CBFH Meeting

Biennial Meeting

ESC Working Groups on Cellular Biology of the Heart & Myocardial Function

25 - 27 November 2023
Naples, Italy

Handbook Programme

ESC Working Group Cellular Biology of the Heart

ESC Working Group Myocardial Function

Supported by Federico II University

Index

Welcome

Dear colleagues,

The ESC Working Groups on Cellular Biology of the Heart and Myocardial Function are pleased to announce that their biennial joint meeting is finally back and will be held in the fantastic venue of Naples on 25th - 27th November 2023.

This meeting, held every two years and presenting cutting-edge scientific outcomes in cardiovascular basic and translational science, has become a tradition, attracting the most talented and promising early career researchers with unique opportunities to interact with established scientists from around Europe.

For this purpose, an intense three-day scientific programme will update you in subjects from basic science to translational medicine in cardiac cellular biology and heart failure. The mechanisms and basic principles of heart failure and cardiovascular disease will be explored in depth to drive forward research and innovation, particularly in topical areas where much remains to be discovered, such as heart failure with preserved ejection fraction and cardiac regeneration.

Specific topics will include: mechanisms and novel regulators in heart failure remodeling and function; (post)transcriptional regulators in the heart; chemotherapy-induced cardiotoxicity, mitochondria and cardiac metabolism; cellular senescence; inherited cardiomyopathies; diabetes and atherosclerosis.

An expert faculty will deliver the scientific programme of some of the most prominent European names in the respective areas. A significant part of the meeting will be dedicated to short oral and poster presentations selected from submitted abstracts, allowing the exchange of ideas and experience among basic and translational researchers.

Alongside opportunities for education and learning, complimentary social events increase the possibilities for networking between young clinicians/scientists and experienced researchers, clinicians and faculty, and will create unique opportunities for developing new ideas and future collaboration. Continuing in this vein, a dedicated session has been conceived and organized by the Scientists of Tomorrow (SOT).

The beautiful city of Naples, thanks to its millenarian history and breathtaking landscape with Mount Vesuvius in the background, is the perfect setting to inspire a memorable event.

We very much hope you will be able to share in this exciting, cutting-edge meeting with us.

Dana Dawson
Chairperson of the ESC Working Group Myocardial Function

Cinzia Perrino
Chairperson of the ESC Working Group Biology of the Heart

ESC Working Group Cellular Biology of the Heart

ESC Working Group Myocardial Function

ESC Working Group on Cellular Biology of the Heart

Nucleus 2022-2024

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Federico II University, Cardiology, Naples, Italy

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Switzerland

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The Netherlands

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SoT representative, Spain

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Germany

Prof. Paul Evans
United Kingdom of Great Britain and Northern Ireland

Prof. Matthias Thielmann
Germany

ESC Working Group on Myocardial Function

Nucleus 2022-2024

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University of Aberdeen, Cardiovascular Medicine, Aberdeen, United Kingdom of Great Britain and Northern Ireland

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University Of Muenster, Muenster, Germany

Past-Chairperson
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Portugal

Prof. Jolanda van der Velden, FESC
The Netherlands

Prof. Serena Zacchigna
Italy

ESC Working Group Cellular Biology of the Heart

ESC Working Group Myocardial Function

Meeting schedule

Saturday, 25th November 2023

13:25 - 13:30	WELCOME
13:30 - 15:05	SESSION 1: Diving deep into mitochondrial functions and diseases
14:20 - 15:05	ABSTRACT SESSION 1: Abstract presentations
15:05 - 15:20	WORKSHOP: How to get an article accepted at Cardiovascular Research Presented by: Sean Davidson (United Kingdom)
15:20 - 16:30	COFFEE BREAK AND MODERATED POSTER SESSION I
16:30 - 18:05	SESSION 2: Cardiac injury, protection, and regeneration
17:20 - 18:05	ABSTRACT SESSION 2: Abstract presentations
18:05 - 18:35	KEYNOTE LECTURE 1: Epigenetic modulation of cardiac disease

Sunday, 26th November 2023

08:30 - 10:25	SESSION 3: Are Scientists of Tomorrow already scientists of today?
10:25 - 11:30	COFFEE BREAK AND MODERATED POSTER SESSION II
11:30 - 12:50	SESSION 4: Cardiovascular alterations in diabetes
12:20 - 12:50	ABSTRACT SESSION 3: Abstract presentations
12:50 - 14:00	LUNCH
14:00 - 15:35	SESSION 5: Single-cell RNA sequencing - a powerful new way to look inside the heart
14:50 - 15:35	ABSTRACT SESSION 4: Abstract presentations
15:35 - 16:30	COFFEE BREAK AND MODERATED POSTER SESSION III
16:30 - 18:05	SESSION 6: Loading the heart: effect of mechanical stimuli on heart function
17:20 - 18:05	ABSTRACT SESSION 5: Abstract presentations
18:05 - 18:35	KEYNOTE LECTURE 2: Biologics and cardiac disease: challenges and opportunities

Monday, 27th November 2023

08:55 - 10:30	SESSION 7: Genome editing and translational control – innovative approaches to treating cardiovascular disease
09:45 - 10.30	ABSTRACT SESSION 6: Abstract presentations
10:30 - 11:15	COFFEE BREAK
11:15 - 12:30	AWARD SESSION: Best abstracts in translational science
12:30 - 12:45	CLOSING & FAREWELL

Scientific Programme

Saturday, 25th November 2023

13.25 - 13.30	**WELCOME by the chairs of the ESC WG of Myocardial Function & Cellular Biology**
13.30 - 15.05	**Session 1: Diving deep into mitochondrial functions and diseases** **Chairpersons:** *Luc Bertrand (Belgium), Michele Ciccarelli (Italy)*
13.30 – 13.50	**Mito ROS, and the NO/sGC/cGMP pathways in doxo-induced cardiotoxicity** *Nina Kaludercic (Italy)*
13.50 – 13.55	Discussion
13.55 – 14.15	**Mitochondrial redox control in hereditary cardiomyopathies** *Christoph Maack (Germany)*
14.15 – 14.20	Discussion
14.20 – 15.05	**ABSTRACT SESSION 1: Abstract presentations** **Chairperson:** *Thomas Thum (Germany)*
14.20 – 14.30	**Oral presentation 1** Impaired cardiac and skeletal muscle energetics following anthracycline therapy for breast cancer *David Gamble (United Kingdom)*
14.30 – 14.35	Comments and discussion
14:35 - 14:45	**Oral presentation 2** Fatty acid-mediated protein acetylation contributes to the regulation of pathophysiological cardiac glucose metabolism *Laurent Bultot (Belgium)*
14.45 – 14.50	Comments and discussion
14:50 - 15:00	**Oral presentation 3** Early impairment of mitochondrial quality control in Fabry heart *Jessica Gambardella (Italy)*
15.00 – 15.05	Comments and discussion
15:05 - 15:20	**Workshop: How to get an article accepted at Cardiovascular Research** **Presented by:** *Sean Davidson (United Kingdom)*
15:20 - 16:30	**COFFEE BREAK AND MODERATED POSTER SESSION I** **Moderated poster chairs:** *Sophie van Linthout (Germany) and Paul Evans (United Kingdom)*
16.30 - 18.05	**Session 2: Cardiac injury, protection, and regeneration** **Chairpersons:** *Petra Kleinbongard (Germany), Sean Davidson (United Kingdom)*
16.30 – 16.50	**Protecting the heart from ischaemia and reperfusion injury with a novel activator of PI3Kalpha** *Sean Davidson (United Kingdom)*
16.50 – 16.55	Discussion
16.55 – 17.15	**Injury models to decipher the molecular basis of cardiac regeneration** *Christian Bär (Germany)*

17.15 – 17.20	Discussion
17.20 – 18.05	**ABSTRACT SESSION 2: Abstract presentations** **Chairpersons:** *Paul Evans (United Kingdom) and Petra Kleinbongard (Germany)*
17.20 – 17.30	**Oral presentation 1** The small mimetic peptide of endogenous Selenoprotein T (SELENOT), PSELT, as a novel cardioprotective candidate against cardiac hypertrophy and heart failure (HF) *Anna De Bartolo (Italy)*
17.30 – 17.35	Comments and discussion
17.35 – 17.45	**Oral presentation 2** Role of Hippo Pathway in the development of endothelial dysfunction in response to metabolic stress *Maurizio Forte (Italy)*
17.45 – 17.50	Comments and discussion
17.50 – 18.00	**Oral presentation 3** Mechanical load affects the proliferation of multiple cell types in the heart *Giulio Ciucci (Italy)*
18.00 – 18.05	Comments and discussion
18.05 - 18.35	**Keynote lecture 1** **Chairperson:** *Cinzia Perrino (Italy)* **Epigenetic modulation of cardiac disease** *Gianluigi Condorelli (Italy)*

Sunday, 26th November 2023

8.30 - 10.25	**Session 3: Are Scientists of Tomorrow already scientists of today?** **Chairpersons:** *Gemma Chiva-Blanch (Spain), Carolina Balbi (Switzerland)*
8.30 – 8.50	**Energizing the failing heart: metabolic mechanisms of SGTL2i** *Mahmoud Abdellatif (Austria)*
8.50 - 8.53	Discussion
8.53 – 9.13	**Extracellular vesicle modulation for cardiac repair: hype or hope?** *Sveva Bollini (Italy)*
9.13 - 9.16	Discussion
9.16 – 9.36	**Novel molecular targets for cardiac repair in post-ischemic heart** *Monika Gladka (Netherlands)*
9.36 - 9.39	Discussion
9.39 – 9.59	**The role of interleukin-11 in cardiac disease** *Mark Sweeney (United Kingdom)*
9.59 - 10.02	Discussion
10.02 – 10.22	**Chromatin remodeling in heart failure with preserved ejection fraction** *Francesco Paneni (Switzerland)*

10.22 - 10.25	Discussion
10.25 - 11.30	COFFEE BREAK AND MODERATED POSTER SESSION II **Moderated poster Chairs:** *Jolanda van der Velden (Netherlands), Mahmoud Abdelatif (Austria), Arantxa Gonzalez (Spain), Gabriele Schiattarella (Italy)*
11.30 - 12.50	Session 4: Cardiovascular alterations in diabetes **Chairpersons:** *Frank Lezoualc'h (France), Dana Dawson (United Kingdom)*
11.30 – 11.50	**Reticulum-mitochondria Ca2+ coupling in diabetic cardiomyopathy** *Mélanie Paillard (France)*
11.50 – 11.55	Discussion
11.55 – 12.15	**Novel therapeutics for treatment of diabetes and atherosclerosis** *Mirela Delibegovic (United Kingdom)*
12.15 – 12.20	Discussion
12.20 – 12.50	ABSTRACT SESSION 3: Abstract presentations **Chairpersons:** *Frank Lezoualc'h (France), Dana Dawson (United Kingdom)*
12.20 – 12.30	**Oral presentation 1** Protein O-GlcNAcylation contributes to the development of cardiac dysfunction in obese diabetic mice *Michele Russo (Italy)*
12.30 – 12.35	Comments and discussion
12.35 – 12.45	**Oral presentation 2** PI3KC2α controls cardiac contractility through regulation of β2-adrenergic receptor recycling *Sophie Cnudde (Italy)*
12.45 – 12.50	Comments and discussion
12.50 - 14.00	LUNCH
14.00 - 15:35	Session 5: Single-cell RNA sequencing – a powerful new way to look inside the heart **Chairpersons:** *Maurizio Pesce (Italy), Elisa Liehn (Denmark)*
14.00 – 14.20	**RNA therapeutics: from basic science to clinical Trials** *Thomas Thum (Germany)*
14.20 – 14.25	**Discussion**
14.25 – 14.45	**Mapping the cardiac vascular niche in heart failure** *Mairi Brittan (United Kingdom)*
14.45 – 14.50	Discussion
14.50 – 15.35	ABSTRACT SESSION 4: Abstract presentations **Chairperson:** *Maurizio Pesce (Italy)*
14.50 – 15.00	**Oral presentation 1** Maturing hiPSC-cardiomyocytes in cardiac microtissues for accurate preclinical in vitro modelling of LQT1 syndrome *Giulia Campostrini (Netherlands)*
15.00 – 15.05	Comments and discussion

15.05 – 15.15	**Oral presentation 2** Human cultured cardiac tissue slices to study disease mechanisms and test drugs in hypertrophic cardiomyopathy *Ali Nassar (Netherlands)*
15.15 – 15.20	Comments and discussion
15.20 – 15.30	**Oral presentation 3** Cardioprotective Effects of Dexrazoxane in Doxorubicin-Induced Cardiotoxicity Using Human Myocardial Tissue Slices *Jort Van Der Geest (Netherlands)*
15.30 – 15.35	Comments and discussion
15.35 - 16.30	**COFFEE BREAK AND MODERATED POSTER SESSION III** **Moderated Poster Chairs:** *Ida Gjervold Lunde (Norway), Bianca Brundel (Netherlands) and Ines Falcao-Pires (Portugal)*
16.30 - 18.05	**Session 6: Loading the heart: effect of mechanical stimuli on heart function** **Chairpersons:** *Serena Zacchigna (Italy), Peter Reiner (Austria)*
16.30 – 16.50	**Cardiac fibroblasts and mechanosensation in heart development, health and disease** *Maurizio Pesce (Italy)*
16.50 – 16.55	Discussion
16.55 – 17.15	**The sarcomeric cytoskeleton at the basis of cardiovascular disease** *Marie-Louise Bang, (Italy)*
17.15 – 17.20	Discussion
17.20 – 18.05	**ABSTRACT SESSION 5: Abstract presentations** **Chairperson:** *Nazha Hamdani (Germany)*
17.20 – 17.30	**Oral presentation 1** Lamin C variants induce arrhythmia in Drosophila melanogaster *Stan W van Wijk (Netherlands)*
17.30 – 17.35	Comments and discussion
17.35 – 17.45	**Oral presentation 2** Role of the alarmin S100A9 on HFpEF development *Isabel Marie Voss (Germany)*
17.45 – 17.50	Comments and discussion
17.50 – 18.00	**Oral presentation 3** A Chromatin Signature by SETD7 Drives Inflammation in Cardiometabolic Heart Failure with Preserved Ejection Fraction *Sarah Costantino (Switzerland)*
18.00 – 18.05	Comments and discussion
18.05 - 18.35	**Keynote lecture 2** **Chairperson:** *Carlo Gabriele Tocchetti (Italy)* **Biologics and cardiac disease: challenges and opportunities** *Serena Zacchigna (Italy)*
19:30	**Conference Cocktail in the venue**

Monday, 27th November 2023

08.55 - 10.30	**Session 7: Genome editing and translational control – innovative approaches to treating cardiovascular disease** **Chairperson:** *Jolanda van der Velden (Netherlands)*
08.55 – 09.15	**Genome editing to analyze cardiovascular diseases** *Katrin Streckfuß-Boemeke (Germany)*
09.15 – 09.20	Discussion
09.20 – 09.40	**Translational control and heart failure** *Mirko Vòlkers (Germany)*
09.40 – 09.45	Discussion
09.45 – 10.30	**ABSTRACT SESSION 6: Abstract presentations** **Chairperson:** *Dana Dawson (United Kingdom)*
09.45 – 09.55	**Oral presentation 1** Unveiling molecular mechanisms involved in cardiac metastasis development by spatial transcriptomics *Daniela Lorizio (Italy)*
09.55 – 10.00	Comments and discussion
10.00 – 10.10	**Oral presentation 2** Green chemistry, red flags: multiparametric cardiotoxicity screening of phytochemicals using hiPSC-CMs-MEA Assay *Laura-Sophie Frommelt (Italy)*
10.10 – 10.15	Comments and discussion
10.15 – 10.25	**Oral presentation 3** The role of GRK2 in Radiation-induced cardiomyopathy (RIHD) *Cristina Gatto (Italy)*
10.25 – 10.30	Comments and discussion
10.30 - 11.15	**Coffee break**
11.15 – 12.30	**AWARD Session: best abstracts in translational science** **Chairpersons:** *Cinzia Perrino (Italy) Dana Dawson (United Kingdom)* **Judges:** *Ioanna Andreadou (Greece) Zoltan Giricz (Hungary), Ange Maguy (Austria), Wolfgang Linke (Germany), Jolanda van der Velden (Netherlands)*
11.15 – 11.25	**Oral presentation 1** Complete Genetic Correction of Duchenne Muscular Dystrophy using Chromosome Transplantation in induced Pluripotent Stem Cells *Ilaria Rao (Italy)*
11.25 – 11.30	Comments and discussion

11.30 – 11.40	**Oral presentation 2** High-throughput contractility and kinome analysis identified EGFR/IGF1R and cell cycle kinases signaling as modulators of relaxation in healthy and hypertrophic cardiomyopathy cardiomyocytes *Diederik Kuster (Netherlands)*
11.40 – 11.45	Comments and discussion
11.45 – 11.55	**Oral presentation 3** Investigating the cross-talk between cardiomyocytes and endothelial cells to promote cardiac revascularization and regeneration. *Roman Vuerich (Italy)*
11.55 – 12.00	Comments and discussion
12.00 – 12.10	**Oral presentation 4** NAT10 inhibition in Cardiolaminopathy with Remodelin rescues the functional phenotype of LMNA-mutated cardiomyocytes *Cecilia Thairi (Italy)*
12.10 – 12.15	Comments and discussion
12.15 – 12.25	**Oral presentation 5** Peripheral blood immunophenotype: a novel etiological and prognostic marker in biopsy-proven myocarditis? *Cristina Vicenzetto (Italy)*
12.25 – 12.30	Comments and discussion
12.30 - 12.45	Closing & Farewell

CBFH MEETING VENUE:

The meeting will take place at the Conference Centre of the University of Naples Federico II, which is located in Via Partenope, 36, 80121.

The Conference Centre of the University of Naples Federico II is the result of a growing demand from both inside and outside the University to use some of the historic venues for the organization of events. Important buildings such as the historic Aula Magna. The Aula Magna of Via Partenope have been restored to their former splendor thanks to extensive restoration work. These halls, together with the Carlo Ciliberto Hall and the Blue Hall in Monte Sant'Angelo and the splendid Villa Orlandi in Anacapri, offer a high level of comfort and innovative technical equipment to ensure functionality at every stage of the conference.

CATERING AT THE UNIVERSITY (Venue)

Saturday 25
15:20 Coffee Break

Sunday 26
10:25 Coffee Break
12:50 Lunch
15:35 Coffee Break
19:30 Conference Cocktail

Monday 27
10:30 Coffee Break

For people traveling to Naples, here is some information about Naples that we think you will find useful.

WHAT IS THE WEATHER LIKE IN NAPLES?

Naples has warm temperatures and a pleasant Mediterranean climate.
November in Naples offers mild and pleasant weather, with temperatures ranging from 12°C to 18°C. Be prepared for occasional rain, so bring an umbrella or raincoat.

WHAT ABOUT THE LOCAL CUISINE?

Don't miss the opportunity to savor Neapolitan pizza, pasta dishes, and delicious seafood. Naples is famous for its street food, like pizza margherita and sfogliatella, a sweet pastry.

IS IT SAFE TO TRAVEL IN NAPLES?

Naples is safer compared to many international cities. It is generally secure to walk the streets day and night. Nonetheless, be aware of pickpockets in the most important tourist attractions areas, as well as railway and bus stations, ports etc. You often see police patrolling on streets and in traffic both day and night.

IS IT POSSIBLE TO PAY WITH CREDIT AND DEBIT CARDS?

Most shops, hotels and restaurants in Naples accept major credit and debit cards (Visa, MasterCard, American Express, etc.). Not all taxis accept payment by banker's card, so it is recommended to check in advance.

TOURIST TAX

The Naples Municipality collects a visitor tax that applies to paying accommodation. The tax varies from € 1.50 to € 5.00 per person/day.

EMERGENCY NUMBERS

Police: 113
Medical Emergency: 118
Fire Department: 115

We hope this information helps you make the most of your visit to Naples.

This event is supported by Bayer, Boehringer Ingelheim, the British Heart Foundation, Ionoptics, Naples University, Sial, The Company of Biologists and Visualsonics Fujifilm in the form of educational grants. The scientific programme has not been influenced in any way by its sponsors.

Sponsors

Oral abstracts

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Impaired cardiac and skeletal muscle energetics following anthracycline therapy for breast cancer

Dr. David Gamble[1], Dr. James Ross[1], Dr. Hilal Khan[1], Dr. Andreas Unger[2], Ms Lesley Cheyne[1], Ms Amelia Rudd[1], Dr. Fiona Saunders[1], Ms Janaki Srivanasan[1], Dr. Sylvia Kamya[1], Dr. Graham Horgan[3], Dr. Andrew Hannah[4], Dr. Santosh Baliga[5], Dr. Carlo Gabriele Tocchetti[6], Dr. Gordon Urquhart[7], Prof. Wolfgang A. Linke[2], Dr. Yazan Masannat[8], Dr. Ahmed Mustafa[8], Dr. Mairi Fuller[8], Dr. Beatrix Elsberger[8], Dr. Ravi Sharma[7], Prof. Dana Dawson[1]

[1]University Of Aberdeen, Aberdeen, United Kingdom, [2]Institute of Physiology II, University of Münster, Robert-Koch-Str., Münster, Germany, [3]Biomathematics and Statistics Scotland, Aberdeen, UK, [4]Department of Cardiology NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, Scotland, United Kingdom., Aberdeen, UK, [5]Department of Trauma and Orthopaedic Surgery. Aberdeen Royal Infirmary, Foresterhill, Aberdeen, UK, [6]Department of Translational Medical Sciences (DISMET), Center for Basic and Clinical Immunology Research (CISI), Interdepartmental Center of Clinical and Translational Sciences (CIRCET), Interdepartmental Hypertension Research Center (CIRIAPA), Federico II University, Naples, Italy, [7]Department of Oncology NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, United Kingdom, [8]Department of Breast Surgery NHS Grampian, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, United Kingdom

Background

Anthracycline-related cardiac toxicity is a recognised consequence of cancer therapies. We assess resting cardiac and skeletal muscle energetics and myocyte, sarcomere and mitochondrial integrity in breast cancer patients receiving epirubicin.

Methods

In a prospective, mechanistic, observational, longitudinal study we investigated chemotherapy-naive breast cancer patients receiving epirubicin versus sex and age matched healthy controls. Resting energetic status of cardiac and skeletal muscle (phosphocreatine/gamma adenosine triphosphate (PCr/γATP) and inorganic phosphate/phosphocreatine (Pi/PCr), respectively) was assessed with 31P-Magnetic Resonance Spectroscopy. Cardiac function and tissue characterisation (magnetic resonance imaging and 2D-echocardiography), cardiac biomarkers (serum NT-pro- BNP and high-sensitivity Troponin I) and structural assessments of skeletal muscle biopsies were obtained. All study assessments were performed before and after chemotherapy.

Results

Twenty-five female breast cancer patients (median age 53 years) receiving a mean epirubicin dose of 304 mg/m2, and twenty-five age/sex-matched controls were recruited. Despite comparable baseline cardiac and skeletal muscle energetics with the healthy controls, after chemotherapy, patients with breast cancer showed reduction in cardiac PCr/γATP ratio (2.0±0.7 versus 1.1±0.5, p=0.001) and increase in skeletal muscle Pi/PCr ratio (0.1±0.1 versus 0.2±0.1, p=0.022). This occurred in the context of increases in left ventricular end-systolic and end-diastolic volumes (p=0.009 and p=0.008, respec-

tively), T1 and T2 mapping (p=0.001 and p=0.028 respectively) but with preserved left ventricular ejection fraction, mass and global longitudinal strain, and no change in cardiac biomarkers. There was preservation of the mitochondrial copy number in skeletal muscle biopsies but a significant increase in areas of skeletal muscle degradation (p=0.001) in breast cancer patients following chemotherapy. Breast cancer patients demonstrated a reduction in skeletal muscle sarcomere number from the pre-chemotherapy stage compared to healthy controls (p=0.013).

Conclusion
Contemporary doses of epirubicin for breast cancer treatment result in a significant reduction of cardiac and skeletal muscle high energy 31P-metabolism alongside structural skeletal muscle changes.

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Fatty acid-mediated protein acetylation contributes to the regulation of pathophysiological cardiac glucose metabolism.

Mr. Laurent Bultot[1,2], Marine De Loof[1], Natacha Fourny[1], Alice Marino[1], Juliette Assenmacher[1], Eline Stukkens[1], Laura Guilbert[1], Sandrine Horman[1], Christophe Beauloye[1,3], Luc Bertrand[1,2]

[1]Institut de Recherche Expérimentale et Clinique, Pole of Cardiovascular Research, Université catholique de Louvain, Brussels, Belgium, [2]WELBIO department, WEL Research Institute, Wavre, Belgium, [3]Division of Cardiology, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Background: Recent scientific literature highlights an important role of protein acetylation in the development of diabetic cardiomyopathy through among other things a modulation of cardiac metabolism. However, the contribution of fatty acids (FA) to this increase and its effect on glucose metabolism remains largely undefined. We aim to decipher the role of FA in cardiac protein acetylation level and its involvement in the regulation of cardiac glucose metabolism.

Material and Methods: Mice were subjected to two different diets, high-fat (HFD) and western (WD) diets to induce obesity and diabetes. Cardiac hypertrophy and dysfunction were monitored by echocardiography whereas acetylation levels were assessed by western blot. Secondly, isolated cardiomyocytes and perfused hearts were treated with oleate or palmitate to evaluate their effect on glucose uptake. The role of protein acetylation was then examined via the use of lysine acetyltransferase inhibitors.

Results: HFD and WD induced progressive obesity and diabetes, with more rapid and more severe metabolic alterations in HFD mice than WD mice. HFD and WD promoted similar cardiac hypertrophy with progressive cardiac dysfunction. Protein acetylation levels increased rapidly and strongly in HFD compared to WD. Secondly, our results showed that oleate or palmitate treatment of isolated cardiomyocytes and perfused hearts resulted in a similar increase in protein acetylation and inhibition of insulin-stimulated glucose uptake. This effect was independent of an inhibition of the insulin signalling pathway but involved a reduction in GLUT4 translocation. Acetyltransferase inhibitors were able to block these FA-mediated events.

Conclusions: Our results show that diabetic cardiomyopathy development depends on the diet used, a diet enriched in FA promotes a more severe increase in protein acetylation in the heart with a more pronounced systemic alteration of its metabolism. Concomitantly, FA-induced protein acetylation is a crucial event in the regulation of cardiac glucose metabolism.

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Early impairment of mitochondrial quality control in Fabry heart

Dr. Jessica Gambardella[1], Dr Antonella Fiordelisi[1], Dr Federica Cerasuolo[1], Dr Antonietta Buonaiuto[1], Dr Roberta Avvisato[1], Dr Alessandro Viti[1], Dr Eduardo Sommella[2], Prof Pietro Campiglia[2], Dr Roberta Paolillo[1], Prof Cinzia Perrino[1], Prof Antonio Pisani[1], Prof Gaetano Santulli[3], Prof Daniela Sorriento[1], Prof Guido Iaccarino[1]
[1]Federico II University Of Naples, , Italy, [2]Univeristy of Salerno , , Italy , [3]Albert Einstein College of Medicine , , USA

Fabry disease (FD) is caused by impaired alpha-galactosidase (GLA) activity and accumulation of globotriaosylceramide(Gb3). Progressive cardiac hypertrophy and diastolic dysfunction are the main cause of death in FD-patients. The pathogenesis of FD-cardiomyopathy is not fully understood and the limited Gb3 deposits in the heart alongside energetic alterations suggest the involvement of other mechanisms, including mitochondrial dysfunction. We aimed to explore the pathogenetic role of mitochondrial and energetic alterations in FD cardiomyopathy. We employed a humanized mouse model of FD (R301Q-Tg/GLA knockout) to assess the cardiac phenotype in vivo, and embryonic fibroblasts (MEFs) in vitro. FD-MEFs display impaired mitochondrial respiration and reduced ATP synthesis. Accumulation of fragmented and unhealthy mitochondria and increased mitochondrial levels of fission/mitophagy markers (DRP1, MFN-ubiquitination, LC3II) indicate the failure of mitochondrial disposal. Accordingly, reduction of mitophagy-flux and accumulation of autophagosomes occur alongside with the impairment of PGC-1alpha dependent mitochondrial biogenesis. FD-mice display diastolic dysfunction and altered hemodynamic. FD-cardiomyocytes are hypertrophic, and exhibit reduced inotropic response to adrenergic stimulation. Remarkably, alterations of cardiac mitochondria are early, including a frank impairment of mitochondrial respiration with reduced myocardial ATP content, accumulation of disarranged mitochondria and impairment mitochondrial biogenesis. L-Arginine supplementation, which has been shown to support mitochondrial energetics and biogenesis, recovers mitochondrial homeostasis in vitro as well as in vivo, thus preventing the development of cardiomyopathy in FD mice. L-Arginine counteracts the energetic failure of FD cells by promoting mitochondrial function. Our data show that mitochondria quality control and energetic alterations participate to FD cardiomyopathy. The initial lysosomal defect (even before the extra-lysosomal GB3 deposition and related damage) perturbs mitophagy and mitochondrial turnover leading to early mitochondrial failure and energetic disarrangement with consequent maladaptive FD cardiac hypertrophy and dysfunction. Mitochondrial targeting, by Arginine, is a promising strategy to early restore cell homeostasis in FD.

