UMC Utrecht

ESC First Contact Initiative Grant

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Dear ESC Council on Basic Cardiovascular Science,

With this letter, I would like to thank the ESC Council on Basic Cardiovascular Science for awarding me with the 2022 ESC First Contact Initiative Grant. This support gave me the opportunity to visit Stockholm and initiate a collaboration with the lab of professor Samir El Andaloussi, Division of Biomolecular and Cellular Medicine at the Karolinska Institute.

**Background**
The aim in our research group is to stimulate cardiac repair after a myocardial infarction, to reduce the damage which is caused by the infarction and prevent remodeling of the heart to the chronic state of heart failure. Preventing or slowing down the development of heart failure would improve both life expectations and quality of life for patients who suffered from a myocardial infarction. For this aim, extracellular vesicle (EV) therapeutics are studied. EVs are nano-sized particles and make up an endogenous intercellular communication system. They transfer active biomolecules (e.g. proteins, RNA) thereby affecting physiological processes. In fact, preliminary data suggest that EVs derived from cardiac progenitor cells are amongst the most potent vectors for cardiac repair by means of reducing immune responses and fibrosis, increasing cell viability, proliferation, and angiogenesis.
Clinical translation is however challenging. Increasing evidence indicates that EVs present a heterogeneous mixture of vesicle populations, with distinct compositions and functions, hampering clinical translation. Selecting specific subpopulations of EVs that are enriched with specific proteins may enhance the efficiency of the cardiac repair characteristics of EVs. Therefore, studying EV heterogeneity could provide new insights into contributing therapeutic mechanisms underlying EV-mediated cardiac repair.
Our experimental design consists of a highly reproducible method for the separation of EV subpopulations based on customized size exclusion chromatography to study functional EV heterogeneity. Distinct subpopulations of cardiac progenitor cell derived EVs were identified based on differential expression of common EV markers. These EV subpopulations differed in size, appearance, proteomic composition and function.

**Aim and results**
During my stay at the Karolinska Institute I aimed to learn the technique imaging flow cytometry to perform single EV measurements to study specific EV protein marker distributions to increase the knowledge about EV heterogeneity. Single particle analysis would allow for a more tailored approach to study active EV subpopulations for cardiac repair.

To obtain EV subpopulations, I used a two-step size exclusion method that I developed during my PhD in Utrecht. We isolated EVs from both cardiac progenitor cells and mesenchymal stromal cells and separated EV subpopulations. At the Karolinska Institute I performed single particle analysis using imaging flow cytometry to characterize and compare the protein expression of these EV subpopulations derived from two different cell lines. Single EV particle measurements are still very challenging often leading to incomplete characterization. However, the host lab has an extensive expertise in high-sensitivity imaging flow cytometry to characterise EVs. Imaging flow cytometry is a method combining flow cytometry with multichannel imaging of hundreds of thousands of events within minutes including high-information-content analysis driven by deep learning algorithms. This makes it ideal for high EV-content analysis, raising the possibility to identify specific EV subpopulations.
We selected different EV surface protein marker candidates by recently acquired mass spectrometry data and furthermore by the MACSPlex Exosome bead assay performed at the Karolinska Institute during the beginning of my stay. The MACSPlex Exosome Kits allow detection of 37 exosomal surface proteins. Based on this data we could find different expression patterns for over 10 EV protein markers between the analysed EV subpopulations. The found results were consistent compared to our mass spectrometry data and more interestingly, overlap between EV subpopulations derived from different cell types was observed. These results demonstrate the existence of specific EV subpopulations with specific protein marker signatures which are conserved in different cell types.
Overall, I acquired good results which will be used for a scientific publication. Increased knowledge of EV heterogeneity will contribute to a better understanding of the mechanisms of action of EVs on cardiac repair and will improve the clinical translation.

**Use of the grant and personal development**
During my research stay I gained unique experience that I can use in the continuation of my PhD. With the financial support of the ESC I was able to afford my stay and make most out of my time. It was an amazing experience to go abroad to a top research institute to work with a leading group in the EV field. Professor El Andaloussi is an expert in the EV field and his laboratory has vast expertise in EV engineering, the delivery of therapeutics and single vesicle analysis. I learned a lot from speaking with different researchers from the lab and the general meetings which were usually followed by extensive discussions. There was a very open climate, everybody was enthusiastic to tell about their research and to help me to learn new techniques.
I succeeded my goal of my stay, to learn imaging flow cytometry and apply it for my research. In the host institution, I received training from Dr. Görgens which is a well-recognized expert in this technique and demonstrated its potential to analyze single EVs. He helped me a lot to learn the technique, apply it in my own research and to analysis the large datasets. By the end of my stay I could prepare my own protocols and do all experiments individual for my research project.
Altogether, I am convinced that this experience will contribute to my career as a researcher and it helped me to set up new collaborations with the host group. I learned a lot during my visit from the highly experienced staff and diverse projects about EVs and drug delivery approaches.

Yours sincerely,

Simon van de Wakker, PharmD