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Global Spotlights

ERC-funded grant: cardiac regeneration

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The promise of regenerative medicine for restoring heart function

Heart failure is a leading cause of morbidity and mortality in the world, but scientists and clinicians have not yet figured out a way to heal failing hearts. Left-ventricular-assisted devices or heart transplantation is the only solution at advanced stages.[1] The ultimate cause underlying heart failure is the lack of regenerative ability of the mammalian heart.[2–4] While other tissues/organs regenerate by activating proliferation of differentiated cells (liver) or by activating stem cells (skeletal muscle), the heart lacks bona fide stem cells, and cardiomyocytes proliferate very poorly in the adult mammalian heart. Hope relies on the fact that, upon massive acute loss of myocardial tissue, lower vertebrates and newborn mammals do regenerate their hearts by activating cardiomyocyte proliferation (liver-type regeneration). A key factor determining regenerative ability is the abundance of diploid cardiomyocytes, given that these are the only ones with robust proliferative capacity[5] and high abundance of diploid cardiomyocytes is a feature shared by vertebrate hearts with regenerative ability.[6] In contrast, non-regenerative hearts, such as the adult human heart, mostly contain polyploid cardiomyocytes. Yet, a small proportion of adult diploid cardiomyocytes (ADCs) remains in adult hearts and scientists have been on the hunt for strategies to target and activate this population, thereby activating regeneration. Unfortunately, efforts to identify molecular features of ADCs have been largely unsuccessful, precluding the deployment of this strategy.[7,8] The ERC-funded REACTIVA project will now fill this gap by identifying and functionally studying ADCs in the mouse model.

A new strategy to potentiate pro-regenerative cardiomyocytes in mammalian hearts

An improved methodology has allowed isolation of adult mouse ventricular cardiomyocytes and their classification according to the number of nuclei (most polyploid cardiomyocytes are multinucleated in the mouse) (*Figure 1A*). This improved technique produced unprecedented high-quality and in-depth single-cardiomyocyte RNA sequencing, which allowed for the first time to define the molecular signature of ADCs. Transcriptomic comparison between bi- and mononuclear cardiomyocytes within the working myocardium revealed a set of 104 genes that specifically characterized the mononuclear population. Within them, markers of the cardiomyocyte foetal programme, factors that protect from oxidative stress and factors that reduce contractility and calcium cycling were found. These markers show that ADCs seem more immature than their polyploid counterparts, an important observation considering that foetal and neonatal cardiomyocytes are pro-regenerative. The next challenge was how to enable the control of this gene expression signature: was there a hierarchical organization that regulates the expression of these genes and if so, can it be modulated? Transcription factors (TFs) regulate sets of genes by binding specific DNA motifs and, consequently, inducing or repressing their expression. Despite being difficult to profile using single-cell RNA-seq technologies, due to their low transcript abundance, TF activity can be predicted using *in silico* methods. These rely on the construction of prior knowledge networks that describe TF—target gene relationships.[9] Based on the prior knowledge network, putative TFs that regulate a transcriptomic footprint can be found through statistical enrichment of their known target genes. Using this methodology, a polyploidization TF (PTF) predicted to repress the mononuclear cardiomyocytes transcriptional signature was identified (*Figure 1A*).

Genetic labelling of PTF-expressing adult CMs detected a prevalence of binuclear CMs, validating its role as a repressor of the mononuclear gene profile. As proof of the relevance of this finding for cardiomyocyte ploidy, PTF silencing *in vivo* sharply increased the proportion of mononuclear cardiomyocytes, confirming that the identified PTF acts as a master regulator of binucleation. Moreover, these induced diploid cardiomyocytes showed features of immaturity, including increased proliferation and reduced size. The discovery of PTF and its downstream gene regulatory network thus provides new tools to analyse the functional role of ADCs in the mammalian heart during postnatal development, adult homoeostasis, aging, and injury.