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The small mimetic peptide of endogenous Selenoprotein T (SELENOT), PSELT, as a novel cardioprotective candidate against cardiac hypertrophy and heart failure (HF)

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Background: Maladaptive cardiac hypertrophy can increase the risk of several CVDs and significantly contribute to the development of HF. SELENOT is a vital ER-resident selenoprotein, that exerts a crucial role in cardiac differentiation and protection, also acting as a stress-sensing protein. Materials and methods: to move toward translatability, here we designed a small SELENOT mimetic peptide (PSELT), including the redox motif of the full-length protein, and assessed its systemic and cardiac protective action in aged spontaneously hypertensive heart failure (SHHF) rats (the main preclinical model of HF) and against isoproterenol (ISO)-induced cellular hypertrophy in H9c2 and AC16 human cardiomyocytes. Results: PSELT attenuated systemic inflammation by reducing the circulating levels of the proinflammatory cytokines TNF-α and IL-1β, and mitigated HF-dependent cardiac dysfunction by decreasing plasma levels of LDH and BNP, as well as myocardial production of galectin-3 (specific markers of HF). Hemodynamic study revealed that PSELT improves cardiac function at baseline and following ischemia/reperfusion injury, reducing infarct size in Wistar and SHHF rat hearts. Ultrastructural analysis revealed that PSELT rescues cardiac microarchitecture by improving myofibrillar disorganization and restoring desmin cardiac expression, a critical protein for cytoskeletal architecture, in failing heart. Mechanistically, SELENOT was up-regulated in SHHF hearts, while PSELT restored SELENOT expression and mitigated the increased levels of fibrotic marker CTGF and p53 and p21 upregulation (markers of aging and end-stage HF). In rat and human cardiomyocytes, PSELT, but not its inert-form lacking selenocysteine (Sec), I-PSELT, counteracted ISO-mediated cell hypertrophy and reduced ANP and BNP expression levels. In AC16 human cardiomyocytes, PSELT improved ISO-induced ultrastructural alterations particularly regarding Golgi compartment and membrane degeneration restoring the conventional cardiomyocyte architecture. Conclusions: Overall, these findings suggest that failing heart can induce SELENOT upregulation as a protective mechanism, and that PSELT may represent a potential therapeutic candidate to mitigate cardiac hypertrophy and counteract HF development.

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Role of Hippo Pathway in the development of endothelial dysfunction in response to metabolic stress

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The Hippo pathway plays a pivotal role in regulating cell survival and growth. Previous studies demonstrated that increased expression of mammalian sterile 20-like kinase 1 (MST1), a critical component of the Hippo pathway, leads to the apoptosis of cardiomyocytes, resulting in dilated cardiomyopathy and heart failure in mice. However, the specific contribution of MST1 to the development of endothelial dysfunction in response to metabolic disorders is unknown. Our study investigated MST1 activity in human umbilical vein endothelial cells (HUVECs) exposed to high glucose or oxidized low-density lipoproteins (oxLDL). We conducted overexpression and inhibition experiments using adenoviruses that either overexpressed wild-type MST1 (AD-MST1) or a dominant negative MST1 (AD-DN-MST1). We evaluated angiogenesis, cell survival, apoptosis, oxidative stress, RAC1 activity, and nitric oxide (NO) metabolism. We also performed vascular reactivity experiments in mesenteric arteries isolated from mice and exposed to high glucose or oxLDL treatment, with or without MST1 inhibition. MST1 is activated in response to hyperglycemia or oxLDL in HUVECs. Moreover, MST1 overexpression induces apoptosis ($p<0.05$) and impairs angiogenesis ($p<0.05$) and NO metabolism ($p<0.001$). Inhibition of MST1 mitigates the adverse effects of both high glucose and oxLDL treatments and improves the endothelial function of mesenteric arteries exposed to metabolic stress ex vivo ($p<0.05$). Mechanistically, we showed that MST1 overexpression promotes the production of reactive oxygen species (ROS) ($p<0.001$) and activates RAC1-NOX2. Conversely, MST1 inhibition reduces ROS levels ($p<0.05$ vs high glucose; $p<0.001$ vs oxLDL) and inhibits RAC1-NOX2 activation in stressed HUVECs. Finally, we found that inhibition of RAC1 reverses the detrimental effects induced by MST1 overexpression. Our findings suggest that the Hippo Pathway plays a fundamental role in endothelial and vascular dysfunction caused by metabolic stress. Inhibition of MST1 may be a promising strategy for preventing cardiovascular diseases associated with metabolic disorders such as obesity and diabetes.

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Mechanical load affects the proliferation of multiple cell types in the heart

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Introduction: The sudden drop in cardiomyocyte (CM) proliferation early after birth in mammals is paralleled by the loss of angiogenic potential of the heart. Concurrently, the heart is rarely affected by cancer, as both primary cardiac tumors and metastases are rare. This suggests that the same mechanisms that halt the proliferation of cardiac cells could also inhibit the growth of cancer cells in the adult heart. Among the mechanisms that have been claimed to be responsible for the loss of proliferative potential of CMs at birth there is the sudden increase in mechanical load. Here, we investigate whether alterations in mechanical load affect the proliferation of multiple cell types in the heart.

Material and methods: Engineered heart tissues (EHTs) were generated using neonatal rat heart cells, with a modified protocol allowing to alter mechanical load. Both cardiac endothelial cells (ECs) GFP+lung adenocarcinoma (LG1233) cells were included in EHTs. Adult heart unloading in vivo was achieved by heterotopically transplanting a mouse heart into the neck of syngeneic recipient mice, which were injected with EdU twice a week for 1 month. LG1233 cells were injected immediately after heart transplantation.

Results and conclusions: mechanical unloading in EHTs significantly increased the percentage of EdU+CMs, ECs and LG1233 cells at 72 hours. In contrast, increasing afterload reduced cell proliferation and improved CM maturation, characterized by increased cross-sectional area and force of contraction. Consistent with in vitro results, heterotopically transplanted hearts showed a higher number of proliferating CMs and ECs, which were almost absent in native hearts. Finally, LG1233 cells did not grow in native hearts, whereas they massively proliferated in unloaded hearts.

Overall, these data indicate that variations in mechanical load in the heart have a dramatic effect on the proliferation of multiple cell types, including CMs, ECs, and cancer cells.

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Protein O-GlcNAcylation contributes to the development of cardiac dysfunction in obese diabetic mice

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Rationale: Literature shows the role of O-GlcNAcylation in the development of diabetic cardiomyopathy. However, the involved molecular mechanisms remain largely unclear.
Objective: To determine the link between O-GlcNAcylation and the progression of heart failure.
Methods: Mice are subjected to two different diets, high-fat (HFD) and western (WD) diets to induce obesity and diabetes over months. Echocardiographic analysis was assessed to monitor cardiac function and hypertrophy. O-GlcNAcylation levels were assessed by western blot and O-GlcNAcylomic analysis was performed to identify the top modulated proteins involved in the pathophysiological process.
Results: HFD and WD induced progressive obesity and diabetes over months, associated with hypertrophic cardiomyopathy. Interestingly, although HFD-fed mice showed more severe metabolic alterations consistent with the obesity condition, cardiac function progressively declined faster in the WD group. The level of protein O-GlcNAcylation in cardiac tissue significantly correlates with the progression of cardiac dysfunction, which was significantly increased by WD while HFD only promotes mild elevation of the signal. Using an O-GlcNAcylomic by mass spectrometry-based approach developed in our laboratory, we identify up to 1900 O-GlcNAcylated proteins in the hearts of obese animals, part of them being specific to the WD group. Their bioinformatic analysis is ongoing to identify and functionally validate the top O-GlcNAcylated proteins.
Conclusion: HFD and WD induce different cardiac maladaptation due to their difference in protein O-GlcNAcylation profiles. We sought to use our O-GlcNAcylomic approach to identify and validate new targets to use for the development of novel therapeutic approaches.

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PI3KC2α controls cardiac contractility through regulation of β2-adrenergic receptor recycling

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Introduction and purpose: Phosphoinositide 3-kinase C2α (PI3KC2α) is a ubiquitously expressed class II PI3K isoform, which has been previously shown to be involved in vesicular trafficking. However, its role in the myocardium has not been investigated. The primary aim of this study is to elucidate the mechanism by which mechanism PI3KC2α controls cardiac pathophysiology.

Methods: Cardiomyocyte-specific PI3KC2α knockout (PI3KC2α KO) animals were generated by crossing mice expressing a tamoxifen-inducible Cre recombinase under the control of the αMHC promoter with PI3KC2α flox/flox mice. Zebrafish embryos were injected with a PI3KC2α (PI3KC2α morphants) or a control morpholino (control morphants) at 1-cell stage. At 3-day post-fertilization, heart function was assessed. HEK293 with stable overexpression of GFP-tagged β2-AR were transfected with either scramble or PI3KC2α siRNAs.

Results: Our findings indicate that PI3KC2α KO mice display reduced cardiac contractility. Similarly, PI3KC2α morphants zebrafish exhibit lower heart rate and fractional shortening compared to controls, both at baseline and after isoproterenol (ISO) stimulation. A similar unresponsiveness to ISO was found in vivo in PI3KC2α KO mice where chronic treatment with the α-adrenergic receptor (β-AR) agonist failed to induce the classical β-AR-mediated remodeling, characterized by an increase of the left ventricular mass and of cardiomyocyte area. These findings suggest that PI3KC2α plays a critical role in the regulation of β-AR signaling. In agreement, cAMP levels failed to increase in response to ISO treatment in PI3KC2α morphants. Furthermore, silencing of PI3KC2α in HEK293-GFP-β2-AR cells resulted in an increased GFP-β2-AR internalization compared to control cells, both at baseline and after ISO stimulation. Interestingly, preliminary findings indicate that overexpression of constitutively active Q70LRab11 induced a redistribution of GFP-β2-AR at the plasma membrane in PI3KC2α-silenced cells.

Conclusion: Overall, our findings identify a key role for PI3KC2α in the control of cardiac contractility through the regulation of β2-AR trafficking through a Rab11-dependent mechanism.

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Maturing hiPSC-cardiomyocytes in cardiac microtissues for accurate preclinical in vitro modelling of LQT1 syndrome

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Long-QT syndrome type 1 (LQT1), a cardiac arrhythmia often leading to sudden cardiac death, is due to mutations in KCNQ1. Human iPSC-derived cardiomyocytes (hiPSC-CMs) have been widely used to model LQT1, promoting the concept of their use in preclinical studies. Notably, KCNQ1 expression is subjected to genomic imprinting: in the heart both alleles become expressed during development, but initially the paternal allele is repressed.
Here, we used hiPSC-CMs from a LQT1 patient carrying a KCNQ1 mutation on the paternal allele and compared their functional properties with the isogenic corrected line. However, patch clamp analysis showed no difference in action potential duration (APD) between the two lines. We hypothesized that residual KCNQ1 imprinting in hiPSC-CMs due to their immaturity was masking the phenotype. We analyzed different hiPSC-CM lines and showed by ddPCR that all presented unbalanced KCNQ1 allelic expression.
We then included hiPSC-CMs in cardiac microtissues (MTs) with hiPSC-derived cardiac fibroblasts and endothelial cells, previously shown to promote maturation and upregulation of KCNQ1. Here, we observed an increased expression of the paternal allele and a reduction in the expression of kcnq1ot1, the long non-coding RNA inducing paternal allele repression. LQT1 hiPSC-CMs dissociated from MTs showed prolonged APD compared to the corrected line. Particularly, we demonstrated that this was due to a reduction in LQT1 hiPSC-CMs of the ionic current mediated by KCNQ1 (IKs), thus revealing the mutation causative effect.
Our findings show that residual KCNQ1 imprinting in immature hiPSC-CMs can lead to underestimate functional effects of pathological variants carried by the paternal allele. This is overcome by maturation of hiPSC-CMs in tri-cellular cardiac microtissue which promotes KCNQ1 bi-allelic expression and allow dissecting mutation mechanisms. This study brings attention to the epigenetic regulation of hiPSC models and demonstrates the utility of using matured hiPSC-CMs as in vitro preclinical model for cardiac arrhythmias.

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Human cultured cardiac tissue slices to study disease mechanisms and test drugs in hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most prevalent genetic cardiomyopathy. It is characterized by left ventricular hypertrophy, preserved or elevated systolic function and diastolic dysfunction. The genetic basis of HCM is diverse, moreover in half of HCM patients no pathogenic gene variant is identified. Pathomechanisms differ depending on the presence of a mutation and the affected gene, leading to diverse drug responsiveness. There is a necessity for human models that recapitulate cardiac physiology and capture variability between patients.
We use the MyoDish platform (InVitroSys) for long-term culture of cardiac tissue slices from HCM patients that underwent septal myectomy surgery. Tissue is cut in 300µm thick slices using a vibratome and inserted into a biomimetic culture chamber. Tissue is stretched to achieve preload, electrically stimulated at 0.5 Hz at 37°C, and force of contraction is constantly recorded. We hypothesize that the viability, structural integrity, transcriptome and metabolome is maintained after long-term culture. Slices were prepared from 10 HCM patients. All slices were successfully cultured for 28 days. To test the effect of long-term culture we compare slices from different time-points (day 14, and 28) to slices at day 0. Confirming previous studies 1, we observe a relatively large variation in contractile function between patients. We show that tissue slices from HCM patients represent a useful system to capture patient-to-patient variability. This method presents a platform for long-term drug testing allowing for more accurate disease characterization at the patient level, and may guide research to develop more tailored therapies.

1. Fischer, C. et al. Long-term functional and structural preservation of precision-cut human myocardium under continuous electromechanical stimulation in vitro. Nature Communications 10, (2019).

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Cardioprotective Effects of Dexrazoxane in Doxorubicin-Induced Cardiotoxicity Using Human Myocardial Tissue Slices

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Introduction:
Cardiotoxicity poses a significant challenge in drug development, often leading to the withdrawal of approved drugs. To address this issue, there is a critical need for improved models to predict cardiac adverse effects. In line with the FDA's guidelines for animal-free preclinical testing, this study aimed to develop a novel model using human myocardial tissue slices for cardiotoxicity.
Methods:
Human myocardial tissue slices of 300 µm thick were derived from patient tissues (N=5) and cultured under physiological mechanical and electrical conditions. Slices were exposed to doxorubicin (n=11) over a 10-day culture period, and their responses were compared to DMSO-vehicle controls (n=13), by recording contractile force, calcium transients and action potentials. Additionally, to assess potential cardioprotection, a subset of slices was pretreated with dexrazoxane (n=13) 1 day prior to doxorubicin exposure.
Results:
Doxorubicin-exposed slices exhibited reduced mechanical force, increased threshold potential, and impaired ability to follow pacing at higher frequencies. Furthermore, increased beat-to-beat variability of calcium transient amplitude, along with an induction of arrhythmias, was observed, particularly in slices from susceptible patients. Encouragingly, pretreatment with dexrazoxane demonstrated a protective effect against doxorubicin-induced cardiac toxicity.
Conclusion:
In summary, human myocardial tissue slices faithfully recapitulate doxorubicin-induced cardiotoxicity. Importantly, our findings demonstrated for the first time the in vitro cardioprotective potential of dexrazoxane. These findings pave the way for using ex vivo myocardial tissue slices for evaluating cardiotoxicity. Moreover, our work contributes to the growing body of evidence supporting the reliability and value of human myocardial tissue slices as a powerful tool for evaluating the efficacy and safety of novel therapeutic treatments and delivery approaches.

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Lamin C variants induce arrhythmia in Drosophila melanogaster

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Introduction

Atrial fibrillation (AF), the most common progressive cardiac arrhythmia, is associated with serious complications such as stroke and heart failure. Although common risk factors underlie AF onset, in ~15% of the affected population, AF may have a genetic cause. Several AF families carrying variants in cytoskeletal proteins, including the nuclear protein Lamin A/C (LMNA) have been identified. How LMNA variants trigger AF is unknown.

Methods

To elucidate the effect of Lamin A/C variants on cardiac arrhythmicity, Drosophila melanogaster strains expressing equivalent mutations in the Drosophila Lamin C (LamC) gene, an orthologue of human LMNA, specifically in the heart were utilized. In prepupae, heart wall movements of Drosophila LamC variants were recorded before (BTP) and after tachypacing (ATP). M-mode kymographs were made to analyze the heart rate (HR), arrhythmicity index (AI) and fractional shortening (FS). Furthermore, Drosophila were treated with microtubule affecting drugs taxol (50nM), or colchicine (25µM) and the bromodomain and extraterminal protein inhibitor inhibitor RVX-208 (250µM) to study the underlying mechanism.

Results

Lamin C wild type (wt), ΔN, and R205W show a significant reduction in HRATP, but AIATP is not affected. In contrast, Lamin C variants N210K and R264Q show a significant reduction in HRATP and increased AIATP. None of these lines show a significant difference in FS. Pharmacological intervention with RVX-208 and taxol significantly prevents reduction in HRATP, specifically in R264Q. Moreover, taxol shows an antiarrhythmic effect in N210K, but is pro-arrhythmogenic in R264Q.

Conclusion

These results indicate that the Lamin C variants N210K and R264Q have a cardiac arrhythmogenic effect in Drosophila prepupae. The arrhythmogenic effect of the LamC variant was prevented by the microtubule stabilizing drug taxol in N210K, but aggravated in R264Q. This indicates that Lamin C variants trigger various molecular pathways that drive arrhythmogenic effects.

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Role of the alarmin S100A9 on HFpEF development

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Background: A systemic low-grade inflammation induced by comorbidities is recognized to underlie heart failure with preserved ejection fraction (HFpEF)-specific remodelling. Recent evidence states that the innate immunity members S100A8, S100A9 and the NLRP3 inflammasome are important contributors of sterile inflammation in the comorbidities of HFpEF. Though, their specific involvement in HFpEF development has not been explored so far.
Methods: Endomyocardial biopsy-derived fibroblasts (EMB-(F)) from HFpEF and HFrEF patients were screened for S100A8, S100A9 and components of the NLRP3 inflammasome. Murine left ventricle (LV)-derived fibroblasts were stimulated with S100A8/9. Wild type (WT) C57BL/6JN or S100A9 knock-out (ko) mice were exposed to a high-fat diet (HFD) and L-NAME (1 g/L in drinking water) over 15 weeks. Controls received standard diet. Left ventricle (LV) function was characterized via echocardiography and conductance catheter measurements. At the day of sacrifice, the LV, spleen, blood, and adipose tissue were collected for subsequent analyses.
Results: mRNA expression of S100A8, S100A9, and NLRP3 was higher in EMB-F of HFpEF versus HFrEF patients. Stimulation of murine LV-F with S100A8/A9 further showed an increase in expression of collagen 1a1 and of the pro-inflammatory chemokines CCL2 and CCL7. WT HFD+L-NAME mice exhibited a preserved EF, combined with a reduced LV relaxation (dP/dtmin), and trends of elevated LV relaxation time (Tau), reduced stroke work, elevated end-diastolic pressure and E/E´. Those HFD+L-NAME-mediated changes were less pronounced in S100A9ko HFD+L-NAME mice. S100A9ko HFD+L-NAME mice were further characterized by lower C-reactive protein levels, and lower splenic ASC-expressing CD68 and Ly6Chigh (% of CD115+CD11b+) cells versus WT HFD+L-NAME mice. Moreover, S100A9ko HFD+L-NAME mice exhibited lower expression of markers of inflammation in LV and particularly in adipose tissue versus respective WT HFD+L-NAME mice.
Conclusion: These data give first insights into the role of S100A9 in HFpEF development.

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A Chromatin Signature by SETD7 Drives Inflammation in Cardiometabolic Heart Failure with Preserved Ejection Fraction

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Background: Obesity represents one of the most common comorbidities in patients with heart failure with preserved ejection fraction (HFpEF). Histone post-translational modifications by chromatin modifying enzymes (CMEs) are emerging as pivotal regulators of gene transcription. Purpose: To investigate the role of chromatin remodelling in obese HFpEF (obHFpEF). Methods: Gene expression profiling of CMEs (PCR array) was performed in left ventricular myocardial specimens from obHFpEF patients and age-matched control donors. Among CMEs, the methyltransferase SETD7 showed the highest variation in gene expression. Hence, we investigated the role of SETD7 and its chromatin mark H3K4me1 in a murine model of obHFpEF. Mice with cardiomyocyte-specific deletion of SETD7 (c-SETD7-/-) and control littermates (SETD7fl/fl) were generated and subjected to high fat diet feeding and L-NAME treatment for 15 weeks to induce obHFpEF. Selective inhibition of SETD7 by (R)-PFI-2 was performed in skinned cardiomyocytes isolated from left ventricular specimens of obHFpEF patients. Results: CMEs profiling showed SETD7 as the top-ranking transcript in myocardial specimens from obHFpEF patients as compared to controls. ChIP-Seq in CMs showed a strong enrichment of SETD7 and H3k4me1 on the promoter of NF-kB p65 gene. SETD7 and H3k4me1 were upregulated in HFpEF vs. control mouse hearts, showed enrichment on NF-kB p65 promoter and were associated with IL-1β and IL-6 upregulation. In HFpEF mice, cardiomyocyte-specific deletion of SETD7 protected against hypertrophy, diastolic dysfunction and lung congestion while improving exercise tolerance. At the molecular level, SETD7 deletion blunted H3K4me1 enrichment on p65 promoter thus preventing the upregulation of inflammatory genes. In cultured cardiomyocytes exposed to palmitic acid, SETD7 inhibition by (R)-PFI-2 prevented H3k4me1-driven p65 upregulation, whereas SETD7 overexpression mimicked HFpEF features. Of clinical relevance, (R)-PFI-2 reduced passive stiffness in skinned CMs isolated from obHFpEF patients. Conclusions: Pharmacological targeting of SETD7 may represent a new strategy to prevent myocardial inflammation in obHFpEF.

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Unveiling molecular mechanisms involved in cardiac metastasis development by spatial transcriptomics

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Background: Cardiac metastasis is considered a relatively rare malignancy, although an increasing prevalence is expected in the future due to the increasing human aging. Several cases of cardiac metastases remain untreatable, given the anatomical location that often hampers both surgical eradication and bioptic collection for biological characterization. Here, we use spatial transcriptomics to provide an unbiased molecular characterization of cardiac metastases, and identify novel targets for their treatment.
Material and methods: We performed spatial transcriptomics on samples of primary tumor, cardiac, and extra-cardiac metastasis from three different patients. First, we checked RNA integrity by means of DV200 analysis. Spatial transcriptomic analysis was performed by using the GeoMX technology (Nanostring), which provides whole-transcriptome data along with spatial distribution information of the preselected samples from the candidate regions of interest (ROIs). ROIs were selected on the formalin-fixed paraffin-embedded sections by means of geometric shapes in combination with pan-cytokeratin or PMEL antibody staining to specifically select tumor cells. Differential gene expression analysis and Gene Set Enrichment Analysis (GSEA) were performed on the entire transcriptome.
Results and conclusions: Differential expression analysis between cardiac metastases and extra-cardiac tumors (primary tumors + extra-cardiac metastases) identified 92 differentially expressed genes (DEGs). Of these, 53 genes were significantly overexpressed in cardiac metastases, while 39 genes were overexpressed in the extra-cardiac samples, regardless of the tumor origin. Unsupervised hierarchical clustering of protein-coding genes clearly separated the samples according to their anatomical location, in particular cardiac metastasis from extra-cardiac tumors. The DEGs included several known genes involved in cell migration, tissue remodelling and cell metabolism, suggesting a potential role of these signaling pathways in the development of cardiac metastasis. These results shed light on the mechanisms that allow cancer cell growth in the heart and pave the way to the development of targeted therapies to treat this malignancy.

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Green chemistry, red flags: multiparametric cardiotoxicity screening of phytochemicals using hiPSC-CMs-MEA Assay

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Background: Cardiotoxicity remains a major challenge in drug development, as adverse cardiac events can lead to severe health consequences, late-stage project terminations or even market withdrawals of drugs. Traditional animal-based assays often struggle to predict human cardiac safety reliably. To address this, human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in combination with advanced screening platforms, like multi-electrode arrays (MEA), have emerged recently as valuable tools for preclinical drug screenings. This innovative approach offers a more physiologically relevant and sensitive platform for evaluating compound-induced cardiac effects and detecting potential cardiotoxic effects at an early stage. Here, we assess the effectiveness of the hiPSC-CMs-MEA assay in detecting both acute and chronic electrophysiological, cardiotoxic, and proarrhythmic effects caused by Prestwick's phytochemicals. Material and Methods: Commercial hiPSC-CMs were seeded in 24-well MEA plates and exposed to a single dose (1µM) of Prestwick's phytochemicals, comprising 320 well-known and novel plant-based compounds. MEA recordings were conducted at multiple time points: 30min, 2hrs (acute phase), 24hrs and 48hrs (chronic phase). Several parameters related to electrophysiology, contractility and viability were measured using the Maestro Edge MEA. The screened compounds were ranked for overall cardiotoxicity by integrating the measured parameters into the Toxicological Prioritization Index application. Results and Conclusion: The hiPSC-CMs MEA assay successfully detected various phytochemical-induced cardiotoxicities, including arrhythmic events (EADs, ectopic beats, rhythmic abnormalities) and changes in field potential (56 compounds), contractility (29 compounds), and viability parameters (12 compounds). Of note, classic pro-arrhythmic drugs (e.g., quinidine, E-4031), whether already present in the library or employed as positive controls, consistently received high scores in the expected categories, indicating high accuracy of the assay. This study showcases the hiPSC-CM-MEA assay's ability to accurately identify cardiotoxic effects in preclinical studies not only of approved cardioactive drugs but also of novel compounds. Funding Sources: This research was funded by the ITAT1047-InCardio-Interreg Project.

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The role of GRK2 in Radiation-induced cardiomyopathy (RIHD)

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BACKGROUND: Ionizing radiation (IR) is the most common therapy for intrathoracic and chest wall tumors, but also are responsible for radiation-induced heart disease (RIHD), a primarily late and sometimes severe side effect inducing mitochondrial impairment. However, the biological mechanisms associated with it are poorly understood. G protein-coupled receptor kinase 2 protects against RIHD by promoting mitochondrial fission/fusion through MFN interaction and phosphorylation. The study aimed to evaluate the role of myocardial GRK2 in mitochondrial repair mechanisms upon IR exposure in mice. MATERIALS AND METHODS: The study involved 3-month-old mice with GRK2 selective myocardial knock-out, exposed to X-rays, and evaluated for cardiac size and function after IR exposure, before euthanization. RESULTS: IR exposure caused a reduction of the ejection fraction (EF) in GRK2fl/fl mice at 3h with recovery after 24 post-IR. In αMyhCRE-GRK2fl/fl, the EF was markedly depressed at 3h post-IR with only partial recovery at 24 h post-IR. To evaluate the role of GRK2 during mitochondrial stress after IR, we performed western blot (WB), proteomic, and transmission electron microscopy (TEM) analysis. In particular, WB analysis revealed a decrease of DRP-1, PINK-1, and PARKIN expression, respectively, and increased LC-3 expression in αMyhCRE-GRK2fl/fl mice. The proteomic analysis confirmed the under-expression of several mitochondrial proteins in the two different genotypes and mostly in cardiac GRK2 knock-out mice. After IR, the most dramatic difference in terms of total proteome was observed between control and GRK2 knock-out mice after 3 hours post-IR, where 25% of them were mitochondrial. TEM analysis revealed anomalies in mitochondrial morphology and cristae remodeling in GRK2 knock-out mice after 3 hours post-IR that are only partly restored after 24 hours. CONCLUSION: GRK2 plays a pivotal in protecting cardiac function during IR exposure; its removal appears deleterious in acute conditions, worsening the mitochondrial response to IR.

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Complete Genetic Correction of Duchenne Muscular Dystrophy using Chromosome Transplantation in induced Pluripotent Stem Cells

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Duchenne Muscular distrophy (DMD) is a degenerative neuromuscular X-linked disease affecting 1 in 3,500-5,000 male newborns, whose life expectancy is about 25-30 years. DMD is generally caused by out-of-frame mutations in the dystrophin gene (DMD), leading to the absence of dystrophin protein, which plays an essential role during muscle contraction and stretching. Loss of dystrophin makes myocytes more susceptible to stretch-induced damage and necrosis. The major cause of death is DMD-related cardiomyopathy, resulting in respiratory or cardiac failure. In spite of the progress in gene editing, for many human diseases associated to gross mutations, conventional gene therapy can only partially solve the defect. In this study, we aimed to fully correct DMD gene defects by validating a novel approach of gene correction, called Chromosome Transplantation (CT), defined as the perfect replacement of an endogenous defective chromosome with an exogenous normal one, restoring a correct diploid karyotype.
To achieve our purpose, we will use CT to correct DMD-induced pluripotent stem cells (iPSCs), which represent a potential source of autologous cells for transplantation. Corrected iPSCs are differentiated toward mature cardiomiocytes (CMs) by two-dimensional cell culturing. To confirm the phenotypic rescue, we conducted electrophysiological analysis using current and voltage clamp techniques. In conclusion, our study demonstrates the successful generation of corrected DMD-iPSCs through CT, followed by their differentiation into functional CMs. This advancement holds promise for potential autologous therapies for DMD.

