Goumans MJ.Nat Protoc. 2009;4(2):232-43. doi: 10.1038/nprot.2008.229.

Figure:

A) Cell debris immediately after isolation

B) Cells at the first passage (14 days after isolation)

C) Immunohistological staining for c-kit positive cells in left atrial appendage tissue, HE staining

D) Left atrial appendage section, HE staining

E) C-kit positive cells, immunofluorescence staining