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First Contact Initiative Grant Report

I would like to thank Prof. Jeremy Pearson and the ESC Council on Basic Cardiovascular Science for awarding me with this grant in July 2018.

It provided me with the invaluable opportunity to visit the laboratory of Prof Roger Foo at the Genome Institute Singapore and National University of Singapore in spring 2019.

My postdoctoral research at Maastricht University focuses on novel molecular mechanisms and biomarkers for sub-types of heart failure. In particular, toxic cardiomyopathy (tox CMP, induced by cardiotoxic chemotherapies) and heart failure with preserved ejection (HFpEF). Both these forms of heart failure, which are specific in their inciting etiology and pathophysiology respectively, currently lack early diagnostic biomarkers and effective therapies.

I have identified a novel long non-coding RNA (lncRNA) that is upregulated in HFpEF in females specifically. More than 70 % of HFpEF clinical cohorts are female and this sex difference is still poorly understood. The novel lncRNA is upregulated in ventricular tissue in human patients as well as in preclinical animal models of HFpEF. Moreover, it is detectable in the circulation (plasma) and we have promising pilot data indicating it could be an early diagnostic biomarker for HFpEF in females.

This lncRNA is antisense to an important myocyte-specific and contractility regulating protein-coding gene, much like *Mhrt* (Han et al., 2014). Through interaction with chromatin remodeling protein, *Mhrt* regulates gene expression in the heart in disease and its overexpression is protective against pathological remodeling.

My primary research project in Maastricht is to characterize this novel lncRNA and uncover its role in the pathophysiology in heart failure, as a circulating biomarker as well as its therapeutic potential.

I had recently identified a manuscript from the laboratory of Roger Foo, describing the landscape of circular RNAs in the human heart (Tan et al., 2017). Included in their data set, was some circular RNAs identified that were transcribed from my lncRNA of interest.

With the help of Dr Benson Lim, Wilson Tan and Lavenniah Annadoray, I had the opportunity to learn from their extensive expertise and skills in identifying circular RNAs at the level of bioinformatics analysis and molecular biology. This included digesting RNA using RNase R to

which circular RNAs are resistant, identifying back-splice junctions and designing primers to amplify back-spliced RNA products only.

Bioinformatically, we identified four circular RNAs that are potentially transcribed from my lncRNA gene of interest in human hearts with different degrees of and aetiologies of heart failure. Two of these – circRNA-11 and circRNA-6 - I validated successfully by RTqPCR in a cohort of human heart tissue RNA, some control healthy donor hearts as well as explanted hearts from end-stage dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM) patients that have had a heart transplant (Figure B).

I then tested the resistance of circRNA-11 and circRNA-6 to RNase digestion, compared with classical linear RNA transcripts (e.g. 18S) (Figure A).

I also examined the localisation of these circRNAs as well as the linear lncRNA by isolating nuclear RNA from myocardial tissue (from a healthy donor control heart) separately from cytoplasmic RNA. The linear RNA as well as the circRNA, though to a lesser degree, where all nuclear-enriched (Figure C).

Furthermore, both of these circular RNAs identified was also detectable in human plasma by RTqPCR (Figure D). Circular RNAs are emerging as highly stable and specific circulating biomarkers of human disease (Zhang et al., 2018). Estimated stability is at least 2.5 times longer than linear RNAs (Enuka et al., 2016).

Following up from this visit to Roger Foo's laboratory, the knowledge I have gained and the skills I have developed, I will analyse circRNA-11 and circRNA-6 in cardiac biopsies and plasma in external patient cohorts such as those from Maastricht University Medical Center (MUMC+) heart failure and HFpEF clinics and overlay with clinical data.

I will also identify circRNA function with the help of knockdown and inhibition functional studies *in vitro* and *in vivo*.

In addition, with the knowledge imparted from members of the Foo lab, I will examine circular RNAs in biopsies and plasma from toxic CMP patients. These are patients that have undergone cancer treatment with cardiotoxic chemotherapies. In particular, treatment with anthracyclines such as doxorubicin. It is predicted that anthracycline treatment can increase the risk of cardiomyopathy by up to 30 % in cancer sufferers. However, as an anti-cancer therapy, they are highly effective.

Identifying biomarkers to predict which cancer patients are most at risk of long-term cardiotoxic effects could then be used in the clinic to personalise both the cancer treatment regime as well as to tailor medication programmes to try to reduce the risk of cardiovascular insult after cessation of chemotherapy. Previous investigations seeking to identify protein or microRNA (short, linear RNAs)-based biomarkers for cardiotoxicity prediction, diagnosis and prognosis in early stages have not yielded consistent positive results that have withstood external validation.

The MUMC+ heart failure clinic has a number of patients that have cardiac dysfunction following anthracyline treatment and I will examine circulating circRNAs in toxic CMP compared with non-toxic CMP at a similar stage of severity along with sex-matched and age-matched control.

From the data generated during the ESC FCI grant period and, importantly, from future experiments enabled from the skills acquired and techniques learnt at my home institution, two significant publications in the field of molecular cardiology are expected to be generated. The ESC and this grant will be acknowledged.

Moreover, the preliminary data and newly acquired expertise will contribute to junior postdoctoral fellowship applications that I will apply for in the next year, to enable me to continue my academic career in this field in European institutions.