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High-throughput contractility and kinome analysis identified EGFR/IGF1R and cell cycle kinases signaling as modulators of relaxation in healthy and hypertrophic cardiomyopathy cardiomyocytes

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Impaired relaxation is central to diseases such as heart failure with preserved ejection fraction and hypertrophic cardiomyopathy (HCM). However, therapies that improve cardiac relaxation are scarce, partly due to a limited understanding of modulators of cardiomyocyte relaxation. We hypothesized that relaxation is regulated by multiple unidentified proteins and that dysregulation of kinases contributes to impaired relaxation in HCM.

We optimized and increased the throughput of unloaded shortening measurements and screened a kinase inhibitor library in isolated adult cardiomyocytes from wild-type mice. 157 kinase inhibitors were screened. To assess which kinases are dysregulated in patients with HCM and could contribute to impaired relaxation, we performed a tyrosine and global phosphoproteomics screen and kinase activity analysis using HCM patient myocardium. Identified hits from these 2 data sets were validated in cardiomyocytes from a homozygous MYBPC3c.2373insG HCM mouse model.

Screening of 157 kinase inhibitors in wild-type (N=33) cardiomyocytes (n=24 563) resulted in the identification of 17 positive inotropes and 21 positive lusitropes, almost all of them novel. The positive lusitropes formed 3 clusters: cell cycle, EGFR (epidermal growth factor receptor)/IGF1R (insulin-like growth factor 1 receptor), and a small Akt (α-serine/threonine protein kinase) signaling cluster. By performing phosphoproteomic profiling of HCM patient myocardium (N=24 HCM and N=8 donors), we demonstrated increased activation of 6 of 8 proteins from the EGFR/IGFR1 cluster in HCM. We validated compounds from this cluster in mouse HCM (N=12) cardiomyocytes (n=2023). Three compounds from this cluster were able to improve relaxation in HCM cardiomyocytes.

We showed the feasibility of screening for functional modulators of cardiomyocyte relaxation, parameters that we observed to be modulated by kinases involved in EGFR/IGF1R, Akt and cell cycle signaling. Integrating the screening data with phosphoproteomics analysis in HCM patient tissue indicated inhibition of EGFR/IGF1R signaling as a promising target for treating impaired relaxation in HCM.

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Investigating the cross-talk between cardiomyocytes and endothelial cells to promote cardiac revascularization and regeneration.

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Background: The adult heart is unable to regenerate after ischemic damage. Recent work performed in our laboratory has demonstrated that the loss of cardiomyocyte (CM) proliferation after birth is paralleled by a loss of angiogenic potential. However, the mechanisms that inhibit angiogenesis in the adult heart remain elusive. We hypothesize that terminal CM differentiation after birth results in the paracrine inhibition of endothelial cell (EC) proliferation after birth.

Material and Methods: To verify our hypothesis, we co-cultured cardiac ECs with CMs at different stages of differentiation. Next, we exploited existing RNA sequencing dataset to generate the interactome between ligands produced by CMs and receptors expressed by cardiac ECs. We selected the most promising anti-angiogenic factors by gain and loss of function studies, and finally knocked-out the corresponding genes in Cas9 mice to rescue the angiogenic potential of the adult heart. In parallel, we tested the capacity of a pro-proliferative miRNA (miR199a), known to induce CM de-differentiation, to induce EC proliferation and angiogenesis when delivered to the adult heart, in combination with VEGF-A, using adeno-associated viral vectors.

Results: Adult CMs impaired EC proliferation. We identified 20 membrane and secreted factors that establish 236 ligand-receptor interactions between CMs and ECs. Among these, gain and loss of function experiments pointed to the relevant role of KCNJ11, CD74 and DSG2. Their knock-out in CMs in vivo resulted in significant EC proliferation and neo-vessel formation. CM-de-differentiation by miR199a promoted EC proliferation and neo-vessel formation upon VEGF-A stimulation.

Conclusions: Collectively, this work shows that fully mature CMs contribute to the low angiogenic potential of the adult heart, likely explaining the failure of therapeutic angiogenesis approaches attempted so far. Innovative strategies that either target CM-EC cross-talk or induce partial CM de-differentiation may open new therapeutic avenues in the field of cardiac regeneration.

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NAT10 inhibition in Cardiolaminopathy with Remodelin rescues the functional phenotype of LMNA-mutated cardiomyocytes

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Mutations of Lamin A/C gene (LMNA) are common causes of LMNA-dependent cardiomyopathy, a form of dilated cardiomyopathy typically manifesting with conduction disorders and arrhythmias. Remodelin – a small molecule inhibitor of N-acetyltransferase 10 – has been shown to ameliorate the phenotype of laminopathic mice and improve nuclear abnormalities of either progeric or Lamin A/C-depleted cells. However, there is a lack of evidence on its role and its potential therapeutic effect in human cardiomyocytes (CMs). To fill this gap of knowledge, we evaluated the effect of Remodelin on CMs differentiated from induced pluripotent stem cells carrying LMNA mutations (p.K219T and p.R190W). Previous studies from our group showed that CMs carrying those LMNA mutations (LMNA-CMs) have a pro-arrhythmic electrophysiological profile which well represents the impaired electrical excitability of laminopathic patients. Here we demonstrated that Remodelin treatment is able to rescue the altered electrophysiological parameters in LMNA-CMs, re-establishing the correct peak sodium current density and action potential properties. Coherently, Remodelin treatment also boosts gap junctional conductance. We hypothesized that the modulation of microtubule network by N-acetyltransferase 10 in LMNA-CMs could be at the basis of the observed effect, as it might promote relocalization to the plasma membrane of Nav1.5 channel and Connexin 43, important for proper cardiac excitability and conduction. Studies addressing Remodelin effect on α-tubulin acetylation and microtubule organization as well as Nav1.5/Connexin 43 quantification at the plasma membrane are ongoing. Moreover, a beneficial effect, in terms of cardiac conduction and action potential, was also observed in control CMs after exposure to Remodelin, suggesting that the treatment modulates other cardiac biological processes, including metabolism and contractility, as emerged from RNA sequencing experiments. In conclusion, although specific underlying mechanisms are yet to be demonstrated, our study reinforces the evidence indicating N-acetyltransferase 10 inhibition as a promising therapeutic target for LMNA-dependent cardiomyopathy.

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Peripheral blood immunophenotype: a novel etiological and prognostic marker in biopsy-proven myocarditis?

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Background: Myocarditis is an inflammatory disease of the myocardium with viral or immune-mediated/autoimmune etiology. Definite etiological diagnosis relays upon histological, immunohistological and molecular evaluation of endomyocardial biopsy (EMB). About 50% of myocarditis cases resolve spontaneously, while 25% of virus-negative cases may require immunosuppression (IS) to reduce the risk of evolution to dilated cardiomyopathy, heart transplantation or death. Prognostic stratification is incomplete and IS is not always efficacious. This study aims at identifying new etiological and prognostic biomarkers in peripheral blood.

Material and Methods: Sixty-four EMB-proven myocarditis patients and 7 healthy controls (HC) were enrolled. Peripheral blood mononuclear cells were purified by Ficoll stratification, and the immune cells distribution was evaluated by flow cytometry. Variables were analysed clustering patients according to EMB results and IS response by with the Mann-Whitney or Kruskall-Wallis tests. Funding source: Ricerca Finalizzata RF-2019-12370183 (Italian Ministry of Health).

Results: Myocarditis patients had higher levels of peripheral Th17 cells than HC ($p=0.005$), and lower levels of plasmacytoid dendritic cells and less mature NK cells ($p=0.004$ and $p=0.033$, respectively). The γδ-TCR positive Th17 cells subpopulation identified viral myocarditis patients and cases without other extracardiac autoimmune diseases ($p=0.021$ and $p=0.011$, respectively). Higher Th1 cells levels defined lymphocytic myocarditis ($p=0.044$), while higher CD3+/CD56+ cells and CD3-/CD56-/CD16high/CD62L+ cells levels identified patients with eosinophilic/polymorphonuclear myocarditis ($p=0.05$ and $p=0.02$, respectively). Patients unresponsive to IS had lower Treg cells than HC ($p=0.016$) and higher CD3+/CD57+ cells levels than IS responsive cases ($p=0.011$). Notably, T helper CXCR3+/CCR4+ cells (linked to cardiac migration) level was higher in IS unresponsive patients compared to HC and IS responsive cases ($p=0.022$ and $p=0.041$, respectively).

Conclusion: Our data demonstrate a skewed immunological peripheral blood cells distribution towards "inflammatory signatures" in myocarditis, suggesting new potential non-invasive immunological biomarkers.

Poster abstracts

1

Abstract retracted by authors

2

Circulating factors from STEMI patients contribute to post-injury vascular endothelial damage

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Background: While primary percutaneous coronary intervention (PCI) is the treatment of choice in patients with ST-segment elevation myocardial infarction (STEMI), this treatment does not fully prevent late adverse cardiac remodeling. Infarcted myocardial tissue releases damage-associated extracellular vesicles (EV) that mediate tissue inflammation and may regulate myocardial remodeling. Here, we investigated the role of circulating factors, in particular EVs, in STEMI patients, with a focus on endothelial cell (EC).
Methods: Peripheral blood samples were obtained from STEMI patients (n=30) within 4 hrs of onset of pain before PCI and from age- and gender-matched healthy control (CTRL; n=30) with documented absence of coronary artery disease. Raw serum was characterized by atomic force microscopy (AFM) single-particle morphometry. Serum and serum-derived Large EVs (EV-L) were used for in vitro experiments on human aortic endothelial cells (hAEC).
Results: Short-time culturing (3hr) of hAEC with STEMI patients' serum results in endothelial damage, measured as increase of reactive oxygen species (ROS), p66SHC, and PAI-1 expressions, compared to cells cultured with serum from CTRL subjects. Furthermore, long-time culturing (7 days) with STEMI patients' serum led to endothelial-to-mesenchymal transition (EndMT), with induction of alpha-smooth muscle actin (αSMA) expression and extracellular matrix (ECM) proteins secretion. Interestingly, AFM analysis showed a 7.5-fold increase in EV-L in STEMI vs. CTRL serum. Experiments with STEMI and CTRL-derived EV-L on endothelial cells partially recapitulate serum effects, with increase in ROS and induction of EndMT.
Conclusions: Our preliminary data showed a detrimental effect on the endothelium of circulating factors in STEMI patients, probably mediated by Large Extracellular Vesicles. Results from in vitro experiments evidence their function in inducing endothelial damage and activation of EndMT; two important features in the progression of pathological myocardial remodeling.

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Three-dimensional co-culturing of stem cell-derived cardiomyocytes and cardiac fibroblasts reveals a role for both cell types in Marfan-related cardiomyopathy

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Pathogenic variants in the FBN1 gene, encoding for the extracellular matrix protein fibrillin-1, result in Marfan syndrome (MFS), affecting multiple organ systems, including the cardiovascular system. Myocardial dysfunction has been observed in a subset of patients with MFS, as well as in various MFS mouse models. However, there is limited understanding regarding the intrinsic consequences of FBN1 variants on the cardiomyocytes (CMs). To elucidate the CM-specific contribution in Marfan-related cardiomyopathy, cardiosphere cultures of CMs and cardiac fibroblasts (CFs) are employed. CMs and CFs were derived through the differentiation of human induced pluripotent stem cells (iPSCs) from MFS iPSCs with pathogenic variants in FBN1 and the corresponding CRISPR-corrected iPSC lines (Cor).
Cardiospheres containing MFS CMs show decreased FBN1, COL1A2 and GJA1 expression. MFS CMs cultured in cardiospheres have fewer binucleated CMs in comparison with Cor CMs. 13% of MFS CMs in cardiospheres are binucleated and 15% and 16% in cardiospheres that contain co-cultures with respectively MFS CFs and Cor CFs, compared to Cor CMs, that revealed up to 23% binucleation when co-cultured with CFs. The sarcomere length of CMs, as a marker of development, shows that CF interactions with CMs result in a significant increase in sarcomere length for MFS CMs. Nuclear blebbing was significantly more frequent in MFS CFs, which correlated with increased stiffness of the nuclear area compared to Cor CFs.
Our cardiosphere models for Marfan-related cardiomyopathy identified a contribution of CFs in Marfan-related cardiomyopathy and suggests that abnormal early development of CMs may play a role in the disease mechanism.

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Multicellular human cardiac organoids as a versatile 3D platform to study cardiovascular pathophysiology in vitro

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There is an urgent need for sustainable and complex in vitro models to sufficiently recapitulate the physiology and pathophysiology of the human heart. Human cardiac organoids (hCO) resemble a self-organized and -assembled 3D culture platform that displays heart-like characteristics. In this context, we established a reproducible multicellular cardiac organoid-approach. hiPSC-derived cardiomyocytes (CMs), fibroblasts, endothelial cells and adipose tissue derived stem cells form a functional and contracting structure, spontaneous or controlled by electrostimulation. By immunostaining we demonstrate that the organoids self-assemble into a specific architecture where the hiPSC-CMs remain in the outer core and non-CMs assemble inside the organoid's core. Utilizing this versatile system, we generated hypertrophic cardiomyopathy hCOs based on patient-derived hiPSC-CMs and initially treated these hCOs with Mavacamten which is the first-in-class small inhibitor of the cardiac myosin ATPase approved for treatment of HCM patients with hypercontractility. Chronic as well as acute treatment thereby led to an impact on the hCO contractility. In contrast, healthy hCOs respond to phenylepinephrine-isoprenaline stimulation with increased contractility resembling the positive chronotropic characteristics of this compound. In addition, these hCOs can be used to model cardiovascular diseases such as a myocardial infarction. By cultivating hCOs under hypoxic conditions and norepinephrine stimulation, we showed that this leads to impaired calcium handling as well as altered fibrosis related gene expression. Additionally, hypoxic hCOs displayed a larger population of dead cells due to non-viable oxygen concentrations in the organoid core. Moreover, stimulating healthy hCOs with Endothelin-1 led to a hypertrophic phenotype with upregulation of ANP and BNP expression. The latter indicates that the hCOs can be used to study cardiac hypertrophy in a multicellular in vitro system. In summary, our preliminary data highlight the beauty of the multicellular hCOs to serve as a functional, versatile drug screening platform as well as to model and study cardiovascular pathophysiology.

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Hypercholesterolemia changes metabolomic and proteomic profile of plasma- and cardiomyocyte-derived extracellular vesicles

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Introduction: Extracellular vesicles (EVs) are involved in the pathomechanism of hypercholesterolemia (HC), a risk factor for cardiovascular diseases. However, whether and how HC changes the structure, cargo, and possible functions of EVs is unknown. Therefore, we aimed to analyze the effect of HC on the characteristics of circulating and cardiomyocyte (CM)-secreted EVs.

Materials and Methods: EVs were isolated with Vezics technology from platelet-free plasma of male Wistar rats fed with high-cholesterol or control chow. The metabolome of both plasma and EV samples were analyzed. AC16 human CMs were treated with Remembrane® HC supplement or with its vehicle or kept in control conditions and then EVs were isolated from cell culture supernatant. Samples were analyzed with nanoparticle tracking analysis, and atomic force microscopy. CM-EV proteomics was measured with liquid chromatography-tandem mass spectrometry. Monocyte activation was measured in THP1-ASC-GFP cells treated with CM-EVs.

Results: HC diet induced hyperlipidemia in rats and reduced the amount of certain phosphatidylcholines in circulating EVs, independently of their plasma level. HC treatment significantly increased EV secretion of CMs and greatly modified EV proteome, with enrichment of several proteins involved in tissue remodeling. CM EVs, regardless of the treatment, did not induce the activation of THP1 monocytes.

Conclusions: HC strongly affects the metabolome of EVs. Furthermore, it induces secretion and modifies the proteome of CM EVs that potentially induce tissue remodeling. EVs of HC-CMs do not affect monocyte activity.

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6

The cGAS-STING pathway as a potential therapeutic target in pathologic progression of aortic valve stenosis.

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Background

Calcific aortic valve disease (CAVD) is the most frequent heart valve disorder, significantly increasing with age. We have recently discriminated between the phenotype of aortic valve interstitial cells (VICs) from two valve pathology models – valve insufficiency and valve stenosis – for the extent of cellular senescence on epigenetic-mediated calcification. In this regard, an interesting pathway under investigation is that dependent on the cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS)-stimulator of interferon genes (STING), which is activated by cytoplasmic DNA that promotes the activation of interferon regulatory factor 3 (IRF3), with consequent upregulation of type I interferon.

Methods:

We employed single cell-RNA sequencing to assess the whole transcriptome in the different populations of cells in stenotic vs. insufficient valves. In total VICs from 4 patients with heart valve stenosis and 4 patients with heart valve insufficiency were profiled with identification of 7 clusters and 5 genes differentially expressed. To validate mechanistically the relevance of cGAS/STING for sVICs senescence, we treated the cells with the RU.521, a cGAS inhibitor known to reduce interferon expression and, thus, the level of cellular senescence.

Results

The bioinformatics analysys of sVICs vs. IVICs scRNA-seq analysis revealed a striking difference in the expression of several GO pathways related to IFN-I in sVICs compared to iVICs. Validation of the IFN-I transcriptional signature (including the IFI6, IFI27, IFITM3, ISG15, IFIT1 genes) by RT-qPCR confirmed the scRNA-seq findings. Interestingly, the assays performed on sVIC treated with RU.521 inhibitor showed an overall reduction of the cellular senescence level, showing the implication of cGAS/STING in the pathologic process of stenotic valves.

Conclusion

These findings reveal for the first time, the implication of the cGAS/STING pathway in pathologic progression of aortic valve stenosis. Modulation of the pathway by RU.521 or other interfering drugs may be a new strategy to treat senescence-associated CAVD.

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An in-vitro engineered living pericardial tissue suitable for pediatric and adult aortic valve reconstruction

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Background

The number of heart valve procedures is expected to triple by 2050 with an increase in the impact on public health. Here, we introduce the possibility to produce living pericardial-based leaflets culturing cells into a decellularized animal pericardium material using a bioreactor-based procedure.

Materials and Methods

Porcine pericardium was decellularized with an aldehyde and xeno-antigen residue free procedure. Recellularization of decellularized matrices with human adipose stem cells (hADSC, Lonza) was performed under perfusion flow using 0.65x106 cells per bioreactor under an initial flow rate of 3 ml/min for 72 hours followed by a rate of 0.03 ml/min for additional 18 days. Samples were harvested and prepared for immunofluorescence staining using α-SMA, Vimentin and proliferating cell nuclear antigen (PCNA). Mass spectrometry was performed after 14 and 21 days of culture.

Results and Conclusions

Immunofluorescence analysis showed increased human valve-like proteins production at late stages of culture, as confirmed by untargeted proteomic analysis. Expression of α-SMA, a pro-pathologic marker for valve interstitial cells was mainly present in cells layering on the surface of the pericardium. According to PCNA staining, the proliferation rate of the cells penetrating the scaffold was lower than in cells covering the scaffold surface; conversely, elastin and pro-Collagen I expression was higher in the cells in the inner part of the pericardium. Our results show that culture conditions described here induce a valve-like phenotype in hADSCs colonizing the matrix. We are now in the progress of scaling up the recellularization procedure and adapt it to a GMP-compliant setting using autologous stem cells and tailored bioreactors (capable of housing scaffolds of up to 5 cm in diameter) to deliver a fully recellularized pericardial tissue amenable for surgical reconstruction (Ozaki procedure) of a patient-tailored aortic valve.

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A signature of mechanically-regulated long non-coding RNAs establishes a gene regulatory network for pro-fibrotic cardiac fibroblasts programming

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We have recently shown that mechanical signaling cooperates with pro-fibrotic stimuli in cardiac fibroblasts evolution into myofibroblasts in vitro, and it has a role in post myocardial infarction remodeling in vivo. This depends on the mechanically-controlled activity of YAP/TEAD transcriptional network, as demonstrated by treating cells and animals with Verteporfin (VTP), a specific inhibitor of YAP/TEAD. In the present work, we identified a signature of long non-coding RNAs (lncRNAs), controlled by YAP/TAZ that positively or negatively correlate with cardiac fibrosis. We report the analysis of differentially expressed (DE) Poly-A+ lncRNAs, emerging from RNA-sequencing performed on RNA extracted from human cardiac-derived stromal cells treated or not with VTP. Bioinformatic analysis identified a signature of 222 lncRNAs that were DE in the comparison between treated and control cells, out of 1770 total lncRNAs expressed in our cells. Functional inference based on neighborhood analysis between lncRNA and spatial proximal target genes revealed an enrichment of Gene Ontology Biological Processes that were positively or negatively associated with the cellular treatment. By performing correlation analysis, we identified clusters of specific lncRNAs that were putatively co-regulated with genes involved in pro/anti-regulation of fibrosis and the Hippo pathway. A lncRNAs ranking was thus established based on their average degree of correlation (Pearson›s r). A signature containing 5 lncRNAs that most strongly correlated positively or negatively was finally identified based on those lncRNAs that were in common to both pathways, as putative candidates expected to modulate the fibrotic process in human cardiac-derived stromal cells.

Our results suggest the existence of a lncRNAs signature connected to the control of the Hippo signaling and inflammatory/pro-fibrotic pathways, thus opening a way to an RNA therapeutics strategy to reduce the burden of cardiac fibrosis and heart failure. We are currently designing gain/loss of function experiments to mechanistically demonstrate these findings.

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Abstract retracted by authors

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Long non-coding RNA Cyrano influences cardiomyocyte proliferation in vitro and cardiac regeneration in vivo

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The tremendous loss of cardiomyocytes after an injury cannot be compensated for due to the low regenerative capacity of the heart. One promising regenerative strategy focuses on the stimulation of the low intrinsic proliferative capacity of cardiomyocytes. In this context, we focused on long non-coding RNAs (lncRNAs) which are regulators of versatile cellular functions. By analyzing publicly available transcriptome data of regenerating and non-regenerating hearts, we identified the lncRNA Cyrano. Cyrano is strikingly well conserved from fish to humans and is predominantly expressed in the brain and the heart with highest abundancy in cardiomyocytes.

In line with our hypothesis, transcriptome analysis of Cyrano-silenced human iPSC-derived cardiomyocytes revealed changes predominantly in gene sets related to proliferation-associated pathways. Cyrano depletion led to an increase in Ki67+ cardiomyocytes in vitro in accordance with an increased number of cardiomyocytes. This was further confirmed after Cyrano knockdown in cardiomyocytes of a cyclin B1 reporter cell line, which marks proliferating cardiomyocytes more specifically as Ki67. Also here the loss of Cyrano resulted in a higher number of cyclin B1+ cardiomyocytes. To understand the molecular mechanism, RNA pulldown followed by mass spectrometry was performed identifying different proteins, of which some are known in the context of cell cycle regulation.

To investigate Cyrano's anti-proliferative phenotype in vivo, we applied the myocardial infarction model in neonatal mice, a sensitive model to assess pro- and anti-proliferative effects. In Cyrano knockout and control neonates the left anterior descending artery was ligated on postnatal day 5. Echocardiographic analysis one week after infarction revealed better recovery of cardiac performance in knockout neonates compared to controls. To understand the beneficial effect of the Cyrano loss, histological assessment of the hearts is ongoing.

In summary, silencing the well-conserved cardiomyocyte-enriched lncRNA Cyrano stimulated cardiomyocyte proliferation in vitro and improved cardiac function after myocardial infarction in vivo.

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Cardiovascular and Non-Cardiovascular Prescribing and Mortality Outcomes after Takotsubo Syndrome

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Background: Takotsubo syndrome is an increasingly common cardiac emergency with no known evidence-based treatment. The aim of the study was to investigate cardiovascular mortality and medication use after takotsubo syndrome.

Methods: In a case-control study, all patients with takotsubo syndrome in Scotland between 2010-2017 (n=620) were age, sex and geographically matched to individuals in the general population (1:4, n=2,480) and contemporaneous patients with acute myocardial infarction (1:1, n=620). Electronic health record data linkage of mortality outcomes and drug prescribing were analysed using Cox proportional hazard regression models.

Results: Of the 3,720 study participants (mean age, 66 years; 91% women), 153 (25%) patients with takotsubo syndrome died over the median of 5.5 years follow up. This exceeded mortality rates in the general population [374 (15%)]; hazard ratio [HR] 1.78 [95% confidence interval 1.48-2.15], p<0.0001), especially for cardiovascular (HR 2.47, [1.81-3.39], p<0.001) but also non-cardiovascular (HR 1.48 [1.16-1.87], p=0.002) deaths. Mortality rates were lower for patients with takotsubo syndrome than those with myocardial infarction (31%, 195/620; HR 0.76 [0.62-0.94], p=0.012), which was attributable to lower rates of cardiovascular (HR 0.61 [0.44-0.84], p=0.002) but not non-cardiovascular (HR 0.92 [0.69-1.23], p=0.59) deaths. Despite comparable medications use, cardiovascular therapies were consistently associated with better survival in patients with myocardial infarction but not in those with takotsubo syndrome. Diuretic (p=0.01), anti-inflammatory (p=0.002) and psychotropic (p<0.001) therapies were all associated with worse outcomes in patients with takotsubo syndrome.

Conclusions: In patients with takotsubo syndrome, cardiovascular mortality is the leading cause of death, and this is not associated with cardiovascular therapy use.

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Investigation of long non-coding RNAs in the regenerating mouse heart

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Background: Developing novel therapeutics for cardiovascular disease is a priority. Within the first week of life, mouse hearts can fully regenerate after myocardial infarction (MI), including recovery of normal cardiac function. The underlying molecular mechanisms are poorly understood. Long non-coding RNAs (lncRNAs) have emerged as powerful regulators of biological processes. Therefore, this study aims to identify cell type-specific lncRNAs of the regenerating mouse heart and to investigate a candidate lncRNA in a neonatal mouse model of MI.

Materials and Methods: FACS and RNA-seq approaches were used to identify cell type-specific lncRNAs of the mouse heart (1 day versus 5 days postnatum). MI in newborns was induced by permanent left anterior descending artery (LAD) ligation and confirmed by echocardiography, which also assessed cardiac functional parameters one or two weeks after MI. Hearts were harvested for molecular and histopathological analysis.

Results and Conclusions: RNA-seq of neonatal endothelial cells (ECs) and fibroblasts revealed 1175 EC-specific and 378 fibroblast-specific lncRNAs, while 263 lncRNAs were significantly altered in whole heart tissue. LncRNA H19 was significantly altered in both EC and whole heart datasets and was selected for in vivo experiments due to its remarkable species conservation. Ejection fraction improved in both H19 knockout (KO) and H19 wild type (WT) mice, but H19KO mice regenerated significantly less. Increased levels of collagens and Mmp9 were detected by q-RT-PCR in H19KO mice one week after MI. No histological evidence of scarring was found in LAD-ligated H19WT mice, whereas H19KO animals showed clear signs of fibrotic healing two weeks after MI.

In summary, our RNA-Seq data seem to be a good source for the identification of (non-coding) RNAs relevant for cardiac regeneration. The in vivo data indicate that H19 is essential for full cardiac regeneration after ischemic injury in neonatal mice.

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Circulating cell-free mitochondrial DNA is inversely associated with heart failure in type 2 diabetes mellitus patients

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Background: Cell-free nuclear (cf-nDNA) and mitochondrial (cf-mDNA) DNA are released from damaged cells in type 2 diabetes mellitus (T2DM) patients, contributing to adverse cardiac remodeling, vascular dysfunction, and inflammation. The purpose of this study was to correlate the presence and type of cf-DNAs with HF in T2DM patients.

Methods: A total of 612 T2DM patients were prescreened by using a local database, and 240 patients (120 non-HF and 120 HF individuals) were ultimately selected. The collection of medical information, including both echocardiography and Doppler imagery, as well as the assessment of biochemistry parameters and the circulating biomarkers, were performed at baseline. The N-terminal brain natriuretic pro-peptide (NT-proBNP) and cf-nDNA/cf-mtDNA levels were measured via an ELISA kit and real-time quantitative PCR tests, respectively.

Results: We found that HF patients possessed significantly higher levels of cf-nDNA (9.9 ± 2.5 µmol/L vs. 5.4 ± 2.7 µmol/L; p = 0.04) and lower cf-mtDNA (15.7 ± 3.3 µmol/L vs. 30.4 ± 4.8 µmol/L; p = 0.001) than those without HF. The univariate log regression exhibited that NT-proBNP (OR = 1.09; 95% CI = 1.05–1.16; p = 0.001); cf-nDNA > 7.6 µmol/L (OR = 1.05; 95% CI = 1.02–1.08; p = 0.02); cf-mtDNA < 21.4 µmol/L (OR = 1.03; 95% CI = 1.01–1.06; p = 0.04); and LAVI (OR = 1.05; 95% CI = 1.02–1.09; p = 0.02) were predicted with respect to HF in the T2DM patients. The multivariate log regression showed that the discriminative potency of cf-nDNA >7.6 µmol/L (OR = 1.07; 95% CI = 1.03–1.12; p = 0.01) was higher that the NT-proBNP (odds ratio [OR] = 1.10; 95% confidence interval [CI] = 1.04–1.19; p = 0.001) for HF.

In conclusion, we established that elevated levels of cf-nDNA, originating from NT-proBNP, were associated with HF in T2DM patients.