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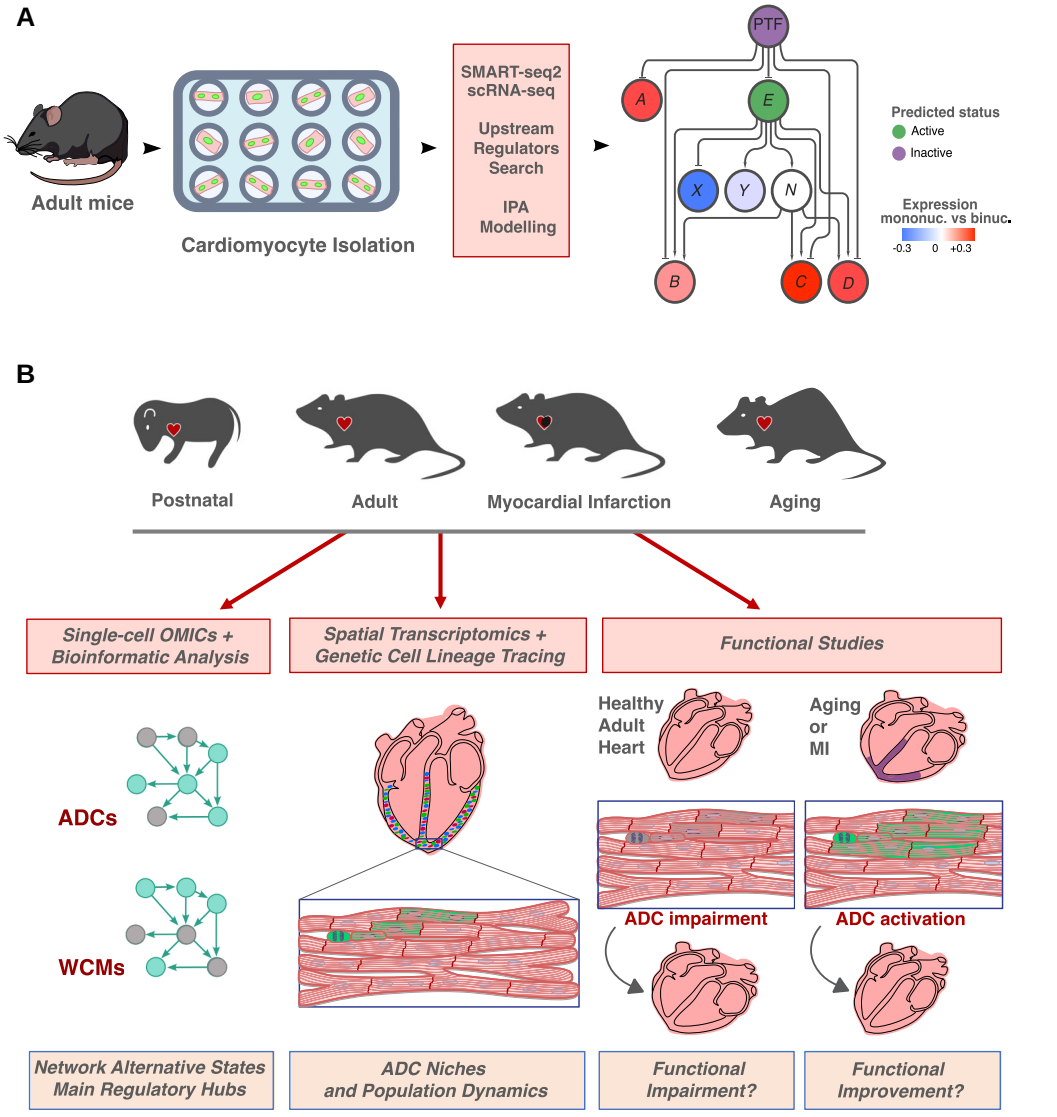


Figure 1 The identification of the adult diploid cardiomyocyte gene expression signature, its regulation, and its relevance in cardiac physiology, pathology, and regeneration. (*A*) Single-cell RNA-seq of isolated adult mouse cardiomyocytes with annotated number of nuclei allowed the identification of a working myocardium mononuclear cardiomyocyte gene signature. Through upstream regulator analysis using Ingenuity Pathway Analysis (QUIAGEN Inc.), a polyploidization transcription factor (PTF), repressor of the diploid gene signature, and its mechanistic network were identified. (B) Aims and working plan of the REACTIVA project: integration of single-cell RNA-seq, spatial transcriptomics, genetic lineage tracing, and functional data during mouse postnatal development, adulthood, aging, and myocardial infarction to obtain high-quality models of the gene regulatory network controlling cardiomyocyte polyploidization in mouse (left), identify niches and population dynamics specific of ADCs (middle), and determine the effect of PTF functional modulation on cardiac regeneration and function (right). WCMs, working cardiomyocytes

Harnessing the regenerative potential of adult diploid cardiomyocytes

The project hypothesizes that ventricular working myocardium ADCs constitute an endogenous progenitor population with potential for both self-renewing and generating new polyploid hypertrophic cardiomyocytes (working cardiomyocytes). The new pathway defined allows for the first time to identify ADCs in the mammalian heart, trace their contribution to the cardiomyocyte population, and specifically impair or enhance their proliferative activity in different physiological or pathological situations.

The project will thus aim to fully characterize in the mouse model the ADC gene regulatory network and its function during cardiac differentiation, homoeostasis, aging, and myocardial infarction/regeneration (*Figure 1B*). In a first aim, single-cardiomyocyte transcriptomic and proteomic data will be used to profile ADCs from homoeostatic, aged or injured hearts (*Figure 1B*). By leveraging literature-based prior knowledge networks,[10] multi-omic data will be used to infer a cardiomyocyte gene regulatory network and explore how it is modulated in diploid vs. polyploid cardiomyocytes during various biological and pathological processes. These data will be complemented by studying the PTF interactome and regulation, to obtain a full picture of the molecular regulation of CM ploidy. In a second aim, ADC markers will be used to genetically trace ADCs, and

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combine this information with that of spatial transcriptomics to determine ADC diversity, molecular/cellular niches in the myocardium, self-renewal, and progenitor activities (*Figure 1B*). In a third aim, the project will analyse whether ADC function is required for heart homeostasis (*Figure 1B*). For this, genetic impairment of ADC proliferation in uninjured mice will be performed to determine whether ADCs are required for heart function in short- and long-term experiments. In parallel, ADC proliferative activity will be stimulated, to interrogate whether this represents a benefit for the aging or the injured heart.

Expectations are that the REACTIVA project will provide a full understanding of the regulatory networks and environments that control the identity of diploid vs. polyploid cardiomyocytes in the mouse working myocardium. The project will also allow to control the transitions between these populations and explore their potential manipulation for heart regeneration.

Declarations

Disclosure of Interest

The authors are co-inventors in a patent application related to the manuscript.

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