Another outcome of this award is that it has strengthened collaboration and interactions between the home and host laboratories. In the field of translational biomarker research, having access to numerous patient cohorts, in particular spanning different countries and continents for external validation of findings, strengthens the conclusions as well as increase the chance for implementation of outcomes in the heart failure clinic. In this way, I hope to identify effective biomarkers for difficult-to-diagnose and difficult-to-treat forms of heart failure for patients.

Yours sincerely,

Dr Emma Louise Robinson

References

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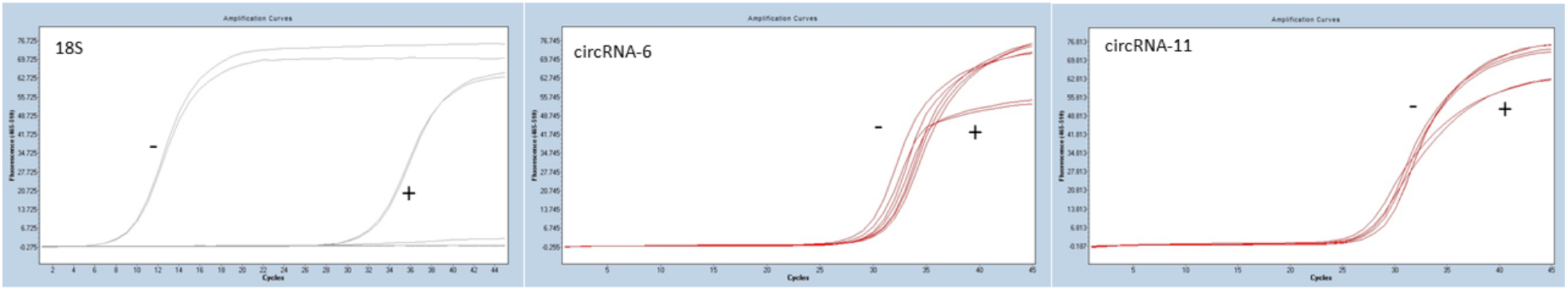
A



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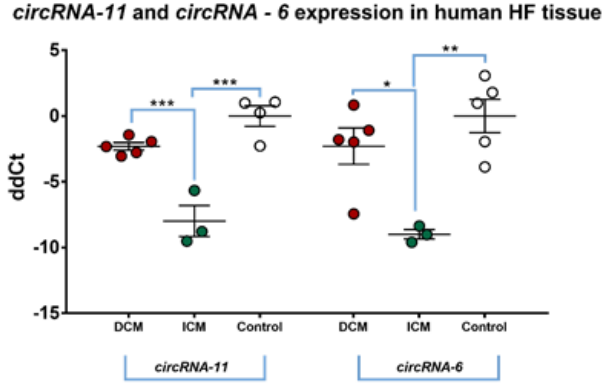

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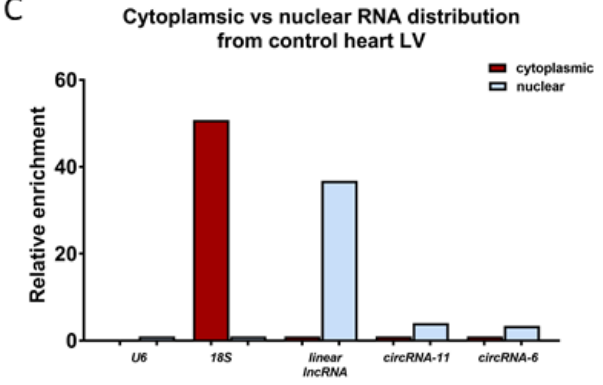

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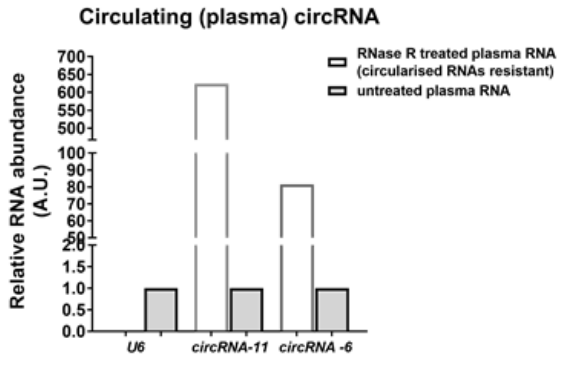

A RT-qPCR amplification curves (Roche Lightcycler LC480) for 18S, circRNA-6 and circRNA-11 with Rnase R treated RNA as the starting material (+) and untreated RNA (-) as the starting material from a pool of human left ventricular tissue RNA (control, ICM, DCM). Indicidual data points displayed and each point indicates one biological replicate. Each point is the mean of three technical replicates.

B Expression of circRNA-11 and circRNA-6 in human LV tissue from healthy donor controls, dilated cardiomyopathy (DCM) and ischemic cardiomyopaythy (ICM) patients. Values are displayed as ddCt (comparative Ct method of qPCR analysis) and statistical analysis performed by one-way ANOVA. ***= p< 0.001, **=p<0.01, *=p<0.05.

C Relative circRNA expression in RNA extracted from nuclear vs cytoplasmic fractions from a healthy donor control heart (n=1).

D Relative circRNA abundance in plasma from a healthy donor, with data displayed from RNA with and without Rnase R treatment prior to cDNA synthesis (n=1).