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The long non-coding RNA Down Syndrome Critical Region 9 is involved in Nitric Oxide Deficiency in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is characterized by impairment in nitric oxide (NO) production in Pulmonary Artery Endothelial Cells (PAEC).
To investigate the molecular underlying mechanisms , commercially primary human PAEC (hPAEC) were incubated for 24 hours at atmospheric or 40 mmHg-higher pressure in a dedicated chamber. NO production was evaluated using diamino-fluorescein diacetate-FM. Cell viability and proliferation were assessed with MTS assay, flow cytometry, cell cycle analysis and scratch assay, while endothelial nitric oxide synthase (e-NOS) phosphorylation and abundance were evaluated by western blot. RNA profile was explored by next-generation sequencing (NGS), analysed by ShinyGO vo.6.1 and key data were validated by RT-PCR in hPAEC and in ECs derived from induced pluripotent stem cells (iPSC-EC) from patients with PAH-causing mutations in BMPR2.
High-pressure exposure led to a decrease in NO in hPAEC, not due to an impairment in cell viability or proliferation. Furthermore, NO deficiency was not reversed by the addition of exogenous L-arginine, the principal e-NOS substrate. Western blot revealed a decrease in phosphorylated e-NOS in the high-pressure condition, despite an increase in total e-NOS expression. Among 11,486 DEGs, the long non-coding RNA Down Syndrome Critical Region 9 (DSCR9) was the most upregulated upon incubation of hPAEC at high-pressure. In silico analysis (LncRRIsearch) revealed its possible involvement in the e-NOS pathway. Furthermore, DSCR9 was enriched in Gene Expression Omnibus microarray datasets from patients with PAH (GSE90943, GSE151971, GSE117261) and RT-PCR confirmed its upregulation, as well as CAMK2 and NOS3 modulation, in hPAEC from PAH patients and in BMPR2-mutated hIPS-EC.
In conclusion, this study provides novel evidence of a role of DSCR9 in impaired NO production by PAEC in PAH.

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Identification of the lncRNA Chheaf-1 as regulator of cardiac function in heart failure

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Cardiovascular diseases are a leading cause of mortality worldwide. Cardiac hypertrophy, a prevalent condition that can lead to heart failure, is highly regulated by non-coding RNAs. In our study on mice challenged to pressure overload (TAC), we identified over 150 dysregulated long non-coding RNAs (lncRNAs) that are important for maintaining cardiac homeostasis. Among them, we discovered a novel lncRNA called Chheaf-1 (Cardiac Hypertrophy and Heart Failure -1), which modulates the expression of its neighbouring gene Rtn4 (Nogo-A) to regulate hypertrophic onset. To asses this interaction we generated a constitutive KO strain, Chheaf-1-/- . Chheaf-1-/- mice, exhibited decreased cardiac function when subjected to pressure overload. Interestingly, Chheaf-1-/- mice also showed reduced levels of Nogo-A and impaired autophagy, which were restored by treating them with myriocin, a ceramide synthesis inhibitor therefore highlighting the protective effect of Chheaf-1 for the heart. Chheaf-1 influences Rtn4 expression through H3K27Ac deposition. We finally identified a human counterpart for Chheaf-1, suggesting novel potential translational applications.

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A solution to no perfusion: a novel method for cardiomyocyte isolation in rodents

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Background
Ex vivo investigation of individual cardiomyocytes provides vital information for translational, clinical and mechanistic research. However, due to the complexity of the isolation steps to preserve cardiomyocyte viability and function, this procedure remains rare, with the Langendorf isolation method being the current gold standard. We have developed a novel, cutting-edge method without Langendorf or syringe perfusion for the isolation of individual cardiomyocytes from rodents.
Materials and Methods
Using healthy 15 week old male Zucker rats, we have successfully isolated cardiomyocytes without perfusion, and by using a particular cutting method we can avoid using a vibratome, allowing for a fast procedure. Cardiomyocyte morphology was assessed using immunocytochemistry coupled to confocal microscopy of WGA and phalloidin staining. Cardiomyocyte function was assessed under field stimulation through sarcomere length contraction recording and calcium transient analysis using a Calcium and Contractility system (IonOptix, the Netherlands).
Results
This novel method allowed isolation of individual and viable cardiomyocytes. Morphology assessment revealed distinct rod-shaped cells with an average sarcomere relaxation length of 1.8µm. The cardiomyocytes retained functionality ex vivo, and stably contracted under field stimulation as shown by sarcomere fractional shortening and maximal contraction velocity. The cardiomyocytes tolerated calcium reintroduction and demonstrated good calcium-handling as shown by calcium transient analysis including calcium amplitude (peak height) and time to baseline.
Conclusions
This new method represents a viable option for cardiomyocyte isolation in rodents without perfusion, therefore allowing for division of the heart for other forms of analysis, and potentially reducing both time and the number of animals required for experimentation. Further investigation is required to assess this method in other species.
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Human cardiomyocyte derived extracellular vesicles regulate cardiac fibroblast activation through miR-24

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Introduction: Cardiac fibrosis involves the increased deposition of collagen type I and activation of cardiac fibroblasts (CF) into myofibroblasts. The contributions of the microenvironment to CF activation are not well understood. Extracellular vesicles (EVs) are nanoparticles released by cells, carrying proteins, lipids, and nucleic acids, which modify the cellular activity of recipient cells. Here, we aim to identify the role of EVs derived from cardiomyocytes (CM) on CF cellular activity in healthy and pathological (MI) conditions, focusing on miR24 activity.

Methods: In silico analysis identified miR24 as a putative miRNA with a role in the regulation of CF activation and proliferation. The investigation of miR›s role in human CF was assessed following direct miR24 transfection by immunofluorescence and western blot analysis. miR24 presence in human iPS-CM derived-sEV was confirmed by RealTime-PCR.

Results: miR24 transfection on hCF demonstrated the role of this miR in the maintenance of the quiescent status of hCF, reducing proliferation and activation mediated by TGFβ treatment. hCM-sEV recapitulated miR transfection, while treatment with sEV-derived from cardiomyocytes damaged with hypoxia (MI-hCM) treatment resulted less efficient. Interestingly, MI-hCM-sEV showed lower levels of miR24 suggesting the pivotal role of these miR.

Conclusion: These data demonstrate the role of hCM-sEV in the regulation of hCF, possibly through miR-24. Further analysis, including the use of a 3D beating human heart organoid, will help to understand the hCM-hCF's cross-talk.

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Hypothermic and Cryogenic Preservation of Cardiac Tissue-Engineered Constructs

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Background

Cardiac tissue engineering (cTE) has already advanced towards the first clinical trials, investigating safety and feasibility of cTE construct transplantation in failing hearts. However, the lack of well-established preservation methods poses a hindrance to further scalability, commercialization, and transportation, thereby reducing their clinical implementation.

Material and Methods

In this study, hypothermic (short-term) preservation (4°C), (long-term) vitrification (-150°C), and (long-term) cryopreservation (-196°C) were investigated as potential solutions to extend the cTE construct implantation window. The cTE model used consisted of induced-pluripotent stem cell-derived cardiomyocytes embedded in a natural-derived hydrogel and supported by a polymeric melt electrowritten hexagonal scaffold. Constructs, composed of cardiomyocytes of different maturity, were preserved for four days, using several commercially available preservation protocols and solutions. Their viability, function (BPM and calcium handling), and metabolic activity were investigated after rewarming.

Results and Conclusions

Our observations suggest that cardiomyocytes› age did not influence post-rewarming viability, however, it influenced construct function. Hypothermic preservation with Hypothermosol® ensured construct viability and function. Furthermore, vitrification outperformed cryopreservation, but both viability and function were severely reduced after rewarming. In conclusion, whereas long-term preservation remains a challenge, hypothermic preservation with Hypothermosol® represents a promising solution for cTE construct short-term preservation and potential transportation, aiding in off-the-shelf availability, ultimately increasing their clinical applicability.

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the Ca[sup]2+[/sup]-activated K[sup]+[/sup] channel K[sub]Ca[/sub]3.1 is expressed in human stenotic aortic valve fibroblasts

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Background: Aortic stenosis (AS) is characterised by the accumulation of fibrotic extracellular matrix and calcific mineral within the aortic valve (AV) leading to reduced leaflet mobility, left ventricular outflow obstruction, and subsequent myocardial fibrosis. Myofibroblasts are the key cells implicated in fibrosis owing to their pronounced and persistent production of extracellular matrix and contractile activity. KCa3.1 channels are expressed by myofibroblasts and promote pro-fibrotic activity in rodent hearts and several human organs. However, the role of KCa3.1 in AS has not been explored. We aimed to examine the expression and function of the KCa3.1 ion channel expression in human AS. Material and Methods: AVs were collected from patients with severe AS undergoing surgical AV replacement. AV fibroblasts were isolated from the AV tissue and cultured. To determine KCa3.1 channel expression, qRT-PCR, immunohistochemistry, immunofluorescence and patch clamp electrophysiology were utilised. The function of KCa3.1 was examined using the selective KCa3.1 channel blocker senicapoc. Results: IHC/IF staining confirmed KCa3.1 protein expression in both AV fibroblasts and tissue. Patch clamp electrophysiology revealed functional KCa3.1 ion channels. KCa3.1 currents elicited by the channel opener 1-EBIO were blocked by senicapoc. RT-PCR confirmed basal expression of KCa3.1 mRNA (KCNN4) in AV fibroblasts that was increased by TGFβ1 stimulation. Furthermore, senicapoc reduced TGFβ1-induced collagen type 1 and α-smooth muscle actin mRNA expression in AV fibroblasts. Conclusions: We demonstrate for the first time that the KCa3.1 ion channel is expressed in cultured AV fibroblasts and in AS valve leaflets, and may therefore contribute to the development of AS. Moreover, TGFβ1-induced upregulation of the channel suggests the biological effects of TGFβ1 may be linked with KCa3.1 channel activity. Blocking KCa3.1 may represent an unexploited therapeutic target in reducing pro-fibrotic myofibroblast activity in AS. Funding sources: University of Leicester funded PhD studentship

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Next-generation sequencing revealed de-regulated microRNAs in long-COVID patients showing specificity for cardiovascular tissues

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Background: Several microRNAs (miRs) have been found as possible prognostic markers of acute coronavirus-19 disease (COVID). However, the diagnostic role of miRs in long-COVID has not been reported yet. We aimed to identify circulating miRs in long-COVID patients to use as a possible biomarker for diagnostic purposes.
Material and Methods: From our long-COVID patient database and biobank we selected plasma samples of 20 symptomatic long-COVID patients (≥3 symptoms from 3 different organs), 20 post-COVID symptomfree controls and 20 healthy controls (no history of COVID infection). Differentially expressed (DE) miRs were identified and their specificity were assessed by using tissue specificity index via miRNATissueAtlas2.
Results: Seventeen miRs (10 up-, and 7 down-regulated) were significantly DE between long-COVID patients and healthy controls, 53 between symptomfree post-COVID patients and healthy controls (Figure 1A). Table 1 depicts the log fold changes (logFC) and false discovery rates (FDR) of up- and downregulated DE miRs of the long-COVID and healthy groups. MiR-1277-5p and miR-3651 had the greatest positive logFC, while miR-203a-3p the most negative logFC. Likewise, miR-1277-5p was the most upregulated when comparing symptomfree post-COVID to healthy controls (logFC 2.81, $p<0.001$, FDR 0.001). MiR-3561 was mostly upregulated (logFC 1.14, $p=0.004$, FDR 0.111) between symptomatic and asymptomatic post-COVID patients. Four of the upregulated miRs had moderate to high tissue specificity indices to heart tissues (ventricle, atria, atrial appendage) and arteries, suggesting cardiovascular involvements as possible pathomechanisms in long-COVID, which is yet to be fully understood.
Conclusion: Ten miRs were found to be upregulated in long-COVID patients compared to healthy controls, showing distinctive tissue specificities for heart, artery, lung tissues; therefore could be candidate miRs for diagnosis of long-COVID. Further experimental validation and clinical correlations of the selected miRs are needed to confirm their role as potential long-COVID biomarkers.
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Abstract retracted by authors

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Investigating the uptake of small extracellular vesicles (sEVs) in the heart and their mechanism of cardioprotection

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Background: Small Extracellular Vesicles (sEVs, also known as exosomes), are nanosized membrane-bound vesicles that have been shown to exert cardioprotective effects in animal models of acute myocardial infarction (AMI). Although biodistribution studies have shown that sEVs are localised in the heart after administration, it is still unclear which cardiovascular cell types contribute to the uptake. Additionally, Phosphatidylserine (PS), a common constituent of the vesicular lipid membrane, has been postulated to have cardioprotective properties. We hypothesise that sEVs are taken up preferentially by cardiac endothelial cells rather than cardiomyocytes and phosphatidylserine (PS) presenting liposomes (PS+-liposomes) reduce the infarct size in AMI.
Materials and Methods: sEVs were isolated from cell media after ultrafiltration and size exclusion chromatography (SEC) from genetically modified HEK293 cells to express a Nanoluciferase (Nluc) sequence on the membrane-bound specific marker CD63 (CD63-Nluc), which allows their luminescent tracking in vivo and in vitro. Human coronary artery (HCAECs) and cardiac microvascular endothelial cells (HCMECs), human umbilical vein endothelial cells (HUVECs) and cardiomyocytes were used for sEVs uptake experiments at 1h, 4h, and 24h. The presence of sEVs was verified by nanoparticle tracking analysis (NTA), DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay) and luciferase assay.
Results: The overexpression of CD63-Nluc and their luminescent capability was verified by luciferase assay, DELFIA, and immunofluorescent assay. HCAECs, HCMECs, and HUVECs internalised sEVs in a dose dependent manner, with the highest uptake recorded after 4h, but cardiomyocytes internalized much less.
Conclusion: A preliminary conclusion of this work to far is that the internalisation and biodistribution of sEVs is cell type specific. Experiments are now being conducted to assess biodistribution after intravenous administration of sEVs in mice, and to assess the effect of PS+-liposomes on infarct size in a mouse model of AMI.
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Markers of metabolic syndrome linked to cognitive decline

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BACKGROUND
Insulin resistance can affect multiple tissues and organs, from the classic "triumvirate" (myocyte, adipocyte, and hepatocyte) to possible effects on organs such as the central nervous system. This review explores shared pathophysiological mechanisms between MCI and MS and establishes a hypothesis of a possible MCI role in the development of IR and the appearance of MS.
OBJECTIVE
The objective of the study was to investigate new biomarkers for early diagnosis of MS and cognitive decline as a follow-up. A cardiological, neuropsychological and neurological study was conducted among 75 Bulgarian participants. All data and samples derived from the University Hospital of Pleven. Participants were collected from July 2018 to July 2019. Beta amyloid in the blood, procalcitonin (PCT), NT-proBNP as predictors of cognitive impairment in patients with MS were identified.
METHODS
Clinical, anthropometric, biochemical, neuropsychological, cognitive and statistical data processing. Plasma amyloid beta (Aβ) levels, procalcitonin, NT-proBNP in MS were investigated in participants with MS and in healthy controls.
RESULTS
In the present study, an inverse relation between NT-proBNP and diastolic blood pressure, waist circumference, triglycerides, HDL- and LDL cholesterol was found. Plasma levels of Aβ42 and Aβ40 were found to be reduced in MetS participants. Regression analysis showed a positive relationship between NT-proBNP and systolic blood pressure ($p < 0.001$) and fasting blood glucose ($p < 0.05$).
CONCLUSIONS
There was a positive association between PCT levels, decreased levels of Aβ42 and Aβ40, as well as elevated NT-proBNP and cognitive impairment in people with MS. A concentration of NT-proBNP of 60 pg / ml or greater could be an indicator of metabolic abnormalities and early cognitive decline. Large-scale studies and longer follow-up periods will be necessary to establish a direct and accurate causal relationship between MS and MCI pathologies.

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Impaired endothelial function in Duchenne muscular dystrophy-associated cardiomyopathy: insights from human induced pluripotent stem cell-derived endothelial cells

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BACKGROUND: Duchenne muscular dystrophy (DMD) is a genetic disorder caused by dystrophin gene mutations, leading to progressive weakening of skeletal and cardiac muscles. While respiratory failure was previously the main cause of death, DMD-associated cardiomyopathy is now the leading cause of premature mortality in affected individuals. Understanding the mechanisms driving DMD cardiomyopathy is crucial, and some potential therapeutic avenues remain not fully explored. Endothelial dysfunction has been proposed as a contributing factor in DMD pathogenesis based on studies involving skeletal muscle biopsies from affected individuals and animal models. However, whether endothelial dysfunction also occurs in the hearts of DMD patients remain elusive.

MATERIAL AND METHODS: To address this knowledge gap, we generated a patient-specific in vitro model of DMD endothelium by differentiating DMD and control human induced pluripotent stem cells into endothelial cells (hiPSC-ECs). In this model, we investigated key features of endothelial dysfunction, including expression of endothelial-specific markers, inflammation, oxidative stress, and nitric oxide production, to explore their involvement in DMD cardiomyopathy.

RESULTS AND CONCLUSIONS: Our characterization of DMD hiPSC-ECs revealed dysregulated mRNA expression of genes crucial for endothelial function. Specifically, downregulation of vWF, critical for primary haemostasis, and upregulation of CD31, involved in regulating inflammation. We also detected increased levels of TNF-α, IL-1β, IP10 and TGF-β in DMD hiPSC-ECs, indicating a pro-inflammatory state. Functionally, these cells exhibited higher production of reactive oxygen species, indicative of oxidative stress, along with lower nitric oxide production. In conclusion, our studies identify hallmarks of endothelial dysfunction in hiPSC-ECs derived from a DMD patient with associated cardiomyopathy. These include impaired expression of endothelial-specific genes, a pro-inflammatory phenotype, oxidative stress, and reduced nitric oxide production. These insights demonstrate the existence of autonomous cellular defects in DMD hiPSC-ECs and set the endothelium as a potential new therapeutic target in DMD.

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Unravelling the metabolic rewiring in the context of doxorubicin-induced cardiotoxicity: fuel preference changes from fatty acids to glucose oxidation

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Background
Doxorubicin (DOX), a potent chemotherapy drug, can lead to cardiotoxic effects, causing a decline in heart function within the first year of treatment due to accumulation within mitochondria and metabolic disruption. Previous research has linked phosphoinositide 3-kinase gamma (PI3Kγ) to DOX-induced cardiotoxicity, with impaired autophagy and accumulation of damaged mitochondria.
Material and methods
We administered DOX to 10-week-old wild-type (WT) and kinase-dead (KD) mice, which express inactive PI3Kγ, with three doses. Metabolomic analysis was conducted on day 3 to assess metabolic alterations. To investigate autophagy›s role, we used AAV9 to inhibit ATG7 in 8-week-old mice. In neonatal mouse cardiomyocytes, we measured glucose uptake and GLUT-4 translocation to the plasma membrane with acute DOX treatment (1μM for 3h). Oxygen consumption was analyzed in myocardial slices using OROBOROS instrument.
Results
Metabolomic analysis of DOX-treated WT hearts revealed an accumulation of TCA cycle metabolites due to a cycle slowdown, with reduced levels of pyruvate, unchanged abundance of lactate and increased Acetyl-CoA. Moreover, the activity of glycolytic enzymes was upregulated, and fatty acid oxidation downregulated, with increased glucose oxidation. In agreement, oxygen consumption was increased in after pyruvate supplementation, with the formation of cytotoxic ROS rather than energy production. These metabolic changes were fully prevented in KD hearts. Interestingly, they failed to increase glucose oxidation in response to DOX even with autophagy inhibition, indicating that PI3Kγ likely controls the fuel preference after DOX through an autophagy-independent mechanism. In vitro experiments showed that inhibition of PI3Kγ inhibits pyruvate dehydrogenase (PDH), the key enzyme of Randle cycle regulating the switch from fatty acids to glucose usage, while decreasing DOX-induced mobilization of GLUT-4-carrying vesicles to the plasma membrane and limiting the ensuing glucose uptake.
Conclusions
These results demonstrate that PI3Kγ promotes a maladaptive metabolic rewiring in DOX-treated hearts, controlling PDH activation and GLUT-4-mediated glucose uptake.

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Empagliflozin's cardioprotection: evidence for the improvement of microvascular injury and cardiac function upon myocardial ischemia reperfusion

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Background: Empagliflozin (EMPA) is cardioprotective in preclinical studies of myocardial ischemia-reperfusion (IR) injury independently of the presence of diabetes and the expression of the sodium glucose co-transporter 2. The off-target mechanism of protection and the application of EMPA in a translational setting remains elusive. Purpose: We aimed to identify which cardiac cell types are affected by EMPA and compare the cardioprotective effect of EMPA pretreatment and treatment after the IR injury which is clinically relevant. Methods: C57Bl6 male mice were randomized into 1)Sham, 2)Control-IR (ischemia/reperfusion) and 3)EMPA (10mg/kg/day for 6 weeks) groups. Mice underwent 30'I/2hR or sham operation. Cardiomyocytes (CMs) were isolated, endothelial cells (ECs) and fibroblasts (FBs) were sorted and 3'RNA sequencing was applied. The infiltration of immune cells in the heart was examined at the 2nd and 7th day of reperfusion. We repeated the experimental protocol and added EMPA-Post group. EMPA was given 1h and for 2 consecutive days after reperfusion. At this timepoint, infarct size (IS) and microvascular injury (MVI) via thioflavin S staining were determined. Cardiac magnetic resonance was performed to evaluate cardiac function. Results: IR significantly alters gene expression in ECs and FBs but not in CMs compared to Sham. EMPA pretreatment significantly alters the ECs transcriptome and restores the gene expression related to extracellular matrix and ECs junction/adhesion molecules. The effect of EMPA on ECs was supported by the reduced infiltration of neutrophils and inflammatory monocytes in the myocardium. EMPA pretreatment and EMPA-Post reduces IS and MVI at 2 days of reperfusion. EMPA pretreatment completely restores cardiac function while EMPA-Post improves global longitudinal strain. Conclusions: EMPA pretreatment affects ECs which is associated with reduced MVI, IS and improved cardiac function. EMPA-Post reduces IS, MVI and shows an intermediate improvement in cardiac function implying that translation in the clinical practice is promising. Funding: Boehringer Ingelheim.

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Circulating and Cardiac Small Nucleolar RNA SNORD3A a novel mediator of Heart Failure.

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Background. Despite optimal therapy, heart failure (HF) remains a relentless and deadly disease. Human cardiac biopsies are invaluable resources to identify cardiac signaling pathways, however blood fractions and peripheral blood mononuclear cells (PBMCs) might be used as a source of surrogate biomarkers of signaling pathways activation in human HF.

Material and Methods. RNA sequencing analysis (RNASeq) was performed in paired human myocardial left ventricle (LV) and PBMC samples obtained from patients with HF and healthy subjects. Five identified transcripts, KCNQOT1, MIAT, SCRN1, MALAT1 and SNORD3A, were then validated by quantitative real-time PCR in PBMCs and in LVs. Next, we analyzed the expression of the Small Nucleolar RNA (snoRNA) SNORD3A in LVs and PBMCs from C57BL/6 mice with pressure overload-induced HF by transverse aortic constriction (TAC) and in sham-operated animals. To further investigate the role of SNORD3A in cardiomyocyte function and survival, SNORD3A antisense oligonucleotides (SNOaso) and scramble control sequences (GFPaso) were synthesized and tested in cardiomyoblasts H9C2 cells under normoxic or hypoxic conditions to evaluate SNORD3A transcription levels, cell death and protein synthesis.

Results and conclusions. SNORD3A expression was significantly increased in both LVs and PBMCs from HF patients compared to control subjects. Consistent with these results, SNORD3A levels were increased both in PBMCs and LVs from TAC mice compared to sham. In vitro hypoxia significantly increased SNORD3A expression levels in H9C2 cells, compared to normoxia. After SNOaso transfection, SNORD3A transcripts were downregulated, and they were further reduced following 3-hour-hypoxia. Depletion of SNORD3A levels increased cardiomyocyte death and reduced protein synthesis in H9C2 cells. Our data suggest that SNORD3A might be involved in the regulation of cardiomyocyte survival in HF and could be useful for diagnostic and prognostic applications in HF.

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Role of Mitochondrial A-kinase anchoring proteins in cardiac function and gut microbiota composition during aging.

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Background: Mitochondrial A-kinase anchoring proteins (mitoAKAPs) locally amplify cAMP/PKA signaling to mitochondria, regulating mitochondrial functions and oxidative stress. While mitochondrial dysfunction and oxidative stress are involved in aging and in the regulation of gut barrier function and microbiota composition, the role of mitoAKAPs in gut-heart axis during aging is still poorly understood.
Materials and Methods: Cardiac function was evaluated by transthoracic echocardiography in 6-month-old (6m) and 24-month-old (24m) wild-type (wt) mice and in genetically modified mice with partial deletion of the Akap1 gene (Akap1+/-). To evaluate gut barrier integrity, we analyzed expression levels of intestinal junction proteins occludin (Ocln), zonulin (Tjp1) in colonic samples. To investigate gut permeability in vivo we analyzed circulating levels FITC dextran D4000 (D). Gut microbiota composition was analyzed by Illumina Mi-Seq analysis. Finally, faecal microbiota transplantation (FMT) was performed to test whether modification of gut microbiota composition might affect cardiac function.
Results and Conclusions: A reduction in % left ventricular shortening (FS%) is observed in 6m and 24m Akap1+/- mice compared to wt mice. This finding was associated to increased intestinal permeability, as indicated by reduced mRNA levels of Ocln and Tjp1, and increased D traslocation across intestinal epitelium into blood in 24m Akap1+/- mice compared to wt. Through microbial signature analysis, we identified in 24m Akap1+/- mice a significant increase of Ruminococcus Torques species and a significant decrease in Blautia producta. After FMT, feces from 24m Akap1+/- mice induced cardiac dysfunction in 6m wt mice, while feces from 6m wt mice ameliorated cardiac dysfunction 24m Akap1+/- mice. Thus, mitoAKAPs play a crucial role in modulating cardiac and gut function during aging. Modulation of gut microbiota composition influences intestinal permeability and cardiac function. MitoAKAPs could represent an important diagnostic and therapeutic target for cardiac and intestinal dysfunction.

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Hydrolytic activity of mitochondrial F1FO-ATP synthase as a target for myocardial ischemia reperfusion injury

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Background: F1FO-ATP synthase is the mitochondrial complex responsible for ATP production. During myocardial ischemia, it reverses its activity, hydrolyzing ATP leading to cardiac injury. Purpose: We aimed to discover novel inhibitors of ATP hydrolysis and access the draggability of the target within ischemia(I)/reperfusion(R) injury. Methods: The inhibitory effect on ATP hydrolysis was evaluated on isolated murine heart mitochondria and at a cellular level on H9C2 cells where their ability to maintain membrane potential was monitored. The previously described ATP hydrolase inhibitor BTB06584 (BTB) was used in vitro and in vivo as a reference. Three novel compounds were selected for in vivo evaluation namely 1117, 1119 and 31. In the first cohort, C57Bl6 mice were randomized into 5 groups, receiving intravenously: 1) vehicle, 2) 1117, 3)1119, 4) 31 and 5) BTB, prior to 30 min of I and 2 hours of R and infarct size (IS) was determined. In a second cohort, the best candidate was given at the 5th minute of I and IS was measured to test the intervention in a more translational setting. Finally, the cardioprotective mechanism was investigated at the 10th min of R. Results: Three candidates inhibited the ATP hydrolysis with better IC50 values than BTB. In vivo, the three novel inhibitors reduced IS compared to the control group with compound 31 having the most remarkable cardioprotective effect while BTB did not result in attenuation of IS. Compound 31 reduced IS when given after the induction of I, indicating that its cardioprotective effect is pertained in a translational protocol. Regarding the mechanism of cardioprotection, compound 31 preserved ATP levels increased activation of PKA, activated the RISK pathway and reduced apoptosis. Conclusion: We discovered an ATP hydrolysis inhibitor that reduces myocardial IS in vivo. Its cardioprotective mechanism involves the activation of PKA signaling and alleviation of apoptosis.

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Empowering A.I. in characterizing the human primary pacemaker at single cell resolution

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Background: The cardiac conduction system orchestrates the electrical activity of the heart, with the sinus node (SN) in the right atrium serving as the primary pacemaker. Understanding the electrophysiology of the SN is crucial for heart failure, arrhythmias, and aging. Due to its anatomical properties and sample scarcity, the cellular composition of the human SN has been historically challenging to study. Here, we employed a novel A.I. deep learning method to characterise the human SN at single cell resolution.
Materials and Methods: To characterise the human SN, a recently published deconvolution tool named Bulk2Space was used to generate scRNA-Seq data from existing bulk RNA-Seq data. As a proof of principle, the tool was validated by deconvoluting previously generated human atrium RNA-Seq data using publicly available mouse scRNA-Seq data as reference. Subsequently, the deconvolution method was applied to RNA-Seq data from the human SN. Deconvolution was performed in Python whereas scRNA-Seq analysis was performed in R, using the Seurat package.
Results and Conclusions: Bulk2Space was able to recapitulate the single cell composition of the human right atrium using existing mouse scRNA-Seq data. Clustering analysis revealed 18 cell populations, with cardiac myocytes being the most abundant. Each cell population generated was highly similar to its published experimental counterpart, demonstrating that Bulk2Space can be used to deconvolute human RNA-Seq data using data from widely used species. Within the human SN, 11 cell populations were identified, including a population of pacemaker cardiomyocytes expressing pacemaking marker genes (HCN1, HCN4, CACNA1D) and transcription factors (SHOX2 and TBX3). Moreover, the connective tissue of the SN was characterised by adipocyte and fibroblast populations, as well as key myeloid and lymphoid cells. In conclusion, we have shown a novel method to unravel the unique single cell composition of the SN. Founding sources: Leducq Foundation grant 19CV03; British Heart Foundation.

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Molecular mechanisms of cardiac lipophagy and dysfunction; a driver of diabesity-linked heart failure

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Background
Myocardial lipid droplet (LD) accumulation is an early pathological development in 'diabesity' related heart failure (HF); however, mechanisms involved remain poorly understood. We hypothesise that deficits in processes regulating LD breakdown (lipophagy) contribute towards LD accumulation and the development of cardiometabolic dysfunction and that the protein Eps-homology domain protein 2 (EHD2) is a novel regulator of lipophagy. EHD2 is a GTPase which binds ATP to form oligomers that can cause membrane tubulation, a property we suggest is crucial for autophagosomal LD engulfment.
Materials and Methods
Cardiomyocytes (H9C2 and human iPSC-cardiomyocytes) were transfected with an EHD2-GFP plasmid (Lipofectamine-3000) with deconvolution (Olympus IX83 Inverted) and live cell fluorescence (Zeiss Axio-Observer Z1) microscopy used to measure co-localisation (Imaris) with lipid droplets (Oil Red O, LipoTox). Experiments included serum starvation and treatments with inducers and inhibitors of lipophagy. Standard qPCR and Western blotting techniques were used to quantify mRNA/protein expression.
Results
In a model of obesity-linked diabetes, EHD2 is down-regulated (0.48±0.1; P=0.01). EHD2 co-localises with LDs and, upon lipophagy induction, there is a 1.7-fold±0.04 (P=0.0278) increase in co-localisation events. EHD2 down-regulation results in a ~1.75-fold+/-0.17 (P=0.0266) increase in lipid droplet content and 3D analyses show LD volume is increased by ~21+/-0.05% (P=0.0204). Specific lysosomal inhibitors cause EHD2 down-regulation (~56+/-0.02%; P=0.00039) indicating a novel role for EHD2 mediating autophagosome-lysosomal fusion.
Conclusions
We have defined a novel role of EHD2 as a regulator of lipophagy in cardiac cells. We propose that larger and more LDs, due to EHD2 down-regulation, may contribute towards impaired lipid homeostasis including altered mitochondrial cross-talk affecting a shift towards β-fatty acid oxidation and cardiac energy inefficiencies. Delineating mechanisms of lipophagy in the heart in the context of diabesity will provide important new directions targeting LD catabolism as a therapeutic strategy.
Funding Sources
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Abstract retracted by authors

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Natural activators of autophagy protect the heart from DOX-induced cardiomyopathy

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Background: Heart failure is a common cause of mortality in cancer patients treated with doxorubicin (DOX), a chemotherapy drug known to inhibit autophagy in cardiomyocytes and induce myocardial damage. Natural activators of autophagy (NAA), including trehalose and spermidine, have demonstrated potential in restoring autophagic flux and treating cardiovascular ailments in mouse models.
Materials and Methods: C57BL/6J WT mice were provided with trehalose or spermidine in their drinking water and subjected to three weekly injections of DOX, resulting in a cumulative dose of 15 mg/kg. Six weeks post-initial DOX administration, echocardiographic, histological, and biochemical assessments were conducted. Additionally, in vitro experiments were conducted using neonatal rat primary cardiomyocytes.
Results: DOX-treated mice exhibited reduced systolic function compared to controls (Fractional Shortening [FS]: 44±1.05% vs. 34.1±2.33%, n=6-8). However, trehalose administration preserved left ventricular fractional shortening (FS: 34.1±2.33% vs. 45.1±0.75%, n=6-8). Autophagy-deficient Beclin1+/- mice displayed reduced FS and were not protected by trehalose administration (35.4±2.48% vs. 45.1±0.75%, n=7-8). DOX-treated mice developed fibrosis (0.1±0.08% vs. 8.6±3.5%, n=5), but trehalose reduced this effect in DOX-exposed mice (8.6±3.5% vs. 2±0.68%, n=4-5). DOX administration increased damaged-mitochondria disposal (2.6±0.74 vs. 9.4±1.7 mitophagic bodies per TEM field, n=13), and trehalose further enhanced this effect (9.4±1.7 vs. 24.1±1.45, n=13). In neonatal primary cardiomyocytes, DOX treatment reduced autophagy (51.9±4.57 vs. 29.9±3.52 red dots per cell, n=34-51), while trehalose restored autophagic flux (29.9±3.52 vs. 121.5±8.02 red dots per cell, n=33-34). ‹MitoTimer› mice exposed to DOX exhibited increased mitochondrial biogenesis (0.8±0.38 vs. 3.3±0.9, n=5-6), whereas co-administration of DOX and trehalose prevented this effect (3.3±0.9 vs. 0.3±0.12, n=5-6). Similarly, the autophagy activators spermidine (FS: 29.4±2.03 vs. 39±3.22, n=5-6) and TAT-Beclin1 (32,8±3,62 vs. 40,9±1,81, n=7-10) preserved systolic function and elevated myocardial autophagy.
Conclusions: The induction of mitophagy by NAA protects the heart from DOX-induced cardiomyopathy by eliminating damaged mitochondria.
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In vitro and in vivo characterization of cardiotoxicity in light chain amyloidosis

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Background: Light chain amyloidosis (AL) is a plasma cell dyscrasia in which cardiac involvement determines the prognosis. Cardiotoxicity of light chains (LCs) complicates the management of heart failure in AL and has not been addressed in the clinical praxis.
Purpose: We aimed to characterize the mechanism of LC induced cardiotoxicity in vitro and in vivo and identify possible protective therapies.
Methods: The clonal LCs from seven patients with AL and cardiac involvement and the respective isotype LCs from two healthy volunteers (HV) were biotechnologically produced. Primary adult ventricular murine cardiomyocytes (pAVMCs) were isolated and treated with the LCs for evaluation of cell death. C57BL6 mice received the LCs via intramyocardial injection (IMI). Echocardiography, histology, electron microscopy and investigation of the cardiotoxicity mechanism was performed on the 4th week post IMI.
Results: Five out of seven AL derived LCs significantly increased cardiomyocyte death in pAVMCs compared to non-clonal LCs derived from HV and cardiotoxicity was correlated to their highest amyloidogenic potency. In vivo, the λ type LCs significantly reduced ejection fraction compared to vehicle and isotype control groups while the κ type did not alter systolic function. None of the animals bared amyloid deposits. The AL-LCs induced different molecular responses in vivo. The AL-κ increased apoptosis via the CHOP-Bax pathway. The AL-λ increased Beclin-1 mediated autophagy without inducing apoptosis. We identified that the common upstream mediator of AL-LCs cardiotoxicity in vivo is the overexpression of the endoplasmic reticulum stress (ERS) markers, IRE-1a and Bip. In vitro, we confirmed that the AL-LCs induce pAVMCs death through ERS. Treatment with the IRE-1a inhibitor STF-083010 and the molecular chaperone tauroursodeoxycholic acid restored the LCs' induced cardiotoxicity.
Conclusions: The IMI of AL-LCs proved the cardiotoxicity of LCs in vivo in the absence of amyloid deposits. We identified ERS as a target to alleviate AL-LCs toxicity.

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Specific, graded cleavage of elastic titin in living mouse hearts reveals compensatory potential but rapid decline in cardiac function above 50% cleavage

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To detect the effect of titin stiffness loss on living heart function, we used a mouse model that allows specific, graded cleavage of elastic titin. In this titin-cleavage (TC) mouse, a tobacco etch virus protease (TEVp) recognition site was cloned into elastic titin. Cardiac-specific titin cleavage was achieved by systemically injecting AAV9-TEVp plasmid under a cTnT-promoter; AAV9-eGFP plasmid was injected as a control. Six days post-injection, 22.2±2.9% (N=3) of cardiac titin were cleaved in heterozygous and 39.8±1.6% (N=4) in homozygous TC mice, increasing to 62.6±2.6% (N=2) ten days post-injection. Two weeks post-injection, 30.9±6.1% (N=3) of titin were cleaved in heterozygous and 57.4±2.1% (N=5) in homozygous TC mice, suggesting saturation of titin cleavage. Microscopic analyses of >50% titin-cleaved heart tissue revealed sarcomeres with disrupted or missing I-bands, thinned diameters, and wavy Z-discs; A-bands were preserved longer. Degenerated areas and aggregates were present in cardiomyocytes; fibrosis was abundant. Titin cleavage by 20-30% resulted in Z-disk waviness but little other damage. Western blotting demonstrated increased ubiquitination of cleaved cardiac titin, upregulation of E3 ligases, and autophagy activation, most of which became significant only above ~50% titin cleavage. Passive force and Ca2+-dependent active force of permeabilized cardiac fiber bundles isolated from >50%-cleaved AAV9-TEVp hearts were significantly reduced compared to control samples. Echocardiography of homozygous, but not heterozygous, TC-Halo mice revealed worsening systolic and diastolic heart function (cardiac output; stroke volume; systolic and diastolic volumes; LV internal diameters; E/E') compared to AAV9-eGFP injected mice, which began to manifest 6 days post-injection and persisted until 14 days post-injection. Mouse voluntary running distance also dropped significantly during this period. Collectively, echocardiographic parameters remained nearly unaltered until ~55% of titin became cleaved. These findings reveal the important role of titin in cardiac contractility but also a high compensatory potential of the titin-cleaved heart.

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A ‘musical chairs’ approach to untangle the sources of myocardial passive stiffness reveals the dominant role of titin in concert with cytoskeletal filaments and the extracellular matrix

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Background: The passive stiffness of the cardiac walls is crucial for the heart’s pump function, however, increased myocardial stiffness is also a hallmark of heart failure with preserved ejection fraction. Various mechanical elements determine and regulate myocardial stiffness, including the extracellular matrix and cardiomyocyte proteins; however, their individual contributions are controversially discussed and difficult to quantify. Here, we aimed to dissect the passive stiffness contributions of the microtubules, the sarcolemma, titin, and actin in the healthy heart by disrupting them one by one using a novel mouse model, which allows for the specific, acute and complete cleavage of the sarcomeric titin springs.

Materials and Methods: Stress-strain relationships were measured in non-activated cardiac fiber bundles or single cardiomyocytes from the homozygous titin cleavage-Halo mouse. This mouse contains a genetic cassette in the titin spring that is cleavable using tobacco etch virus protease (TEVp). The microtubules, sarcolemma, actin and titin were systematically disrupted with colchicine, detergent, gelsolin, and TEVp, respectively, and confirmed biochemically. Changes to the cellular substructure were examined by microscopy.

Results and Conclusions: The distribution of passive forces among individual elements varied with strain. Titin alone contributed over one-half of the elastic forces at 10% strain and just over one-third at 20% strain, ‘losing’ mainly to the matrix and the sarcolemma, whereas actin and microtubules substantially contributed to a similar proportion at all strain levels . The microtubules contributed ~35% to viscous forces at low strain but their contribution at high strain (27%) was matched by titin and the extracellular matrix, with actin and desmin playing lesser roles.

Our findings answer long-standing questions about cardiac mechanical architecture and inform on therapeutic strategies that target myocardial stiffness in heart failure.

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Biochemical Markers of Heart Failure Phenotype in Takotsubo Syndrome

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Abstract

Background: We investigate if renin-angiotensin and endothelin-1 response pathways follow the same pattern of recovery as left ventricular ejection fraction in patients with takotsubo syndrome.

Methods: Ninety takotsubo syndrome patients [n=30 in each of "acute", "convalescent" (3-5 months) and "recovered" (> 1 year) groups] who were on minimal or no medication and were free of any significant cardiac/metabolic co-morbidities, and 30 healthy controls were studied. Serum concentrations of renin, angiotensin converting enzyme, angiotensin II, big endothelin-1, endothelin-1 were measured using commercially available ELISA, and BNP was measured using an immunoassay.

Results: Left ventricular ejection fraction was 38 ± 1.6 % in acute, 63 ± 2.0 % in convalescent and 64 ± 2.6 % in recovered takotsubo syndrome patients. As shown in the Figure, serum renin concentrations are persistently elevated after a takotsubo episode ($p=0.03$ vs controls). Angiotensin converting enzyme levels are significantly depressed during the acute phase compared to convalescent ($p=0.004$), recovered takotsubo ($p=0.02$) or controls ($p=0.03$). Angiotensin II is increased in takotsubo patients ($p<0.001$ vs controls) remaining persistently elevated long-term in the recovered group ($p=0.03$ vs controls). B-type natriuretic peptide concentrations remain elevated after a Takotsubo episode compared to controls ($p=0.003$). Big endothelin-1 levels are unchanged, but endothelin-1 is significantly lower after takotsubo syndrome compared to controls ($p=0.03$).

Conclusions: Despite 'normalisation' of the left ventricular ejection fraction, there is long-term maladaptive activation of renin-angiotensin system in takotsubo syndrome patients. This suggests therapy aimed at modulating this pathway may be beneficial in the long-term

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Titin contributes less to transverse passive stiffness of cardiac fibers than to longitudinal stiffness.

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Background. The giant sarcomere protein titin plays a key role in active and passive cardiac muscle function. We recently demonstrated (Loescher et al., Nature Cardiovasc. Res. 2023; in press) on longitudinally stretched cardiac fiber bundles of a unique titin-cleavage (TC) mouse model that titin contributes over one-half of the tensile elastic forces at 10% strain and just over one-third at 20% strain. Since the heart functions like a pump, with three-dimensional force vectors, it would be interesting to also characterize the contribution of titin to myocardial stiffness in the transverse direction.
Material and Methods. As it is difficult to stretch cardiac fibers in the transverse direction, we applied atomic force microscopy nanoindentation to determine the transverse myocardial stiffness. We used the TC mouse model containing a HaloTag-TEV cassette in titin, which allows the specific and acute cleavage of I-band titin in permeabilized cardiac fibers by TEV-protease (TEVp). Transverse stiffness was measured on vital sections of homozygous TC fibers freshly permeabilized using Triton X-100 or on glycerol-permeabilized fibers, before and after a 25-min incubation with TEVp.
Results. We found that vital sections of permeabilized fibers exhibited substantial heterogeneity of transverse stiffness depending on the site of nanoindentation along the fiber surface. On average, incubation of homozygous TC fibers with TEVp reduced the Young´s modulus by approximately 20% (n=789; N=3). The indentation depth was similarly increased, suggesting softening of the myocardial structure in the transverse direction upon titin cleavage. Similar results were obtained with glycerol-permeabilized fiber bundles. These changes are much smaller than those found with titin cleavage in fibers stretched longitudinally.
Conclusions. Titin contributes to the transverse stiffness of cardiac fibers. However, whereas titin is the main contributor to myocardial passive stiffness in the longitudinal strain direction, it contributes to a much lesser extent to transverse myocardial stiffness.

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Living myocardial slices as a novel experimental model to investigate intercellular communication during right ventricle remodelling

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Background: Right ventricle (RV) hypertrophy and failure is the leading cause of death in patients suffering from pulmonary hypertension (PH). While much is known about left ventricle (LV) hypertrophy and remodelling, many mechanisms of RV remodelling and failure remain unknown, and no cure is available. Extracellular vesicles (EVs) are small vesicles released by all tissues that carry different types of molecules, including small RNAs. Studies have shown that the content of EVs, used for intercellular communication, changes during disease. However, current models are not RV specific. Living myocardial slices (LMS) is a novel biomimetic model consisting of ultra-thin slices of tissue that can be kept viable in culture for multiple days. The LMS culture media can be used to identify RV-specific secreted products during hypertrophic remodelling and disease.
Materials and Methods: Male Wistar rats were injected either 60 mg/kg body weight of monocrotaline (MCT) to induce the development of RV failure or PBS (control). After 4 weeks, rats were sacrificed and the RV free wall was used to generate 4 LMS, all of which were cultured for two days. Media was collected and refreshed each day and, after 48 h, pooled, spun down and concentrated with 100 kDa AMICON ultra concentrators. Concentrated media was mixed with Exo-Quick to precipitate the EVs which were then characterized by Western blot (WB) and nanoparticle tracking analysis (NTA).
Results: NTA shows EVs with a characteristic size between 50 and 150 nm and expressing the typical membrane markers. RNA isolation and subsequent miRNA sequencing identified three miRNAs, miR-126b-3p a known miRNA in RV remodelling, to be depleted in EVs from diseased RV LMS.
Conclusion: RV LMS maintain their RV phenotype and offer a novel method to isolate and analyse tissue-specific EVs and their downstream applications.

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A stem cell model of the RBM20-p.R634L variant from a left ventricular non-compaction patient reveals novel insights in metabolic and endothelial dysfunction

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Background/Methods: Mutations in the splice factor RBM20 account for ~3% of genetic cardiomyopathies. Mutations in the hotspot RS-domain (p.633-637) cause dilated cardiomyopathy or in rarer cases left-ventricular non-compaction-cardiomyopathy (LVNC), but the pathophysiological drivers that govern the heterogeneity in phenotype presentation remain unknown. We generated iPSC-cardiomyocytes (iPSC-CM) of a LVNC patient with RBM20-p.R634L mutation, and explored RBM20-mediated alternative splicing, RBM20- localization, sarcomeric regularity, Ca2+-homeostasis as well as mitochondrial and endothelial function. To investigate the direct impact of the mutation, isogenic rescue-lines (resLVNC) were generated by CRISPR/Cas9.

Results: We investigated the splicing pattern of the RBM20-mutation p.R634L in LVNC-iPSC-CM and observed isoform changes in titin, a 24bp-insertion in ryanodine-receptor 2, in the Ca2+-handling genes triadin and CAMK2δ and the mitochondrial gene IMMT. In addition, RBM20- protein accumulated in the cytoplasm in LVNC-CM. As a possible physiological consequence, we observed severe RBM20-dependent sarcomeric irregularity, shortened Ca2+-elimination time and weakened response to β-adrenergic stimulation. The mitochondrial and metabolic profile of LVNC-CM revealed an increased mitochondrial-membrane-potential with a concomitant increase in respiratory oxygen consumption rate, a possible consequence of the increased systolic Ca2+ content and/or the IMMT missplicing. Isogenic CRISPR/Cas9-repair of the RBM20-mutation (resLVNC) rescued all observed phenotypes. To analyze further the paracrine effects of CM on non-CM in LVNC pathology, LVNC- and resLVNC-endothelial-cells (EC) were generated and incubated either with supernatant of LVNC- or rescue-LVNC-CM. Using scratch assays, both LVNC-EC and the resLVNC-EC demonstrated a dysfunctional migration behavior if treated with supernatant from diseased LVNC-iPSC-CM. This suggests paracrine effects of LVNC-CM on endothelial function.

Conclusion: We show the first iPSC-model of splice-defect associated RBM20-dependent LVNC. In summary, our results suggest that the RBM20 mis-localization and molecular aberrations in splicing convey various physiological impairments. Our results expand the scope of RBM20 mutation-dependent pathogenic mechanisms to metabolic and endothelial dysfunction.

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BACE1-independent BACE1-AS epigenetic mechanisms in cardiomyocytes

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Background

The lncRNA BACE1-antisense RNA (BACE1-AS) is transcribed from the opposite strand of Beta-Secretase-1 (BACE1), which causes β-amyloid accumulation in Alzheimer ‹s disease. BACE1-AS/BACE1 axis was also activated in ischemic heart failure (IHF) patients producing β-amyloid deposition in cardiac tissue. In vitro studies demonstrated that BACE1-AS increase provoked cell apoptosis. However, it remains unclear the sense/antisense interaction mechanisms and the BACE1-independent transcriptional effects of BACE1-AS.

Material and Methods

BACE1-AS-interacting RNAs were investigated in AC16 cardiomyocytes by BACE1-AS-pull-down, identified by RNA-sequencing and the reads mapped by IGV software on GRCh38/hg38 genome. Gapmers transfection or hypoxia-induced BACE1-AS expression were used as loss- and gain-of-function experiments, respectively. Reduced Representation Bisulfite Sequencing (RRBS) allowed to investigate the DNA methylation status produced by BACE1-AS-expressing lentivirus particles. CRISPR-Cas9 approach was used to disrupt BACE1 exon 3 in order to study the BACE1-independent BACE1-AS transcriptional effects. Gene expression quantification was performed by RT-qPCR assays.

Results

698 RNAs were enriched by BACE1-AS pull-down. Among them, a 1152 bases intronic RNA transcript, corresponding to the GH09J089370 genomic enhancer, was identified in the locus of SEMA4D, which is regulated by hypoxia inducible factor (HIF-1) in multiple cells. We found that this transcript was expressed into the nucleus, and have hypothesized that it was an enhancer-RNA (SEMA4D eRNA). Hypoxia induced the expression of both BACE1-AS and SEMA4-D eRNA, as well as of some enhancer-related targets. Both SEMA4-D eRNA and targets expressions were reverted by BACE1-AS and SEMA4-D eRNA gapmers transfection. In addition, the SEMA4D and mRNA targets promoters were found hypomethylated by BACE1-AS over-expression. In BACE1-exon 3 knock-out cells, BACE1-AS over-expression increased the transcription of SEMA4D enhancer's targets.

Conclusions

These data suggest that BACE1-AS can regulate the transcription of mRNAs other than BACE1 and that, independently from BACE1, it regulates, in cardiomyocytes, SEMA4D eRNA transcriptional activity by hypomethylation.

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SERCA2 phosphorylation at the heart of myocardial protection

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Despite advances in cardioprotection, new therapeutic strategies capable of preventing acute myocardial ischemia-reperfusion injury and reducing secondary event of patients are still needed. Here, we discovered that the phosphorylation of SERCA2 at serine 663 (S663) is a clinical and pathophysiological event of cardiac function. We demonstrated that the phosphorylation level of SERCA2 at S663 is increased with damage in both patient and mouse hearts. Mechanistically, we demonstrated that preventing S663 phosphorylation significantly increased SERCA2 Ca2+ pumping activity into the reticulum and protected against hypoxia/reoxygenation-induced cell death, by counteracting the mitochondrial and cytosolic Ca2+ overload in several human cell types, notably hiPSC-CM. To link this specific residue event to a physiological role of SERCA2 in heart, we demonstrated that gene therapy for the phosphoresistant form of SERCA2 at S663 improved the excitation/contraction coupling of cardiomyocytes and significantly reduced infarct size in an in vivo myocardial infarction model, whereas mice expressing a phosphomimetic form of SERCA2 developed a larger infarct size. Together, these findings establish the pathophysiological role and the therapeutic potential of SERCA2 modulation in acute myocardial infarction, based on the hotspot phosphorylation level of SERCA2 on its S663 residue.

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Pro-inflammatory Macrophage Role in the Heart Failure with Preserved Ejection Fraction

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Introduction: Heart failure with preserved ejection fraction (HFpEF) is a global public health problem with no effective treatment available. Pro-inflammatory cardiac macrophages are emerging as key determinants of adverse left ventricular remodeling; however, their role in HFpEF remains poorly understood. Mechanistically, the recruitment and activation of macrophages represent a key event in maladaptive myocardial remodeling in HFpEF patients. Evidence suggests that remodeling processes in HFpEF hearts are orchestrated and amplified by cardiac macrophages which regulate cardiomyocyte function, endothelial cell activation and fibroblast differentiation. Here we sought to determine the role of macrophage inflammation in experimental and human HFpEF.

Methods: Experiments were performed in rat cardiomyocytes (H9c2), transgenic mice, and left ventricular (LV) myocardial samples from patients with HFpEF. H9c2 treated with pro-inflammatory macrophage-like cells (RAW 264.7) conditional media in the presence or knockdown of a top-ranking activator of M1 type macrophages such as nuclear receptor corepressor 1 (NCOR1), were used to elucidate better the involvement of this gene in transcriptional regulation in vitro. The cardiac function of myeloid cell-specific NCOR1 knockout HFpEF mice undergoing a high-fat diet and L-NAME was investigated. LV myocardial samples were used to evaluate the pro-inflammatory activity of macrophages in human HFpEF tissues.

Results: Macrophage marker expression in left ventricular specimens from HFpEF-patients is shifted toward enhanced pro-inflammatory (M1) and decreased regulatory (M2) macrophage markers as compared with age-matched control donors. NCOR1 - an essential co-regulator of gene transcription - was highly expressed in cultured macrophages, which is shown to drive pro-inflammatory transcriptional programs. Moreover, the depletion of the NCOR1 gene in the myeloid line contributed to an improvement in the heart function of HFpEF mice.

Conclusion: The data strengthen the importance of immune regulation in the HFpEF and pave the way for mechanism-based therapies in this setting

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Interrogating Early Molecular Events of Doxorubicin-induced Cardiotoxicity at Single-cell Level to Identify New Therapeutic Approaches

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Doxorubicin (DOX) is a potent chemotherapeutic agent used against various cancers but is notorious for its cardiotoxic side effects, contributing to elevated mortality among cancer survivors. Despite extensive research, the molecular basis of anthracycline-induced cardiotoxicity (AIC) remains elusive, particularly regarding the molecular events that distinguish an early reversible phase to a late irreversible one.

This study aimed to comprehensively profile the early transcriptional alterations triggered by DOX at a single-cell resolution to unveil potential therapeutic targets for cardioprotection against AIC.

According to our established murine model of AIC, BALB/c mice received either saline (Vehicle) or DOX (3 weekly injections of 4 mg/kg). Hearts were collected at two time points: 3 days (acute) and 6 weeks (chronic) after the first injection. Nuclei were isolated from hearts for single-nuclei transcriptional profiling (snRNAseq, 10X Genomics). Downstream bioinformatic analysis was performed using Seurat pipeline.

Echocardiography confirmed DOX-induced systolic dysfunction at 6 weeks, validating AIC establishment. SnRNAseq analysis of 12 hearts identified eight major cell types, including cardiomyocytes (CM). Subclustering of CM unveiled six subpopulations, with a CM_Stressed population showing enrichment in cardiac stress markers (e.g., Nppb and Myh7). Differential abundance analysis indicated enrichment of CM_Stressed population at both 3 days and 6 weeks, revealing DOX-induced transcriptional alterations at early and late stages in CM. Notably, Golgi associated kinase 1B (Gask1B) was significantly upregulated in CM_Stressed at both time points. Preliminary experiments in a zebrafish AIC model demonstrated that Gask1b gene silencing partially rescued DOX-induced fractional shortening decline, implicating Gask1b as a novel player in DOX cardiotoxicity.

We generated a single-nucleus dataset of DOX-treated mouse hearts and identified transcriptional changes driven by DOX in CM at early and late stages of the disease. Furthermore, we identified Gask1b gene, whose role in cardiac pathophysiology was previously unappreciated, as a potential new determinant of DOX cardiotoxicity.

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Investigation of cardioprotective effects of ticagrelor, alone or in combination with remote ischemic conditioning (RIC); a proteomic approach and an in vivo study

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Background: Repeated ischemia-reperfusion (IR) cycles in an organ or tissue, remote from the heart (remote ischemic conditioning; RIC) have been proven to be cardioprotective despite contradicting clinical trial results. We have previously shown that platelets from healthy volunteers subjected to RIC or upon ticagrelor administration possess cardioprotective effects ex vivo. Purpose: We aimed to explore which platelet-related factors contribute to cardioprotection induced by RIC via proteomic analysis and investigate whether ticagrelor exerts additive effects on cardioprotection alone or in combination with RIC, in vivo.

Methods: Venous blood from 18 healthy volunteers (with or without oral pre-treatment of 180 mg ticagrelor) was collected before and 60 min after RIC (3x5'/5' inflation/deflation of blood pressure). Washed platelets and their releasate were isolated and proteomic analysis was performed. Wistar male rats (n=6 per group) were randomized to: 1. Control, 2.RIC (3 cycles x 5'/5' limb IR, during ischemia) 3. Ticagrelor (20 mg/kg, 1h before ischemia) and 4. Ticagrelor+RIC. Rats were subjected to myocardial (30min I/3h R). Infarct size was calculated as infarct to risk ratio (I/R%).

Results: Proteomic analysis of washed platelets showed an induction of early platelet degranulation via the upregulation of calcium-dependent signaling pathways, whereas platelet releasate analysis resulted in the upregulation of innate immune system-related pathways, including heat shock protein-mediated stress response. Moreover, the proteomic profile of platelets obtained from volunteers treated with ticagrelor was characterized by, RhoA-mediated, platelet activation pathways. Ticagrelor, RIC and their combination significantly reduced IR compared to control (12.05 %, 11.45% and 9.32% respectively, vs 26.19% for the control, $p < 0.0001$) in vivo.

Conclusions: Platelets serve as carriers for RIC›s or ticagrelor's cardioprotective signal through releasing potential cardioprotective mediators or by reducing their activation status. RIC and ticagrelor reduce IS in vivo, however, in our experimental setting their combination had no additive cardioprotective effect.

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A human cardiac bioeletronics model: an alternative to animal models for cardiotoxicity risk prediction

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Background: A considerable number of drugs was removed from the pharmaceutical market due to cardiotoxicity. Moreover, adverse drug reactions are a significant cause of morbidity and mortality worldwide and are often associated with various cardiovascular side effects. Preclinical evaluation of drug cardiotoxicity has used animal models, which tend to be expensive, low throughput, and have limitations as not ever faithfully reflect the human pathophysiology, while current two-dimensional cellular models revealed to be not sufficient and specific. The aim of this study is to develop an efficient predictive model for drug cardiotoxicity, able to mimic the morphological, cellular, and electrophysiological complexity of the intact adult human heart, in order to study cardiotoxicity effect.
Material and Methods: to generate a 3D bioengineered replica of human myocardial tissue we started from fresh porcine hearts. Cardiac specimens were isolated from left ventricle using biopsy puncher and sectioned with vibratome. Then, these were decellularized through a novel, serial decellularization treatment. For a standardized removal of cardiac cells and non-ECM proteins, tissue specimens were submitted to an automated, dynamic decellularization with different perfusion devices. Different scaffolds were analyzed for decellularization effectiveness by DNA quantification, histology, histochemistry, and immunofluorescence. Moreover, they were tested for cytocompatibility with human mesenchymal stem cells.
Results: With respect to the standard decellularization protocol results based on the automated, dynamic treatments have shown a superior ability to obtain acellular, biocompatible scaffolds in terms of cell elements' removal and ECM preservation, as well as time- and cost-effectiveness. Indeed, these decellularized scaffolds allow for cell penetration, adhesion, and survival, thanks ECM microstructure integrity and preservation.
Conclusions Further experiments will be focused on generating functional bioengineered myocardial tissues with cardiovascular progenitors derived from the differentiation of human induced pluripotent stem cells. The validation of these bioelectronic platforms will be performed with drugs with known cardiotoxic effect.

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Desmin Dislocation and Sarcomere Disruption in αB-Crystallin Dilated Cardiomyopathy

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Background:
αB-crystallin, a small heat shock protein encoded by CRYAB, plays a critical role in maintaining cellular hemostasis and protecting cells from stress-induced damage. CRYAB variants are associated with clinical phenotypes such as myofibrillar myopathy, cataracts, and dilated cardiomyopathies. Nonetheless, the molecular disease mechanism remains unclear. Insight into compensatory cytoprotective mechanisms activated by distinct CRYAB variants is necessary to predict patient-specific phenotypes and devise potential therapeutic options. This phenotype-genotype translation ultimately contributes to early recognition and optimal management of CRYAB-related cardiomyopathies.

Methods:
We report a longitudinal follow-up of a family (n=3) carrying the pathogenic A527G-CRYAB mutation that ultimately underwent cardiac left ventricular assist device implantation (LVAD) followed by cardiac transplantation in two patients. Explanted heart tissue obtained at the time of LVAD implantation and subsequent heart transplant was histologically analyzed and compared with myocardial tissue from other dilated cardiomyopathy etiologies and non-cardiac diseased myocardial tissue.

Results:
Affected family members exhibit a unique phenotype encompassing posterior pole cataracts and adult-onset dilated cardiomyopathy. Microscopic analyses of excised heart sections unveiled a myocardium characterized by fibrosis and hypertrophic cardiomyocytes containing intracellular aggregates of αB-crystallin, desmin, and an abundant amount of p62 proteins – a distinctive feature of CRYAB-dilated cardiomyopathy. Staining for troponin-I and desmin revealed sarcomere disarray and the absence of desmin filaments at the intercalated disc, potentially indicating disease severity.

Conclusion:
We propose that abnormal protein homeostasis and the deficits in the cytoskeletal network's integrity, are potential contributors to the disease mechanism in CRYAB-dilated cardiomyopathy. Moreover, the interplay between αB-crystallin and desmin seems crucial in maintaining the architecture of the sarcomeres and intercalated discs. Future research should focus on elucidating the functional implications of the CRYAB-A527 variant with the aim of integrating this knowledge into clinical applications.

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Novel genetic variants associated with sudden cardiac death due to primary myocardial fibrosis

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Background: Post-mortem investigations in SCD victims reveal that myocardial fibrosis is a common finding, as more than 90% of the SCD victims have fibrotic accumulation in the myocardium. Our aim was to identify novel candidate genes and variants associated with the presence of myocardial fibrosis in sudden cardiac death (SCD) victims.

Material and Methods: Whole exome sequencing was performed in 127 victims of SCD associated with nonischemic cardiomyopathy with primary myocardial fibrosis as the only pathological finding in autopsy. We sought rare variants (minor allele frequency <0.005) estimated to be pathogenic and present in three or more cases. Geographically matched controls were used in statistical analyses. A computational approach was used to identify protein interactions for identified genes in cardiomyocytes. Associations of the identified variants with cardiac disease endpoints were investigated in the Finnish national genetic study (FinnGen) dataset. Cardiac FFPE samples of cases carrying identified variant and controls were subjected to 3› mRNA sequencing (N=24).

Results: We identified 21 missense and one nonsense variant. Heart enhanced protein interactions were identified in 16 genes. Four missense variants were highly likely to be pathogenic, significantly associated with SCD and primary myocardial fibrosis and were also associated with cardiac diseases in Finnish population. These variants locate in cartilage acidic protein 1 (CRATC1), calpain 1 (CAPN1), unc-45 myosin chaperone A and B (UNC45A and UNC45B). Transcriptomic changes were observed in SCD cases carrying variants in CAPN1, UNC45A and UNC45B .

Conclusions: We identified novel variants and candidate genes predisposing to SCD associated with primary myocardial fibrosis. These variants and genes contribute to regulation of extracellular matrix production and cardiomyocyte function.
Funding sources: Academy of Finland (349331, 333284, 333349), The Finnish Foundation for Cardiovascular Research, Orion Research Foundation.

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Tocilizumab Reduces Neutrophil Extracellular Traps and Associates with Infarct Size in ST-elevation Myocardial Infarction

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Background: Interleukin-6-receptor inhibition with tocilizumab improves myocardial salvage in patients with ST-elevation myocardial infarction (STEMI). Neutrophil extracellular traps (NETs) comprise nuclear material studded with proteins released upon neutrophil activation. We hypothesized that reduced NETs could partly explain the effect of tocilizumab in STEMI. We aim to evaluate the effect of tocilizumab on NETs and investigate the association between NETs and myocardial injury in patients with STEMI.

Material and Methods: We used data from a trial that randomized 199 patients with STEMI to tocilizumab or placebo before percutaneous coronary intervention (PCI). In this substudy, we analyzed blood samples at admission, after 24 hours and 3-7 days for the NETs markers double-stranded deoxyribonucleic acid (dsDNA), myeloperoxidase-DNA (MPO-DNA) and citrullinated histone 3 (H3Cit). We tested the associations between NETs markers and the myocardial salvage index (MSI) and infarct size measured by cardiac magnetic resonance imaging after 3-7 days.

Results: All NETs markers were lower in the tocilizumab than in the placebo group at 3-7 days (all p<0.04). The beneficial effect of tocilizumab on MSI seemed to be partly dependent on reduction of NETs (structural equation modeling: 0.05, p=0.001 (dsDNA) and 0.02, p=0.055 (H3Cit)). Patients with NETs in the three lower quartiles compared to Q4 had smaller infarct sizes at 3-7 days (9.7% vs 17.2% (dsDNA) and 9.8% vs 16.3% (H3Cit), both p<0.004). MSI were higher i Q1-3 compared to Q4 in multivariable linear regression analysis (10.9 [95% CI 0.04, 0.15] (dsDNA) and 8.9 [95% CI 2.0, -15.9] (H3Cit), both p=0.01).

Conclusions: NETs levels were were associated with tocilizumab treatment and myocardial injury. The effect of tocilizumab on MSI might be mediated through NETs.

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A single-cell genomics roadmap and gene regulatory network of multi-lineage human cardiac differentiation

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The ability to culture many cell types from human pluripotent stem cells offers great potential in mimicking organogenesis. The earliest steps of human heart development are inaccessible in vivo, especially since the first cardiac progenitors already arise when the embryo just starts to form its three germ layers during gastrulation. Various cardiac organoid models have been established over the years. These culture systems have move from relatively homogeneous two-dimensional cardiomyocyte cultures to three-dimensional cardiac organoids with increased maturity and inclusion of epicardial cells and other cell types. We are using both multi-lineage stem cell-based 3D models and hydrogel microspheres to model human cardiac tissue differentiation in vitro. We present a compendium of single-cell transcriptomic and chromatin accessibility datasets from multiple cardiomyocyte culture models, in which the cells were directed to either atrial or ventricular cardiomyocyte lineages with the inclusion of epicardial cells. This roadmap enabled us to identify key cardiac progenitor states, as well as the gene regulatory network involved in specific lineage choices. We identify a hierarchy within the transcription factor regulatory network and identify both redundant and unique roles of retinoic acid and transcription regulators in lineage commitment towards atrial cardiomyocytes and epicardial cells. A better understanding of the molecular mechanisms involved in these processes of cardiac lineage commitment and plasticity, will help to further advance the culture systems and increase its applicability as a developmental model and in the study of congenital cardiac diseases.

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Single cell RNA-sequence and machine learning approach to analyze transcriptomes from 2D and 3D differentiated human induced pluripotent stem cell-derived cardiac cells

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) together with advanced cell culture techniques like 3D-engineered heart muscle (EHM) have emerged as promising platforms for translational cardiac research and drug development, but uncertainty surrounding hiPSC-CMs maturation status raise questions about their similarity to cell types from the human heart. Here, we mapped all developmental transcriptomic changes in hiPSCs from pluripotency to 2D beating heart muscle cultures to EHM rings at single cell resolution. We sequenced a total of 62,398 cells across five developmental stages and revealed 32 distinct populations that highlight the diverse developmental origins and display a remarkable cellular heterogeneity. Next, we trained and evaluated a random-forest algorithm to classify hiPSC-CMs to adult heart muscle cell populations using data from the human heart atlas. We further narrowed down candidate transcripts by feature selection, correlation analysis and importance ranking resulting in a final set of 108 features. We were able to distinguish between heterogenous cardiomyocyte populations with 61% accuracy. Finally, RNA velocity was assessed to estimate turning point events of these features, leading to a subset of 8 largely unknown cardiomyocyte maturation driver genes.

To validate that these 8 genes are relevant to the cardiac development the next step will be the knock-down of these genes in hiPSC-CMs, to check the effect on the expression of cardiac markers.

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Lasso-based feature selection identifies active relaxation as the most informative ventricular parameter for proteomic alterations after pressure unloading

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Background: Pressure overload (PO) induces significant impairment of left ventricular (LV) function, while effective pressure-unloading therapies may lead to reverse remodeling, restoring LV structure and function. In patients, ethical concerns hamper serial sampling of the myocardium for molecular-level follow-up after application of pressure unloading therapies. This study aims to identify novel LV functional and morphological parameters closely associated with myocardial proteomic alterations.

Methods: Male and female rats were subjected to surgical aortic constriction (aortic banding, AB) to induce PO, and pressure unloading was achieved through aortic debanding (DB) at week 6. LV remodeling and reverse remodeling were assessed through morphological and functional evaluations using echocardiography and pressure-volume (PV) analysis at weeks 6 and 12. We employed feature selection through Lasso regularization, looking for associations among LV proteomics and functional as well as morphological parameters of PV analysis and echocardiography.

Results and conclusions: Our proteomic analysis identified 3343 proteins. The measurements of Tau (from PV analysis) and LV mass (from echocardiography) emerged as the most closely correlated with the proteomic data. Among them, 415 proteins displayed significant associations with changes in Tau, a robust parameter of LV active relaxation. Tau notably outperformed the other measured parameters, demonstrating a strong correlation with LV proteomic alterations during myocardial remodeling and reverse remodeling. Gene ontology biological process (GO:BP) analysis of these 415 proteins revealed the involvement of epigenetic, transcriptional, post-transcriptional, and post-translational processes. Alterations in active relaxation were closely linked to proteins governing functions such as «cardiac muscle development" and "regulation of ion transmembrane transporter activity" as well. Our findings suggest that parameters of active relaxation may be the most effective metrics for assessing the extent of myocardial remodeling and reverse remodeling in experimental and potentially clinical settings, while LV mass remains the preferred echocardiographic measure to evaluate remodeling.

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Abstract retracted by authors

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Investigating mitochondrial fitness and redox status in arrhythmogenic cardiomyopathy

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Background: Arrhythmogenic Cardiomyopathy (ACM) is a cardiac disease characterized by a progressive replacement of the myocardium with fibro-fatty tissue. ACM can be associated with stressful-life-threatening arrhythmias, eventually leading to sudden cardiac death. Despite genetic predisposition being the main underlaying cause of ACM, the genetic background alone cannot fully explain disease severity and penetrance. Emerging evidence suggests an active role of mitochondria and ROS) signaling in ACM pathogenesis. Thus, the current project employs primary Cardiac Stromal Cells (CStC) from ACM patients to explore whether mitochondrial fitness and redox systems are altered and how they can be linked to disease etiology and progression. Material and Methods: CStCs were cultured either in basal medium (BM) or adipogenic medium (AM), a condition used to model the ACM fibro-fatty phenotype. Mitochondria-related analyses of ultrastructure, membrane potential, network connection, respiratory capacity, ROS production and redox system were performed at day (d) 0 in BM and at d3, d7, d15 of AM exposure. Results and Conclusions: Mitochondria appear visually healthy in ACM both in BM, with no difference in mitochondrial membrane potential, fusion/fission mechanisms, mitochondrial density, and ETC protein expression. Despite that, the mitochondrial network is more fragmented in ACM compared to healthy CStCs (CTR) in BM, which goes along with a decreased mitochondrial respiratory capacity and increased ROS production. Interestingly, we observed mitochondrial and cellular remodeling on d3 of AM and a significant increase of mitochondrial respiratory capacity in ACM compared to CTR cells at d7 of AM. Finally, this study performs a comprehensive analysis of mitochondrial morphology, dynamics, function, and redox status in CStCs from ACM subjects carrying different genetic backgrounds to provide evidence for mitochondrial changes as co-factors in ACM progression. Funding Sources: Joint Project Südtirol-FWF (grant number 23623; Italy-Austria) and Department of Innovation, Research and University of the Autonomous Province of Bolzano (IT).

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Chronic rofecoxib treatment leads to major changes in cardiac protein phosphorylation and expression in rats

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Background and Purpose:
We reported previously that chronic treatment with the Cyclooxygenase-2 inhibitor, rofecoxib, increased acute mortality in rats exposed to ischemia/reperfusion injury (I/R). This manifestation of hidden cardiotoxicity was attributed to the proarrhythmic effect of the drug on the ischemic heart. However, rofecoxib had beneficial effects on ischemic injury by decreasing the infarct size. In the present study, we aimed to identify molecular changes caused by chronic rofecoxib treatment to reveal our previous results´ underlying cardioprotective and cardiotoxic molecular pathways.
Experimental Approach:
Rats were treated with 5.12 mg/kg rofecoxib or vehicle for four weeks. Messenger RNA (mRNA), microRNA (miRNA) deep sequencing data, and proteomic datasets of left ventricular tissue samples were used for an unbiased differential expression analysis followed by in silico molecular network analysis and experimental target validation.
Key results:
Using mass spectrometry and filtering criteria, 26 proteins were identified that exhibited pronounced changes in protein expression or phosphorylation due to chronic rofecoxib treatment. The transcriptomic analysis showed mild alterations in the heart›s mRNA- and miRNA expression. The correlation of miRNAs and their mRNAs did not translate to differential protein expression.
Conclusions and Implications:
This is the first demonstration that chronic rofecoxib treatment affects posttranslational modification and expression of several proteins in the heart. This could account for its hidden cardiotoxic and cardioprotective properties, however, changes in transcription, as well as posttranscriptional regulation by miRNAs, are unlikely to mediate these effects.
This study was supported by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017 National Heart Program) and by a grant from the National Research, Development and Innovation Office of Hungary (NKFIH; K139237) awarded to Anikó Görbe.

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Overexpression of the calcineurin A splicing variant CnAβ1 worsens diastolic function in diabetic cardiomyopathy

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1. Background

The alternative splicing calcineurin A beta variant CnAβ1 has an opposite effect on the heart to that of other calcineurin isoforms. Overexpression of CnAβ1 leads to activation of the serine and one-carbon pathway, increased production of anti-oxidant mediators, preserved ATP production, reduced cardiac remodeling and improved cardiac function in mouse models of systolic dysfunction. However, the potential benefit of CnAβ1 in the context of diastolic dysfunction remains unknown.

Our aim was to investigate the effect of CnAβ1 overexpression on diastolic dysfunction associated with diabetic cardiomyopathy.

2. Material and Methods

We used WT and αMHC-CnAβ1 transgenic mice, which overexpress CnAβ1 in cardiomyocytes under the control of the alpha myosin heavy chain promoter. These mice show strong activation of the serine and one-carbon pathway and have been extensively characterized by our laboratory.

Diabetes was induced by streptozotocin injection in WT and αMHC-CnAβ1 at 8 weeks of age and cardiac function was monitored by echocardiography.

3. Results and Conclusions

Both WT and transgenic mice develop diastolic dysfunction by middle age, indicated by an increase in the isovolumetric relaxation time (IVRT). αMHC-CnAβ1 transgenic mice showed a further delay in IVRT, suggesting worsening of diastolic function ($p=0.07$). Additionally, we observed a reduction in the left ventricle diastolic volume in transgenic mice in the absence of left ventricular hypertrophy, further supporting relaxation impairment. These results suggest that, contrary to its beneficial effect on models of systolic dysfunction, CnAβ1 has a detrimental effect on diastolic dysfunction. Further work will be needed to determine whether this calcineurin A variant is a valid therapeutic target in the context of diabetic cardiomyopathy.

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Sodium myo-inositol cotransporter-1, SMIT1, promotes cardiac hypertrophy and fibrosis in pressure overloaded mouse hearts.

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Background: Sodium myo-inositol cotransporter-1 (SMIT1) belongs to the sodium-glucose cotransporter family and accounts for intracellular accumulation of myo-inositol. SMIT1 is expressed in the heart, where its function remains unknown.
Material and Methods: Here, we demonstrate the contribution of SMIT1 in pathological hypertrophic cardiac remodeling and the progression to heart failure, using a mouse model of pressure overload induced by transverse aortic constriction.
Results: We found that aortic banding failed to induce systolic dysfunction, cardiac hypertrophy and fibrosis in mice lacking SMIT1 (Smit1-/-), in contrast to wild type controls. SMIT1 genetic deletion reduced cardiac O-GlcNAcylation, which normally increases following hemodynamic stress, and restored ions homeostasis preventing the resulting pro-hypertrophic transcriptional reprograming.
Conclusions: This work provides important insights into the role of SMIT1 at the onset of heart failure, opening new avenues for potential therapeutic strategy to prevent or treat pathological hypertrophy and heart failure.

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GRK2 as a novel target to prevent aortic valve calcification

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BACKGROUND Calcific aortic valve stenosis (CAVS) is a common heart valve disease characterized by endothelial dysfunction, inflammation, fibrosis, and calcification. Treatment options include surgical valve replacement or transcatheter intervention. A novel therapeutic strategy could target G protein-coupled receptor kinase 2, which regulates endothelial function and immune responses. This study aimed to evaluate the role of GRK2 in Aortic Valve Calcification (AVC) by in vivo and in vitro studies. MATERIALS AND METHODS To reach our purpose we first evaluated the expression of GRK2 in aortic valve of patient displaying fibrotic or calcific lesions of valve leaflets. Then, we evaluated GRK2 expression in mitochondria fraction from EC isolated from CAVS patients (VEC) vs control ECs upon Angiotensin II (AngII) stimulation (1µM). We also performed histological analysis by using mice with selective endothelial knock-out of GRK2 (Tie2CRE-GRK2fl/fl) compared to control (GRK2fl/fl). Mice has been fed with a standard or a HF/HC diet for 3 months and then subjected to chronic administration AngII (1000 ng/Kg/min) or saline solution by subcutaneous implantation of osmotic pumps for 28 consecutive days, to evaluate the role of Grk2 on the early development of aortic valve lesion. RESULTS Immunofluorescence staining of aortic valve leaflets revealed that GRK2 expression is more abundant in calcific lesion instead of fibrotic valve. In vitro, we observed that AngII can upregulate GRK2 mitochondrial localization in a time-dependent manner in ECs. Otherwise in VEC the stimulation with Ang II is not able to further induce GRK2 mitochondrial localization. Histological analysis revealed that Tie2CRE-GRK2fl/fl/HF/HC/AngII mice display presence of microcalcification, more pronounced than GRK2fl/fl thus demonstrating that the lack of GRK2 in the EC accelerates the calcific degeneration of the aortic valve in mice. CONCLUSIONS In conclusion, our data suggest a direct involvement of GRK2 in the pathogenesis of CAVS by regulating endothelial and inflammatory response.

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The role of LMNA variants as drivers of Atrial Fibrillation

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Introduction

Atrial fibrillation (AF), the most common progressive cardiac arrhythmia, is associated with serious complications such as stroke and heart failure. Although common risk factors underlie AF onset, in ~15% of the affected population, AF may have a genetic cause. Several AF families carrying variants in cytoskeletal proteins, including the nuclear protein Lamin A/C (LMNA) have been identified. How LMNA variants trigger AF is unknown.

Methods

Both HL-1 atrial cardiomyocytes and human iPSC-derived atrial cardiomyocytes expressing LMNA variants p.R331Q, p.Q493X, or ΔExon1, all associated with clinical AF, were utilized. Cardiomyocytes were tachypaced in order to investigate the impact on DNA damage and cytoskeletal organization, via Western blot analyses or immunofluorescence staining. Furthermore, the viscoelastic properties of the nuclei of the different LMNA variants was studied with a microfluidic device and morphological examination with immunofluorescence.

Results

In HL-1 cardiomyocytes, LMNA variants have a significant decreased nuclear circularity and viscoelastic properties by either making the nucleus stiffer (p.R331Q) or softer (p.Q493X) compared to WT. Tachypacing decreases the nuclear size in atrial iPSC-CMs except for ΔExon1. Although the ratio of peripheral/nucleoplasmic Lamin A/C is similar between all conditions, the expression of peripheral Lamin A/C p.R331Q in the nucleus is twice the amount of WT. Furthermore, the atrial iPSC-CM LMNA p.R311Q shows more cytoskeletal disorganization after tachypacing as compared to its isogenic control.

Conclusion

These results indicate that the various LMNA variants have a different impact on the viscoelastic, morphological properties on the nucleus and cytoskeletal structure of atrial cardiomyocytes. The heterogenic effects of LMNA variants on nuclear and cytoskeletal structures suggest involvement of various molecular pathways in AF arrhythmogenicity. The primary focus of future experiments is focused on DNA damage as nuclear integrity and shape are linked to DNA damage.

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Quantity of DNA lesions correlates with electropathology and stage of atrial fibrillation

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Introduction
Previous research revealed DNA damage as a molecular driver of atrial fibrillation (AF). This study investigates the diagnostic value of the quantity of DNA damage as a marker of the degree of electrical conduction disorders and the stage of clinical AF.
Methods
High-sensitivity long-run real-time PCR (LORD-Q) was performed on atrial tissue samples of 83 patients with paroxysmal AF (PAF), persistent AF (PeAF), long-standing persistent AF (LS-PeAF) and controls (sinus rhythm [SR]), to quantify the number of atrial DNA lesions in the mitochondrial (ND1) and nuclear (P53) genome. PicoGreen assay and qPCR were utilized to quantify circulating free DNA (cfDNA) markers (total cfDNA, β-globin, ND1 and P53) in blood samples of 70 patients with AF or SR. High-resolution epicardial mapping was conducted to quantify electrical conduction disorders during SR.
Results
Compared to SR, the number of DNA lesions was significantly and gradually increased in PAF and PeAF followed by a decrease in LS-PeAF. In line, patients with <3 years AF showed an increase and >3 years AF a decrease in DNA lesions compared to SR. In SR patients, the quantity of nDNA damage was significantly correlated to degree of potential fractionation ($r=0.85$), while a higher number of mtDNA lesions was correlated to slower conduction velocity (CV: $r=-0.708$) and lower potential voltage ($r=-0.742$) in AF, especially in patients with <3 years AF (all $P<0.001$). In addition, serum cfDNA levels decreased significantly in patients with >3 years AF compared to <3 years AF. These serum DNA levels significantly discriminate patients with short- from long-term AF.
Conclusion
The quantity of DNA lesions in atrial tissue samples associates with the severity of AF and atrial conduction disorders during SR. Furthermore, serum markers discriminate between short- and long-term AF. This indicates that the quantity of DNA damage has diagnostic value in clinical AF management.

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Cardiac unloading by implantation of left ventricular assist device: Addressing the molecular changes associated with reverse cardiac remodeling

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Background: Heart failure (HF) affects 1-2% of the population of the Western world and heart transplantation (HT) or left ventricular assist device (LVAD) implantation are currently the only treatment options. LVADs are used as either «bridge to transplant» or destination therapy in patients ineligible for HT, although long-term LVAD support is associated with significant complications, limiting survival. However, in a small subset of patients, LVAD support leads to partial or full molecular and functional recovery of the heart, termed reverse cardiac remodeling (RCR), in some cases allowing for LVAD explantation. The aim of the study was to provide insights into the molecular mechanisms associated with unloaded-mediated RCR as well as identify predictive and diagnostic biomarkers for RCR.

Material and Methods: Total RNA-Seq and miRNA-Seq were performed on i) left ventricular (LV) biopsies from HF patients with ischemic (ICM) and non-ischemic (NICM) cardiomyopathy (n=25) obtained during LVAD implantation and subsequent HT vs. biopsies from healthy control (Ctrl) hearts (n=11), and ii) plasma from HF patients before and 1 and 9 months after LVAD implantation (n=20) vs. Ctrl (n=14).

Results and Conclusions: Bioinformatic analysis showed differential expression of 4082 protein-coding genes, 483 lncRNAs, and 262 miRs in ICM vs. Ctrl patients, of which 83%, 99%, and 80% were normalized following LVAD implantation, respectively (FDR ≤ 0.05; |log2FC| ≥ 0.5). Similarly, 2050 protein-coding genes, 335 lncRNAs, and 65 miRNAs were altered in NICM vs. Ctrl patients, of which 56%, 88%, and 52% were normalized after LVAD implantation, respectively. Thus, most dysregulated lncRNAs were normalized after unloading. In plasma, 49 and 75 differential expressed miRNAs were identified in ICM and NICM patients respectively, of which 25% and 100% were normalized 9 months after LVAD implantation. Analysis of lncRNAs in plasma is ongoing. Dysregulated genes normalized after unloading may represent targets for novel therapies promoting RCR.

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Long-term exposure to pesticides induces arrhythmic events and alters energy metabolism in rats

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Background: In agriculture, pesticides are used to control weeds or insect infestation. Residuals of pesticides can commonly be found on fruits and vegetables harvested for human consumption. Although acute exposure to pesticides has been related to various severe diseases, chronic pesticide exposure is poorly studied. Herein we investigate the potential influence of commonly used pesticides on the most common cardiac arrhythmia, atrial fibrillation (AF).

Methods: Over 16 months, pesticides were added to the drinking water of brown rats. Next to control rats (n=6), respectively 12 rats were exposed to the pesticides Glyphosate, Roundup (a pesticide also based on Glyphosate), Paraquat and MCPA. Subsequently, ECG measurements were performed and atrial tissue samples analyzed via proteomics (Control n=4, Glyphosate n=5, Roundup n=5, Paraquat n=5, MCPA n=5) and metabolomics (Control n=5, Glyphosate n=10, Roundup n=9, Paraquat n=9, MCPA n=9).

Results: A significant increase in atrial arrhythmic episodes was observed in rats treated with pesticides (15-70%) compared to non-treated control rats (0%). The proteomics dataset revealed one protein significantly downregulated in rats treated with Paraquat, while in Roundup treatment, 24 proteins were found to be significantly upregulated (FC>1.5). Increased were exemplarily the COX assembly mitochondrial protein I or proteins of calcium ion- and actin filament-binding. Interestingly, no proteomic changes in rats with Glyphosate treatment were found, indicating a separate effect of Roundup additives. Investigating trends observable in combined proteomics and metabolomics data showed enrichment in nicotinate and nicotinamide metabolism, potentially reduced glycolysis, as well as an increase in metabolites of the energy metabolism and thus likely mitochondrial involvement in pesticide-induced AF.

Conclusion: These results demonstrate that long-term exposure to pesticides negatively affect functional integrity of the heart, possibly due to disrupted or altered energy metabolism. Further studies need to be performed in order to identify the underlying mechanisms.

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Impact of tachycardia on cardiomyocytes and endothelial cells in a patient-derived pluripotent stem cell model

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Purpose and aim:

Persistent tachycardia can induce reversible left ventricular dysfunction termed Tachycardia-Induced Cardiomyopathy (TCM). A disturbed Ca2+ handling has been described as an underlying mechanism. However, the influence of tachycardia on other cardiac cell types remains elusive. Here, we aimed to characterize the impact of tachycardia on heart failure development using induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CM) from a TCM patient. Furthermore, this project aims to determine the potential role of endothelial cells (EC) in the development of TCM.

Methods and results:

We generated human iPSC from a patient who suffered TCM-induced heart failure. We differentiated the iPSC to iPSC-CM and subjected them to 24-hour and 7-day culture field pacing under tachycardic (120 bpm) or sinus frequential conditions (60 bpm). Fura-2 AM epifluorescence measurements revealed reduced systolic Ca2+ contents after tachy stimulation, and whole-cell current-clamp measurements demonstrated significantly prolonged action potential duration. Mechanistically, tachypacing significantly increased apoptosis and ROS production in the iPSC-CM compared to sinus rhythm. Moreover, while TCM iPSC-CM showed normal sarcomeric regularity under basal conditions, tachypacing disrupted sarcomeric regularity within 24 hours, persisting after 7 days.

These data show that tachycardic pacing impairs the functional and cellular phenotype of CM. To unravel the effect of tachycardia on the crosstalk between CM and EC, we differentiated iPSC to iPSC-EC and treated them with supernatant from sinus and tachy stimulated iPSC-CM. Using the Tube Formation Assay, we found reduced angiogenesis of the iPSC-EC incubated with the conditioned media of tachypaced iPSC-CM compared to sinus-paced iPSC-CM.

Conclusion:

Our results show an adverse influence of tachycardic pacing on a TCM-cell-line on molecular, cellular, and functional levels. Furthermore, tachycardia-induced paracrine signaling impaired endothelial function, making the crosstalk between CM and EC in tachycardia an interesting target for further investigations.

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Allergic diseases may drive atrial fibrillation: a case report and mechanistic research study

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Background: Low-grade inflammation, due to allergy, may promote impairment of cardiomyocyte function and alter immunosurveillance. Molecular pathways involved are mediated by the bioactive amine histamine via the histamine 1 receptor (H1R). Interestingly, an association between circulating histamine and cardiac arrhythmias has been observed. Whether circulating histamine acts as a trigger for the cardiac arrhythmia atrial fibrillation (AF) has not been fully elucidated.
Methods: A case study, from an AF patient was explored to obtain early evidence for the role of histamine as trigger for AF. In this patient, AF episodes were determined by smartwatch read-outs. In order to investigate the underlying mechanism of histamine and allergies on AF occurrence, serum histamine of 38 patients dependent on the presence of AF history as well as the presence of underlying allergic diseases were analyzed via ELISA (no AF and no allergies (n=10), no AF and allergies (n=10), AF and no allergies (n=10), AF and allergies (n=8)).
Results: The case study describes that a 70-year old patient with a history of paroxysmal AF of 10 years received a 4-week treatment of 5mg/day desloratadine, a selective H1-anihistamine. During the period of the desloratadine intake, all AF episodes of the patient were fully suppressed. Upon termination of drug intake, episodes recurred. Among patients with AF, an upward trend in serum histamine levels was observed in patients with a history of allergies compared to non-allergic patients.
Conclusion: The case study reveals histamine as a trigger AF. In patients with high levels of histamine, a potentially beneficial effect of an antihistamine treatment may be anticipated. Additional studies should elucidate the underlying molecular mechanisms how atrial tissue or circulating histamine levels have an effect on atrial cardiomyocyte functions that drive AF progression.

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Changes in cardiomyocytes organelles communication in experimental model of Wolfram Syndrome

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Background: Wolfram syndrome (WS) is a recessively inherited disease with a low prevalence. This endoplasmic reticulum disease is caused by a genetic mutation in the Wfs1 gene. The role of this gene is to create a functional protein called wolframin. Wolframin is expressed in various organs, such as in the pancreas, brain, but also in the heart, and its functional use in cells is still insufficiently investigated. Due to the symptoms of this disease, scientists are studying the function of wolframin primarily on nerve cells and pancreatic β-cells. However, the issue of this syndrome is relatively little investigated in cardiomyocytes, although wolframin is expressed to a high degree in the heart.
Methods: Experimental group consisted of 4 animals bred from Sprague Dawley rats with an exon5 deficiency of wolframin (age: 4 months, weight 524 ± 11g) and control group consisted of 5 rats bred from Sprague Dawley (age: 4 months, weight 570 ± 19g). To evaluate transmission electron microscope images a stereological analysis using an Electron Microscope Image Analyzer 1.0 program was used with a grid delta of 150nm.
Results: In this study an unsignificant increased trend was observed in mean value of sarcolemma-t-tubule (0,000% vs 0,036%, p=0,089), mitochondria-SR (1,419% vs 3,324%, p=0,092), myofibril-SR (1,625% vs 2,959%, p=0,063) and SR-t-tubule (2,497% vs 3,455%, p=0,080) contacts in knockout subjects compared to control subjects, which could indicate that wolframin affects not only the morphology of the sarcoplasmic reticulum and mitochondria, but also the sarcolemma.
Conclusion: This study shows that WS influences chosen contacts between cardiomyocyte organelles and that wolframin influences sarcoplasmic reticulum (SR), mitochondria and sarcolemma. For the future improvements a deeper understanding of the issue is necessary.

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Abstract retracted by authors

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Identification of dysregulated genes in a murine model of arrhythmogenic cardiomyopathy by single nucleus transcriptome

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Background: Arrhythmogenic cardiomyopathy (ACM) is a rare genetic cardiac disease characterized by the progressive loss of cardiomyocytes coupled with fibrofatty replacement of the myocardium predisposing to arrhythmias and sudden death especially in young patients and athletes. Approximately half of ACM cases are attributed to inherited mutations in one or more genes encoding cardiac intercalated disc components. Among those genes, the most commonly affected are those encoding for desmoplakin (DSP), plakophilin-2 (PKP2), desmoglein-2 (DSG2), desmocollin-2 (DSC2) and plakoglobin (JUP).

Methods: In order to clarify the pathogenic mechanisms and the contribution of the different cell populations to ACM development, we performed single-nucleus RNA sequencing in the heart of 6 month-old mice with cardiomyocyte-specific overexpression of human DSG2 carrying the p.Q558* pathogenic variant and wild type animals.

Results and conclusions. Consistent with previous works, we could recognize the main cell types present in the heart, including cardiomyocytes, endothelial cells, macrophages and fibroblasts. Among the 16 clusters identified in our analysis, two were recognized as cardiac fibro-adipogenic progenitor cells (cFAPs), which have been proved by different studies to be a potential source of fibro-fatty tissue in the disease. Further investigation of genes differentially expressed between the two analyzed genotypes allowed to investigate the behavior of different cell types in normal or pathological conditions. For instance, expression of genes encoding proteins of the intercalated discs appeared downregulated in the murine model likely predisposing to abnormalities in the architecture of these junctions with consequent functional impairments. The obtained data are currently being used to determine novel insights into the cell populations and the mechanisms driving ACM pathogenesis, opening new possibilities to develop novel therapeutic strategies.

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Human endogenous peptide Catestatin and its novel polymorphic variants exert cardioprotection against cardiac hypertrophy

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Background:The chronic stimulation of sympathetic nervous system (SNS) is one of the key pathophysiological mechanisms that occurs in heart failure;during its acute phase, the up regulation of SNS is essential for a compensatory response, while in the long-term, it becomes a key contributor for cardiac dysfunction. Catestatin (CST), an endogenous and bioactive peptide derived from the cleavage of chromogranin A (CgA), exerts anti-hypertensive and cardio-suppressive actions. Material and Methods:Here, we investigated the cardioprotective profile of novel CST peptides obtained strategically substituting certain amino acids in the wild-type CST with alpha-amino isobutyric acid (Aib) residues at different positions [CST wild-type (CST-1) and 4 CST variant peptides CSTs 2-5 (Aib 19-Aib 5, 9-Aib 5,9,13-Aib 5, 9,13,17)], by testing their anti-hypertrophic effect against isoproterenol (ISO) in H9c2 cells and ischemia/reperfusion injury (IRI) in Langendorff settings. Results and Conclusions:Morphological and immunofluorescence analyses revealed that the variant peptides, CSTs 1-3, but not CSTs 4-5, are able to counteract ISO-dependent cardiomyocyte hypertrophy starting from 1 nM, also decreasing the upregulation of β1-AR. While hemodynamic evaluation indicated that all CSTs (5 nM), in particular CSTs 2-3, limit myocardial damage-dependent IRI by improving post-ischemic systolic recovery and attenuating cardiac contracture, infarct size and LDH leakage in coronary effluent. On the other hand, nitric oxide production significantly increased in IRI hearts exposed to CSTs with respect to IRI alone, even if CSTs 1-3 exerted a more pronounced effect. These data suggest that CST-1 and specific CSTs variants with improved cardioprotective properties may represent novel promising therapeutic candidates in cardiac hypertrophy by blocking β1-AR.

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Recombinant antibody fragments derived from Trastuzumab (rFab HER2) as novel ErbB2 receptor modulators: exploring the cardiotoxic profile in H9c2 cardiomyocytes

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Background: Cardiotoxicity is a well-established complication of anticancer treatments that poses a serious threat to life of oncological patients and limits the clinical use of several chemotherapeutic agents. Trastuzumab (TRZ), a monoclonal antibody targeting the human epidermal growth factor receptor 2 (HER2), improves HER2+ tumor outcomes; however, it also associates with left ventricular dysfunction and heart failure, hampering its clinical use. To overcome this limitation, recombinant antibody fragments (rFabs) emerged as promising novel therapeutic approaches.

Materials & Methods: Here we investigated the action of rFabs derived from TRZ (rFab-HER2) by comparing their biological profile with that of TRZ in H9c2 cardiomyocytes. rFab-HER2 was also tested in coupling with human serum albumin nanoparticle (HSA-NP), because of its verified efficacy as a drug delivery system.

Results: Results indicated that rFab-HER2 alone and coupled with nanoparticles (HSA-NPs + rFab-HER2) determine minor cardiomyocyte cytotoxic effects compared to TRZ alone. The ability of rFab-HER2 to bind HER2/ErbB2 receptor, tested by western blot, indicated that cells treated with rFab-HER2 and HSA-NPs + rFab-HER2, like those treated with TRZ, show a down-regulation of the receptor compared to control cells, suggesting that rFabs, although causing less cardiotoxicity than TRZ, act as negative modulators of HER2/ErbB2 in cardiomyocytes.

Since oxidative stress plays an important pathogenic role in the TRZ-dependent cardiotoxicity, we investigated the intracellular ROS generation through the fluorescent probe CM-H2DCFDA and the mitochondrial superoxide generation by MitoSOX Red staining. Results indicate that HSA-NPs + rFab-HER2 increased ROS generation, inducing a redox imbalance in cardiomyocytes, while determining a reduced cell mortality rate compared to TRZ.

Conclusions: Overall, our evidence suggests that rFab-HER2 can be considered a valuable cardiac negative modulator of HER2, as well as a potential candidate for the development of HER2-targeted therapies for the treatment of HER2 overexpressing malignancies with minimal off-target cardiac effects.

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Impact of two-week volume overload on cardiac function and gene expression in normotensive and hypertensive rats

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Background:The long-term impact of volume overload (VO) induced by aortocaval fistula (ACF) in normotensive and hypertensive rats is well described, but not its early effects.The aim of this study was to compare the short-term impact of VO between normotensive and hypertensive rats.

Material and Methods:VO heart failure was induced by ACF in eight-weeks old normotensive (HanSD) and hypertensive Ren-2 transgenic rats (TGR).Two-weeks after ACF, echocardiography and left ventricular (LV) pressure-volume (PV) analysis were performed for morphology and function assessment.Cardiac tissue was harvested for PCR measurement of selected gene expression.

Results:Right ventricular (RV) hypertrophy was more pronounced than LV in both TGR ACF and HanSD ACF.Both strains showed increased maximum velocity in the RV outflow tract and diameter of the inferior vena cava as VO markers.In TGR, but not in HanSD, the anterior LV wall thickness in diastole was decreased after ACF.TGR ACF had more decrease in blood pressure, higher LV filling pressures (E/E› index, E TMT, Ped), and LV mass index than HanSD ACF. However, HanSD ACF had reduced LV ejection fraction and fractional shortening more than TGR ACF. Contractility parameters (ESPVR, PRSW, dP/dt max, VAC) were decreased in HanSD and TGR ACF in a similar manner.These changes were followed by differences in the gene expressions.TGR ACF had more increased Nppa, Nppb, Phospholamban, Na+/H+, Glut1 transport, production of ROS (Cytochrome b245), and fibrotic markers (Col1, Col3) than TGR and HanSD control rats.

Conclusions:Both HanSD and TGR showed signs of VO two-weeks after the ACF creation. These findings were accompanied by gene expression changes typical for HF.Despite similar changes in contractility and ejection fraction, in most of the parameters, early impact of ACF was more pronounced in TGR compared to HanSD,reflecting the pressure component of hemodynamic overload.

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CardioMEMS monitoring system reduces IL-6 levels and improves quality of life in patients with advanced heart failure in the real-world

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Background: Despite several pharmacological advances, the morbidity and mortality in heart failure (HF) remain high and this has led to the development of remote monitoring systems.
Specifically, in this study we have investigated a new therapeutic approach and its effect on IL-6 levels.
Material and methods: We enrolled 7 patients with end-stage HF, who received the combined CardioMEMS/levosimendan strategy to reduce the number of hospitalizations and optimize both tailored adjustment of home therapy and levosimendan infusions. Specifically, CardioMEMS was implanted in the pulmonary artery and IL-6 levels were measured in duplicate by an enzyme-linked immunosorbent assay (microplates coated with a mouse monoclonal antibody against IL-6; Quantikine® HS Kit, R&D Systems, USA).
Results: The 7 patients (69.00±4.88 years; 30% female) were monitored daily by CardioMEMS; if the cardiologist detected a tendency for pulmonary artery diastolic pressure to rise, patients were contacted for home therapeutic changes or for hospitalization and levosimendan infusion. Precisely, following the implantation of CardioMEMS, we observed a 50% reduction in the total number of hospitalizations and a 68.7% reduction in the number of days in hospital.
Moreover, lower pulmonary arterial pressures on CardioMEMS monitoring (pre sPAP 47.86±8.67 vs 1-year post 35.14±9.34 mmHg, p 0.022) and lower echocardiographic E/e› (19.33±5.05 vs 12.58±3.53, p 0.023) were recorded at follow-up. Accordingly, improved patient quality of life was observed with EQ-5D (pre 58.57±10.29 vs. post 84.29±19.02, p 0.008).
Finally, the Quantikine® HS Kit determined elevated IL-6 values at baseline in all patients, with a statistically significant reduction at 6-months FU (p 0.0211).
Conclusions: IL-6 is potentially linked to the pathophysiological cascade of HF. On the one hand, IL-6 could be helpful for predicting HF exacerbation in still-asymptomatic patients; on the other hand, these results lay the foundations for evaluating the effects of IL-6 receptor blockers on HF progression.

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Spirulina-derived-peptide 6 controls hyperglycemia through the modulation of LCPAT1 and GLUTs expression

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Background
Although several new drugs therapies, such as glucagon-like peptide-1 (GLP-1) receptor agonists, DPP-IV inhibitors, and sodium–glucose co-transporter 2 (SGLT2) inhibitors, have shown an important effect on the reduction of hyperglycemia with higher efficacy and safety respect to older generation antidiabetic drugs, to date there is still an extreme difficulty in obtaining adequate metabolic control in type 2 diabetic patients. The purpose of this study was to investigate the possible anti-diabetic role of a novel spirulina-platensis derived-peptide "SP6" isolated from its gastro-intestinal-simulated in vitro digestion.

Material and Methods
Using in vitro Human Umbilical Vein Endothelial Cells (HUVECs) we have assessed the role of SP6 on the impaired process of diabetic wound healing. At the end of the assay, we have collected the cells to perform western blot analysis. To translate in vivo the effects of SP6, we used an experimental mouse model of type 2 diabetes. A 4-week treatment by gavage administration of SP6 was performed. By molecular biology studies and the use of mass spectrometry imaging (MALDI-MSI), the expression of GLUTs levels, DPP-IV activity and GLP-1 levels, and oxidative stress were evaluated.

Results
SP6 treatment evokes a rescue of wound healing, and this effect is linked to the regulation of LPCAT1 and PIM3 modulation. In vivo, we show that daily oral administration with SP6 peptide was able to protect against hyperglycemia-induced endothelial dysfunction. SP6 in vivo treatment was able to regulate the expression levels of GLUT1, GUT2, and GLUT4. In parallel, SP6 treatment was able to inhibit DPP-IV activity by promoting an increase in circulating levels of GLP1. HbA1c levels, bringing its levels back below 6%.

Conclusions
Our data lay the foundation for being able to develop a possible new preventive approach to control pre-diabetic condition and its related complications using a natural-derived peptide.

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Extracellular vesicles characterization in patients with hypertrophic and dilated cardiomyopathies

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Background: According to the 2023 ESC cardiomyopathy guidelines, hypertrophic (HCM) and dilated (DCM) cardiomyopathies are described according to morphological and functional traits of the heart. However, a specific biomarker assessing the evolving nature and aetiology of these conditions is lacking. Extracellular vesicles (EVs), small membranous particles released by cells into biological fluids, hold promise as diagnostic and prognostic biomarkers for cardiac diseases. This study aimed to characterize plasma EVs isolated from HCM (n=19) and DCM (n=15) patients, alongside healthy volunteers (n=13).

Methods: Patients underwent clinical assessment (echocardiography and CMR) and genetic testing (NGS). EVs were isolated via ultracentrifugation from platelet-free plasma. Nanoparticle tracking analysis, transmission electron microscopy, and WB were performed for quantitative and qualitative assessment of EVs. FACS analysis was performed to distinguish circulating EV subpopulations (i.e., cardiomyocytes, platelet, endothelial cells, monocytes, macrophages, neutrophils).

Results: Most patients were male (70.8% HCM, 86.7% DCM and 54% controls). Mean ages were 57.7±15.5 years (HCM), 45.9±16.15 years (DCM), and 47.6±4.5 years (controls). MYH7 and MYBPC3 variants were the most observed in HCM. Patients with DCM carried mainly TTN, SCN5A and DSP variants. EV concentration was significantly different between HCM and controls (5.6*108 EV/ml/cell count in HCM and 1.06*109 EV/ml/cell count in controls). Concerning EV subpopulations, an increase in EVs released from platelets (CD41a+), endothelial cells (CD62E+) and progenitor endothelial cells (CD309+) was observed in HCM compared to controls (for all p< 0.05). A negative association was found between CD309+ EVs and left ventricular mass in HCM (r= 0.93, p<0.007). No differences were found between DCM and controls or HCM concerning concentration and EV subpopulation.

Conclusions: EVs isolated from HCM patients exhibited a peculiar phenotypic pattern (e.g., increased platelet-derived EVs) that may be associated with the microvascular dysfunction in HCM.

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The myo-inositol/SMIT1 axis in heart failure: a potential metabolic actor in the disease pathophysiology and clinical outcome

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Background: Metabolites availability is crucial to the development of heart failure (HF) and cardiac fibrosis. We recently showed that myo-inositol (MYO), transported via sodium/myo-inositol co-transporter 1 (SMIT1), is a metabolite that can induce oxidative stress in cardiomyocytes. Therefore, elevation in MYO could be harmful to the myocardium. However, plasmatic MYO level has never been measured in a large cohort of patients with HF.

Aims: Evaluate MYO level in HF patients and investigate its role in the disease pathophysiology.

Methods: 451 patients were prospectively included in a Belgian discovery cohort, containing control, HFrEF, and HFpEF (HF with reduced and preserved ejection fraction respectively) patients. MYO plasmatic level was measured by mass spectrometry and linked to clinical characteristics and outcomes. Observations were replicated in a confirmation cohort of 285 participants from the Montreal Heart Institute Biobank. MYO impact on human cardiac fibroblast (HCF) activity was assessed in vitro and human myocardial biopsies were used to investigate SMIT1 role in HFrEF.

Results: MYO level was significantly increased in HF patients compared to controls, and even greater in the HFpEF population. High MYO level was associated with decreased renal function, HFpEF profile and FGF-23, a fibrotic marker. Interestingly, abnormal MYO level (> 69 µM) was predictor of poor outcome only in HFpEF patients. We demonstrated that MYO promotes HCF migration, proliferation and myodifferentiation and that SMIT1 expression is dramatically increased in fibrotic myocardial tissue of HF patients.

Conclusion: Plasmatic MYO level is elevated in HF, especially in HFpEF where it is associated with poor clinical outcome and FGF-23. SMIT1 controls HCF activity through the transport of MYO and its expression is elevated in fibrotic heart, suggesting that the MYO/SMIT1 axis could be involved in cardiac fibrosis.

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Parkin and SMAD7 are targeted by miR-181c in human cardiac fibroblasts: ex vivo data and clinical validation in frail older adults with HFpEF and diabetes mellitus

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Background: Heart failure with preserved ejection fraction (HFpEF) is a condition highly prevalent amongst geriatric patients, especially if diabetic. The pathobiology of HFpEF is complex and encompasses a number of mechanisms including endothelial dysfunction, inflammation, oxidative stress, and fibrosis. The microRNA miR-181c has been linked to the response to exercise training in HFpEF patients and has been also associated to diabetic cardiovascular complications. However, the underlying mechanisms have not been fully elucidated.

Aim: We sought to measure circulating miR-181c in patients with HFpEF and diabetes mellitus and identify gene targets pathophysiologically relevant in HFpEF.

Methods: We quantified circulating miR-181c in frail older adults with a confirmed diagnosis of HFpEF and diabetes, and, as control group, we enrolled age-matched subjects without HFpEF and without diabetes. Additionally, we validated in human cardiac fibroblasts the molecular mechanisms linking miR-181c to a pro-fibrotic response.

Results: 51 frail patients were included (34 patients with diabetes and HFpEF and 17 age-matched controls). We observed that miR-181c was significantly upregulated ($p<0.0001$) in HFpEF patients vs controls. We confirmed via luciferase assays in human cardiac fibroblasts that miR-181c is targeting Parkin (PRKN) and SMAD7; mechanistically, miR-181c relieves the pro-fibrotic process from the SMAD7-mediated inhibition, hence its antagonism eventually reduces fibrosis.

Conclusions: We demonstrate that miR-181c levels are significantly increased in frail older adults with diabetes and HFpEF and that miR-181c specifically targets PRKN and SMAD7 in human cardiac fibroblasts.

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Metabolic switch and activation of cardiac fibroblasts in the initial phase of anthracycline cardiotoxicity

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Background

The use of Doxorubicin (DOX) is hampered by cardiotoxicity with asymptomatic diastolic dysfunction as the earliest manifestation. Fibrosis leads to impaired relaxation, but the mechanisms that operate shortly after DOX exposure are not clear. We asked whether the activation of cardiac fibroblasts (CFs) anticipates DOX-induced myocardial dysfunction and evaluated the effects of DOX on CFs metabolism.

Methods

Fischer 344 rats were exposed to 6 injections of 2.5 mg/kg of DOX over 2 weeks. Heart function was assessed by echocardiography and ventricular catheterization. Primary CFs were isolated from hearts of DOX-treated rats after the first injection of DOX. In another set of experiments, CFs were exposed to DOX in vitro. Cell metabolism was measured by a Seahorse-XF Real-Time ATP assay and cell phenotype was determined by immunocytochemistry and molecular biology.

Results

Early effects of DOX consisted of diastolic dysfunction and unchanged ejection fraction. Markers of pro-fibrotic remodeling and histological evidence of CFs transformation were present in the heart immediately after treatment completion. Oxygen consumption rate and extracellular acidification revealed the increased metabolic activity of CFs and the switch to the glycolytic energy production. These effects were consistent in CFs isolated from the hearts of DOX-treated animals and in naive CFs exposed to DOX in vitro. The metabolic switch was paralleled with the phenotype change of CFs towards extracellular matrix-producing cells. Upon DOX, CFs upregulated markers of myofibroblast differentiation and the activation of pro-fibrotic signaling (aSMA, TGFb and phospho-SMAD). CFs upregulated also NOX2, indicating these cells as an intramyocardial source of reactive oxygen in the initial phase of the disease.

Conclusion

The metabolic switch and activation of CFs anticipate the onset of early diastolic dysfunction and represent a novel target in the early phase of anthracycline cardiomyopathy.

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Proteomics of Human Heart Failure: Effects of Mutations, Medications and Comorbidities

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Background. We have previously published a large-scale cardiac proteomics analysis of heart failure (HF) patients with ischaemic heart disease (IHD). No similar proteomics analyses are available on hearts from HF patients with dilated cardiomyopathy (DCM).

Material and Methods. To understand the factors driving patient variability in HF, we compared, using proteomics, left ventricular samples from patients with IHD (n=65), DCM (n=114), and non-failing controls (n=19).

Results and Conclusions. In all HF patients, reductions in myofilament-related proteins accompanied an increase in cardiac concentrations of atrial natriuretic factor (ANF). In contrast to IHD, few proteins changed related to medication usage in DCM patients. Instead, we identified four compositional clusters of DCM patients. The main clinical characteristics defining clusters were hypertension, atrial fibrillation, sex and body mass index. DCM patients carrying titin-truncating variants (19.3%) were enriched in two clusters (3.6-fold, $p<0.001$) and were characterised by a downregulation of thick filament constituents compared to non-carriers. Interestingly, among all quantified proteins, local myocardial ANF levels varied the most between DCM patients and correlated with the abundance of extracellular matrix proteoglycans including versican, but not with collagen content. Previously, we demonstrated that versican accumulation is detrimental for cardiac function. In cultured fibroblasts, ANF stimulation induced versican, but not collagen expression. In mice with a single amino acid mutation preventing the proteolytic processing of versican (VcanE441A) ANF levels were increased compared to WT at baseline, and this difference was exacerbated after angiotensin-2 infusion for 14 days, suggesting that versican and ANF form a previously unrecognised regulatory loop controlling cardiac extracellular matrix remodelling. In conclusion, comorbidities and DCM-causing mutations, but not medications, are associated with distinct proteomics profiles in DCM, even in explanted hearts. The paracrine effect of ANF on proteoglycan synthesis by cardiac fibroblasts could advance our mechanistic understanding of the effects of neprilysin inhibitors in HF.

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Effects of sacubitril-valsartan on ageing-related cardiac dysfunction

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Heart failure (HF) remains a huge medical burden worldwide, with aging representing a major risk factor. Here, we report the effects of sacubitril/valsartan, an approved drug for HF with reduced EF, in an experimental model of aging-related HF with preserved ejection fraction (HFpEF).

Eighteen-month-old female Fisher 344 rats were treated for 12 weeks with sacubitril/valsartan (60 mg/kg/die) or with valsartan (30 mg/kg/die). Three-month-old rats were used as control.

No differential action of sacubitril/valsartan versus valsartan alone, either positive or negative, was observed. The positive effects of both sacubitril/valsartan and valsartan on cardiac hypertrophy was evidenced by a significant reduction of wall thickness and myocyte cross-sectional area. Contrarily, myocardial fibrosis in aging heart was not reduced by any treatment. Doppler echocardiography and left ventricular catheterization evidenced diastolic function in untreated and treated old rats. In aging rats, both classic and non-classic renin-angiotensin-aldosterone system (RAAS) were modulated. With respect to untreated animals, both sacubitril/valsartan and valsartan showed a partial restoration of cardioprotective non-classic RAAS.

In conclusion, this study evidenced the favorable effects, by both treatments, on age-related cardiac hypertrophy. The attenuation of cardiomyocyte size and hypertrophic response may result in a shift of RAAS signaling towards cardioprotective components. However, diastolic dysfunction and cardiac fibrosis persisted despite of treatment and were accompanied by myocardial inflammation, oxidative stress, and endothelial activation.

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Western diet or aging do not aggravate hypertrophic cardiomyopathy disease progression in a heterozygous MYBPC3 c.2373insG mouse model.

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Hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disease, is characterized by hypertrophy and impaired relaxation. HCM is caused by mutations in genes encoding sarcomere proteins, but large heterogeneity in disease onset and severity suggests that second hits are needed for disease development. Aging and obesity are recognized as risk factors in patient cohorts that may contribute to phenotypic expression of HCM. To elucidate the mechanisms through which aging and perturbed metabolic health-related stress trigger development of HCM, we tested whether Western Diet (WD) feeding or aging could induce HCM in mice heterozygous (HET) for the Dutch founder mutation MYBPC3 c.2373insG.

MYBPC3+/2373InsG wild type (WT) littermates received WD for 10 weeks or were aged for 9/18-months. Mice underwent cardiac magnetic resonance imaging. Subsequently, the heart, epicardial fat, liver, and femoral arteries were isolated for further analysis. Initial data are reported below.

In mice exposed to WD, both WT and HET mice exhibited increased body weight and liver enlargement. Neither WD-feeding nor aging induced cardiac hypertrophy in MYBPC3+/2373InsG mice. When examining mitochondrial function, WD-fed mice, regardless of their genotype, displayed a minor decrease in NADH-linked respiration and a modest increase in leak respiration. Age-related increases in total oxidative phosphorylation capacity and respiration in response to fatty acids were evident at younger age in HET mice (9-months) compared to WT mice (18-months). When assessing the peripheral vascular response to acetylcholine, no significant differences were observed between genotypes, irrespective of diet or aging. Aged mice showed increased vascular wall thickness, reduced wall-to-lumen ratio and decreased response to acetylcholine independently from the genotype.

Western diet induced increased body weight, liver enlargement, and decreased mitochondrial function. Aging induced increased oxidative phosphorylation capacity, vascular remodeling and dysfunction. Overall, our initial data indicate that WD or aging do not aggravate disease progression in this HCM mouse model.

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Exploring m6A modification in early human cardiomyocyte development

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Background

RNA modifications, particularly N6-methyladenosine (m6A), have emerged as crucial post-transcriptional regulators influencing gene expression. While recent studies showed that m6A modification plays a key role in dictating cell fate in embryonic stem cells, its role in cardiomyocyte commitment has not been explored. Here, we mapped m6A modifications in the transcriptome of early human cardiomyocyte development to identify key regulators of this process.

Material and Methods

Human induced pluripotent stem cells (hiPSCs) were differentiated into beating cardiomyocytes (hiPSC-CMs) via Wnt signaling modulation. Four cellular stages—pluripotency, cardiac mesoderm cells, cardiac progenitors, and hiPSC-CMs—were analyzed at single-cell resolution to evaluate m6A-methyltransferase expression. Subsequently, we performed m6A RNA immunoprecipitation followed by high-throughput sequencing (MeRIP-seq) to investigate transcriptomic and epitranscriptomic changes in coding and non-coding RNA during hiPSC differentiation. Enrichment analyses of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were conducted to elucidate the biological significance of differentially expressed and methylated transcripts.

Results and Conclusions

We analyzed m6A-methyltransferase and m6A cellular expression levels in each cellular stage and observed higher abundance in earlier stages of human cardiomyocyte lineage specification. Subsequently, we explored the relationship between transcriptomic and epitranscriptomic changes of differentially expressed RNA transcripts during hiPSCs differentiation into hiPSC-CMs and observed the highest number of differentially m6A-methylated and expressed transcripts in the transition from cardiac mesoderm to cardiac progenitor cells. GO and KEGG analyses indicated hyper-methylated upregulated transcripts enriched in muscle cell differentiation, cardiac physiology terms, and calcium and MAPK signaling pathways regulating heart contraction. Interestingly, the distribution analysis of m6A modifications exhibited distinct topological arrangements in lncRNA and mRNA transcripts.

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Protective role of SK1/S1P system in ischemic cardiomyocytes

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Objectives: The sphingosine kinase 1 (SK1)/S1-phosphate (S1P) system is downregulated in post-ischemic hearts. These pathways are pivotal in metabolic reprogramming and proliferation in highly proliferating cells like tumor cells. Based on this premise, we investigated whether and how boosting SK1/S1P signaling in cardiomyocytes affects their proliferation/survival after ischemia.
Materials and methods: In vivo, we used wild-type (WT) C57Bl6 mice who underwent myocardial infarction (MI) for 4-weeks. Seven days post-MI, mice were randomized to Vehicle (saline solution) or S1P (10 µM) treatments. In vitro, H9c2 cardiomyoblasts were transduced with Adenovirus (Ad; 20 MOI) encoding for SK1 or GFP as control.
Results: In MI-mice infused for 3 weeks with S1P, we observed a marked and significant increase in cardiac expression of the hexokinase factor 2 (HK2), a key enzyme of the first stage of glycolysis. In line with these results, we observed an increased proliferation rate of both total cardiac cells and individual cardiomyocytes associated with improved LV ejection fraction (% LVEF), which deteriorated in the MI control group. In addition, infarcted hearts infused with S1P showed a significant increase in the expression of genes that promote glucose metabolism and cell proliferation/regeneration in tumor and muscle cells. Next, in H9c2, in vitro, S1P treatment (250 nM for 3 hours) resulted in a significant increase in glycolysis and a substantial rise in BrdU and Ki67 positive cells compared to control cells. Finally, in H9c2 cells overexpressing SK1 or GFP (Ad transduction) under hypoxic conditions, the cell proliferation rate increased in the SK1 group compared with the GFP-expressing cells.
Conclusions: These data, albeit preliminary, support our central hypothesis that, after an ischemic event, the potentiation of glycolysis through the stimulation of the SK1/S1P system prompts the adult cardiomyocyte to re-enter the cell cycle, thus limiting the loss of cardiac cells.

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The role of OPA1 in risk factor-induced endothelial dysfunction

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Background: Endothelial dysfunction is associated with cardiovascular disorders. Emerging evidence links mitochondrial dynamics disturbances to heart damage and cardiac diseases, making mitochondrial dynamics a potential therapeutic target. Optic Atrophy 1 (OPA1) regulates mitochondrial fusion and exerts cardioprotective properties. However, the role of OPA1 in endothelial cells undergoing cardiovascular stress is unclear. This study examines the influence of OPA1 on endothelial cell function and angiogenesis in response to common cardiovascular risk factors.

Methods: Human umbilical vein endothelial cells (HUVECs) were exposed to conditions mimicking type 2 diabetes (high glucose, HG), hypercholesterolemia (oxidized LDL, ox-LDL), and smoking (cigarette smoke condensate, CSC). OPA1 levels were evaluated by western blot analysis, and Mitotracker and JC1 Assays were performed to evaluate mitochondrial morphology and integrity. Moreover, angiogenic function was assessed by Matrigel Assay. Endothelial-dependent vasorelaxation measurements were obtained from the mesenteric arteries of mice overexpressing OPA1 (Tg-OPA1).

Results: In endothelial cells subjected to stress, OPA1 levels initially increase after 4 hours (CTR vs HG/ox-LDL/CSC: *p<0.05) and later decrease after 6 hours (CTR vs HG/CSC: *p<0.05; CTR vs ox-LDL: **p<0.01), returning to baseline after 8 hours. Mitotracker analysis shows disrupts mitochondrial networks at 2 hours, followed by restoration, enduring to 8 hours. We also found an increase of mitochondrial membrane polarization at 2 hours, partially reduced at 4, 6, and 8 hours. Prolonged exposure (24 and 48 hours) to risk factors increases OPA1 levels, preserving mitochondrial networks. OPA1 silencing reduces HUVEC angiogenic function and exacerbates risk factor-induced angiogenesis inhibition (CTR vs HG/ox-LDL/CSC: **p<0.01). High glucose-induced endothelial dysfunction is reduced in mesenteric arteries of Tg-OPA1 mice.

Conclusion: OPA1 levels in endothelial cells are modulated by cardiovascular risk factors, affecting mitochondrial dynamics and function. OPA1 is crucial for preserving endothelial function under stress, suggesting its potential as a therapeutic target to sustain mitochondrial activity and endothelial function.

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Progerin is an unlikely contributor to natural cardiac aging, but promotes age-related phenotypes in vitro

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Background: Ageing effects in the heart are observed at several levels: functional, structural, cellular, and molecular. In human, main functional and structural age-related changes are well characterised. However, the molecular and cellular mechanisms that modulate them are largely based on animal studies (with translation limitations) or cross-sectional population studies (affected by high inter-individual variability). Relevant models to study these mechanisms longitudinally in humans are lacking. Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder characterised by accelerated ageing. HGPS is caused by the expression of a truncated form of lamin A, progerin, resulting from cryptic splicing. Patients with HGPS present cardiovascular alterations that resemble those of natural ageing. Although progerin has been reported to be expressed in several aged human tissues, its cardiac expression in natural ageing and its ability to induce ageing of human cardiac cells remains to be assessed. These are the aims of this study.

Materials and Methods: We evaluated the expression of LMNA gene and progerin mRNA in the human left ventricle (LV). We also generated a transgenic human iPSC line and characterised phenotypic changes caused by the inducible expression of progerin in committed cardiomyocytes. Finally, we compared our in vitro model with cross-sectional human transcriptomic data.

Results: LMNA and progerin mRNA expression appeared to increase similarly with age in the human LV. This suggests that natural ageing is not associated with a shift towards the pathological transcript variant. However, in committed hiPSC-derived cardiomyocytes, inducible expression of progerin led to the recapitulation of ageing hallmarks and transcriptomic changes present in the human LV.

Conclusions: Progerin is unlikely to be a major inducer of natural cardiac ageing, but may promote ageing features in vitro. Longitudinal mechanistic studies of human cardiac cell ageing (or in other cell lineage) can be advanced with this groundbreaking accelerated ageing tool.

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Abstract retracted by authors

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IMMUNE-MEDIATED MYOCARDIAL FIBRO-FATTY INFILTRATION DURING THE ATRIAL CARDIOMYOPATHY

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Background. Atrial fibrillation (AF), the most common arrhythmia and the first cause of stroke, is often associated with an atrial cardiomyopathy. The epicardial adipose tissue (EAT) has emerged as a major component of the atrial cardiomyopathy. Direct arrhythmogenicity of EAT has been attributed to its role played in fibrosis, inflammation or oxidative and metabolic stress of neighbouring myocardium. Epicardium is reactivated during the formation of the atrial cardiomyopathy induced with a myocardial infarction.

Hypothesis. The immune response is a mechanistic link between EAT and the formation of the atrial cardiomyopathy by favouring fibrosis of EAT and fibro-fatty infiltrations of the atrial myocardium and by modulating recruitment of resident progenitor cells.

Research strategies. In atria, subpopulations of immune cells were analyzed with flow cytometry in a mouse model of myocardial infarction that developped an arrythmia, adipogenic and inflammatory phenotype. To characterize the impacts of immune response on atrial remodelling, a deficient mouse models for monocyte-derived macrophages, CCR2KO was used. Clinical parameters were used together with flow cytometer, histology and immunolabeling. Data obtained in mice will be confronted to results from human specimen atria obtained during cardiac surgery in patients suffering of AF and compared to a control group of patients in sinus rhythm. In human atrial sections, the identification and localization of immune cells subpopulations will be addressed using Spatial transcriptomic approach.

Results. Our studies have shown the contribution of macrophages in atria remodeling that lead to a better understand the link between AF and atrial cardiomyopathy and that open new avenues of research to prevent the atrial cardiomyopathy.

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Dapagliflozin and hydrogen sulfide donor protect the heart in an animal model of heart failure with preserved ejection fraction (HFpEF)

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Background. Clinical studies have shown that patients with and without diabetes may benefit from using sodium-glucose cotransporter 2 inhibitors (SGLT2i) to reduce the risk of heart failure (HF). Several findings have highlighted the critical role of the gasotransmitter hydrogen sulfide (H2S) in the regulation of cardiac and vascular biology. Its role in heart failure with reduced ejection fraction (HFrEF) has been widely investigated while the effect of H2S on HFpEF is less known.

Therefore, in this study, we investigated the effects of SGLT2i dapagliflozin alone or in combination with H2S donor (NaHS) in an animal model of HFpEF.

Material and Methods. Seven-week-old male Dahl salt-sensitive rats were fed with laboratory chow containing 8% NaCl (high-salt diet, HS) or 0.3% NaCl (low-salt diet, LS). After 5 weeks, the animals on HS diet were randomized to dapagliflozin (0.1mg/Kg/day, DAPA) or combination (DAPA+NaHS) for the following 6 weeks. Echocardiography was used to assess systolic and diastolic function. Hearts were collected for molecular and histological analysis.

Results and Conclusions. After six weeks of treatments, echocardiography showed that DAPA alone or in combination with NaHS significantly improved cardiac function and hypertrophy. Functional changes were coupled with a reduced RNA expression of pro-inflammatory and pro-fibrotic genes (TGF-beta and collagen I). Interestingly, these changes were associated with up-regulation of miRNA133a and miRNA1 and downregulation of miRNA21.Our data suggest beneficial effects of DAPA and DAPA+NaHS, by modulating miRNA profile and inflammatory response, are able to improve diastolic function and cardiac remodeling in a model of HFpEF.

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Inhibition of long non-coding RNA Meg3 supports reverse remodeling in a mouse model resembling aortic stenosis and TAVI

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Aortic stenosis (AS) represents one of the most common valve diseases. It is accompanied by progressive left ventricular remodeling and fibrosis transitioning towards heart failure (HF). Transcatheter aortic valve implantation (TAVI) represents the common treatment strategy whereby fibrosis in patients with severe AS is associated with worse outcome. At present, there is no treatment of cardiac fibrosis in those patients, underlining the need for innovative therapeutic strategies. However, for testing such therapies, we first needed to establish an in vivo model resembling AS and TAVI. For proof-of-concept, we combined this model with an adjuvant therapy inducing the inhibition of the long noncoding RNA Meg3.

To establish an in vivo model, we performed an 8-weeks and 12-weeks in vivo experiment in which first pressure-overload induced cardiac hypertrophy was induced by transverse aortic constriction (TAC) surgery. After 4-weeks or 6-weeks the previously performed TAC was removed simulating TAVI (referred to as debanding, DeTAC). Both experiments were echocardiographically and histologically assessed whereby the 12-weeks approach was proven to be an appropriate model since the heart failed to completely recover LV mass, LV wall thickness and ejection fraction. In line, cardiomyocyte hypertrophy and increased heart weight was still present in the DeTAC group. Based on these results we conclude that in the 12-weeks experiment reverse remodeling is incomplete suggesting this debanding approach as an appropriate tool for the investigation of adjuvants supporting cardiac recovery. Since we previously demonstrated that preventive ASO-mediated inhibition of Meg3 decreases cardiac hypertrophy and fibrosis in the TAC mouse model, we here investigated whether MEG3 knockdown can aid in reverse remodeling after debanding. Indeed, anti-Meg3 therapy, enhanced cardiac recovery as indicated by a significantly increased stroke volume. Furthermore, we could show that inhibition of human MEG3 elicits anti-fibrotic response in human cardiac fibroblasts, which was further validated by mRNA-Seq analysis.

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Modulation of miRNA expression in a model of age-related heart failure treated with sacubitril-valsartan and sacubitril/valsartan-dapagliflozin.

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Aim. Pathophysiology of heart failure with preserved ejection fraction (HFpEF) is incompletely understood, because of the heterogeneity of HFpEF patients, the biggest hurdle for the interpretation of the response to therapy. HFpEF patients are generally older, frequently female and have increased incidence of comorbidities. On these premise, our study aims to verify the potential beneficial effects of sacubitril-valsartan (S/V) alone or in combination with dapagliflozin [(S/V)+DAPA]), in an age-related HFpEF model.
Methods. 12-month-old Fischer 344 female rats (OLD) were treated with S/V (60 mg/kg/day) alone [OLD+(S/V)] or with a combination of S/V and DAPA (0.1mg/Kg/day) (OLD+[(S/V)+DAPA]) for 45 weeks. Echocardiography and hemodynamic analysis were used to assess cardiac function.
Results. Echocardiographic and hemodynamic analysis showed that both treatments effectively improved diastolic dysfunction. RT-qPCR showed that miR-21a, 22a, 29a, 499a, mainly associated with cardiac remodeling (hypertrophy and fibrosis), were significantly up-regulated in aged rats and significantly down-regulated after treatment. Moreover, miR-21a up-regulation was significantly associated with TGF-β over-expression in aged rats, whereas its down-regulation, in treated rats, was accompanied by reduced TGF-β expression. An inverse correlation between miR-34 and SIRT-1 expression, involved in apoptosis, cellular senescence and oxidative stress response, was also evidenced. The restoration of Sirt-1 in [OLD+(S/V+D)], could drive several benefits including improved cell viability and attenuated oxidative damage.
Conclusions. Our results showed that long-term treatment with S/V and [(S/V)+D] improve cardiac function. The effects may be related to the regulation of miRNA expression, which may represent innovative therapeutic targets in aging-related HF.

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High-content phenotypic profiling to advance the maturation state of human induced pluripotent stem cells derived cardiomyocytes

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Background

Human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) have a great potential for disease modelling and thus, for developing new treatments. However, their use is hampered by their immature estate. Here, we have developed methods for advanced image-based phenotypic profiling of hiPSC-CMs to investigate the effect of different 2D maturation strategies. The ultimate goal is to elucidate the most time and cost-effective approach to obtain mature hiPSC-CMs.

Material and Methods

Regular and epigenetically primed using pIC (EP) differentiated progenies with >90% TNNT2+ hiPSC-CMs were cultured on regular plates or on PDMS sheets (to imitate the stiffness of the heart) for 8 days. These cultures were maintained either in cardiomyocyte maintenance media (CMM) or in maturation medium (MM). MM contained hormones and factors that pike naturally after birth (T3, and corticoids and IGF-1α) and a factor to provoke a metabolic switch from glycolysis to β-oxidation (as occurs postnatally). Methods were developed to phenotypically characterise the degree of maturation of the cultures using high content imaging (HCI) snd analysis techniques.

Results

Previously existing deep-learning based tools have been optimized to automatically segment multinucleated cardiomyocytes. Also, the staining of several structural, functional and maturation markers have been optimized. With this, a dataset of 12,480 images has been single-cell analysed using user-defined feature extraction methods (CellBlocks). UMAP and PCA analysis show variability among biological replicates that better explains the differences found by the phenotypic features than the different conditions. The combination of EP with MM seems however to yield the more consistent effects in maturation, clustering on UMAP far from the baseline condition, and achieving an increase on TNNI3, a well-known maturation marker

Conclusions

Beyond the robust commitment of hiPSC-derived cardiac progenies as TNNT2+cardiomyocytes, HCI analysis highlights an interexperimental phenotypic heterogeneity of hiPSC-CM that combinatorial maturation approaches might contribute to reduce while promoting maturation.

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Exploiting the therapeutic potential of microRNAs and DNA nanotechnology to create novel cardioregenerative treatments.

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Background: Myocardial infarction, if not fatal, can lead to remodelling of the cardiac tissue and progression to heart failure. Currently no effective therapy to prevent this pathological process exists. MicroRNAs (miR), i.e. miR-199a-3p, have been reported to promote cardiac regeneration after infarction by stimulating cardiomyocyte proliferation. Here we propose to exploit this capacity to develop cutting-edge miR nanotherapies. DNA nanostructures (DNS) capable of carrying and delivering miR-199a-3p are created and characterised.

Material and Methods: DNS have been built by supramolecular polymerisation of the DNA three-way junction construct with complementary DNA-based linkers. DNA sequences were rationally designed to allow the loading of large amounts of the therapeutic miR while achieving an appropriate size for cell internalisation. A luciferase reporter vector containing a miR-199a-3p sponge has been created to assess the functionality of DNS in vitro. Structural characterisation of the DNS has been performed by gel electrophoresis (GE), dynamic light scattering (DLS) and atomic force microscopy (AFM). In cells, the internalisation capacity (Cy3-labelled DNS and flow cytometry) and the RNase H-mediated intracellular release of miR (luciferase reporter assays) has been tested in Hek293 cells.

Results and Conclusions: Nanostructures harbouring the miR-199a-3p have been created and characterized. They have a diameter of around 30 nm and effectively internalize in Hek293 with an uptake capacity that reaches nearly 100% of cells. The miR is actively released through the action of intracellular RNase H and exerts inhibition of the luciferase activity, demonstrating thus its correct processing by the cellular machinery and the interaction with its target sequence. In conclusion, we have created a novel nanocarrier for the delivery of functional miR-199a-3p in a model cell system. Current efforts focus on enhancing the biological stability of the miR-loaded nanostructure in cell culture to maximise its potential therapeutic activity.

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Lymphatic Vessel and Tenascin C crosstalk in Left Ventricular Hypertrophy-Induced Cardiac Remodeling

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Introduction:
Boosting lymphangiogenesis has proven to alleviate inflammation, fibrosis and myocardial edema tafter myocardial infarction (MI). However, the overexpression of the matricellular protein Tenascin-C (TNC) negatively regulates lymphangiogenesis post-MI, contributing to impaired repair and remodeling process. Nevertheless, the dynamic changes in lymphangiogenesis in pressure-overload induced left ventricular hypertrophy (LVH) and its crosstalk with TNC are poorly understood.

Methods:
LVH was induced by transverse aortic constriction (TAC) in adult male C57BL/6 mice, allocated to groups of sham, 1-week and 6-weeks post-TAC. Hearts were processed for histology of Masson's trichrome and immunostainings for lymphatic markers (Podoplanin and LYVE1) and TNC. Alterations of lymphatic vessels were quantified in perivascular areas and total heart tissue. In addition, lymphatic endothelial cells (LEC) were subjected to 48h low (1-3%) or high (18-21%) elongation using FlexCell to mimic stretch overload. The expression of NF-κB and fibrotic markers (Collagen I and III) were assessed by western blot and RT-qPCR. Scratch-wound healing and XTT cell viability assays were performed on LEC after treatment with recombinant human TNC for 24h (1µg/ml or 5µg/ml).

Results:
Region-specific alterations of lymphatic vessels in heart tissue after pressure overload were detected. In addition, 1-week post-TAC, a regression of lymphatic structures was observed exclusively in perivascular areas, along with increased perivascular fibrosis and TNC expression. High stretch conditions in LEC showed increased expression of inflammatory and fibrotic markers, suggesting lymphatic remodelling. Scratch-wound healing and XTT assays revealed prolonged wound healing and a tendency towards reduced viability in LEC after TNC treatment.

Conclusion:
Our study demonstrates dynamic spatio-temporal changes of lymphatic vessels in LVH due to pressure overload. Moreover, TNC appears to be a crucial player in modulating lymphatic vessel growth in LVH. Thus, inhibiting TNC and/or boosting lymphangiogenesis may be a potential novel therapeutic strategy to alleviate fibrosis and remodeling in LVH.

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Abstract retracted by authors

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Abstract retracted by authors

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Exercise training induces right ventricular hypertrophy along with functional improvement without pathological myocardial processes or arrhythmogenicity in a rodent model

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Background: Regular sport activity leads to the adaptation of cardiac structure and function, the athlete's heart. Research projects over the last years have focused on exercise-induced adaptation of the right ventricle (RV), because the disproportionate load on the RV - when compared with the left ventricle - might lead to pathological consequences, such as myocardial interstital fibrosis or chamber dilation. We aimed at providing a comprehensive characterization of exercise-induced RV alterations in a rat model of athlete›s heart.

Materials and Methods: Young, adult rats were divided into control (Co) and exercised (Ex) groups (n=12-12). Exercised rats underwent a 12-week-long swim training program. In vivo electrophysiological study and in vitro cellular force assessments on isolated cardiomyocytes were carried out to investigate electrical and functional RV alterations, respectively. Molecular biological and histological investigations were applied to reveal underlying mechanisms.

Results and Conclusions: Exercise training was associated with increased RV cardiomyocyte diameter along with hyperphosphorylation of protein kinase B (Akt). RV cardiomyocytes from exercised animals showed improved calcium sensitivity and increased maximal force development, that was associated with hypophosphorylation of troponin I. We found increased length of ventricular effective refracter period along with decreased gene expression of potassium channels and could not induce ventricular arrhythmia by programmed stimulation. Picrosirius staining did not reveal fibrosis, while profibrotic and pathological markers remained unaltered. According to our data, regular swim training induced RV hypertrophy, that was associated with functional improvement (improved calcium sensitivity and maximal force), hypophosphorylation of troponin I without characteristic pathological alterations or arrhythmogenicity of RV myocardial tissue.

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Design of DNA-based nanocarriers for the delivery of microRNA therapies against cardiac dysfunction

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Background & goal: DNA nanotechnology leverages Watson-Crick-Franklin highly specific base pair recognition for the construction of complex DNA nanostructures (DNS), which can contain, protect, and specifically deliver functional sequences. Age is a main contributor to cardiac disease. Recently, cardiac microRNA (miR24-2) has been identified upregulated with age in the human left ventricle and to regulate important genes for cardiac function, such as SERCA2. SERCA2 is also downregulated in heart failure and a common aim of current gene therapy for heart failure. Hence, the main goal of this study is to design DNS with deliver capacities of functional anti-miRNA sequences to balance their dysregulation in human cardiomyocytes as potential cardiac therapy.

Materials and methods: Structural and functional characterization of DNS was carried out by PAGE, DLS and AFM techniques. Internalization of DNS was studied by FACS and confocal microscopy. Functional assay was performed with a luciferase reporter system.

Results: Different versions of anti-miR24-2-5p strands-containing DNS were designed. Their structural and physicochemical characterization showed a successful assembly and proper stability up to 48 h in serum. Additionally, specific disassembly in the presence of miR24-2-5p demonstrates their miRNA trapping ability. Biological characterization of DNS in vitro in Hek293 cells and in human induced pluripotent stem cells (iPSC)-derived cardiomyocytes (iCM) showed high unspecific uptake levels with partial degradation after 48h in HEK293, and remarkably lower uptake in iCM, as expected. Nonetheless, DNS demonstrated in vitro biocompatibility in both, Hek293 and in iCM. Furthermore, luciferase reporter assay system demonstrated functional anti-miR24-2-5p activity of DNS in HEK cellular model.

Conclusions: We report the generation and characterization of functional DNS capable to modulate miR-24-2-5p activity in vitro. Our data suggest that lower uptake efficiency by iCM needs to be overcome by DNS functionalization with ligands that promote their efficient and specific uptake by primary cardiac cells.

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Impact of Progerin on cardiomyocyte differentiation in a novel hiPSC model of Hutchinson-Gilford Progeria Syndrome

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The Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare form of systemic laminopathy characterized by an accelerated ageing phenotype. The molecular signature of HGPS is the production of a shorter variant of Lamin A/C known as Progerin that improperly retains the post-translational modification named farnesylation. Once incorporated in the nuclear lamina, the farnesylated progerin negatively interfere with key cellular processes such as DNA repair mechanisms, chromatin remodeling and gene transcription. Patients affected by HGPS suffer of premature death around 14 years of age due to myocardial infarction caused by electrical abnormalities in the cardiac conduction system, suggesting that progerin presence strongly affects the heart. However, the mechanisms through which progerin exerts its toxicity are still unknown.
In this study, we aim to elucidate the role of progerin in the pathophysiology of HGPS at the molecular level in human induced pluripotent stemcells (hiPSC) derived cardiomyocytes (hiPSC-CMs) from a HGPS patient and its isogenic control line. Both hiPSC lines were differentiated into cardiomyocytes (CMs) through temporal modulation of the canonical Wnt-β catenin pathway. Cells were collected during the differentiation at day 0, 3, 10, and 25 to analyze the pluripotent, mesodermal, cardiac progenitor and cardiac stage respectively. Preliminary results indicate an impaired cardiac differentiation ability of HGPS hiPSC-CMs compared to the isogenic control hiPSC-CMs as shown by downregulation of key cardiac markers associated with the upregulation of progerin in HGPS hiPSC-CM. Finally, contractility analysis at day 25 indicated a pro-arrhythmic phenotype for HGPS hiPSC-CMs in line with downregulation of key genes involved in the formation and maintenance of the cardiac conduction system.

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Epithelial-to-mesenchymal transition is essential for epicardial cells to participate in cardiac repair processes

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During development, the epicardium forms as a single cell layer on the outside of the heart. Part of these cells undergo epithelial-to-mesenchymal transition (EMT) and migrate into the myocardium where epicardial-derived cells (EPDCs) participate in heart formation by contributing cells and by producing paracrine factors. In the adult heart the epicardium exists as a dormant layer until ischemic injury leads to a partial reactivation and participation of epicardial cells in the repair process. Therefore, the epicardium is considered an interesting cell source for endogenous cardiac repair.
Aim: to establish if differences between developmental and adult epicardium exist, and in which state (pre- or post-EMT) epicardial cells contribute efficiently to cardiac repair.
Methods: Primary epicardial cells were isolated from human fetal and adult cardiac tissue and cultured as epicardial cells (EPIs) or as EPDCs after inducing EMT with TGFβ3. Cells were processed for RNA-sequencing and cultured to obtain conditioned medium. 240k cells were injected intramyocardially after myocardial infarction (MI) in NOD-SCID mice, followed by functional and histological assessment.
Results: Fetal and adult EPDCs displayed limited transcriptomic differences. In vitro, conditioned medium of both cell sources conveyed cardioprotection to staurosporin-treated cardiomyocytes. Next, we studied the effect of EMT: comparison of adult EPIs to EPDCs revealed that EPDCs have an angiogenic paracrine and transcriptional profile. Transplantation of adult EPDCs resulted in a higher EF (25.4±3.8 vs. 11.1±4.6%, $P<0.005$), lower ESV (139.6±13.5 vs, 91.37±14.2ul, $P<0.05$), and a smaller infarct size 6wks post-MI compared to EPI-recipients. EPDC-injected hearts contained more proliferating cells at 3days, and a higher vessel density at 6wks post-MI. Interestingly, while cell engraftment was equal in both groups at 3days, no cells were retrieved at 6wks supporting a paracrine effect.
Conclusion: epicardial EMT is essential to unleash the predominantly paracrine contribution of epicardial cells in cardiac repair.
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Abstract retracted by authors

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Identification of biological age indicators as potential predictors of physical and cardiac-related conditions

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Background

Ageing stands as a main predisposing risk factor for numerous diseases, including cardiovascular diseases (CVD). Extensive research has delved into the ageing role in CVD, however, variable ageing rates mean chronological age (CA) may not always provide a precise insight into an individual´s physiological condition. Age-related biological indicators, influenced by genetics and environmental factors over time, could provide a more accurate explanation. Consequently, assessing the influence of age on cardiac function raises the necessity for purposeful biological age (BA) indicators for predicting age-related cardiovascular risk. This study aims to establish individual (BAind) and cardiac-specific BA (BAcardiac) indices and present proof-of-concept of their ability to discriminate between individuals' physical and cardiac condition.

Materials and methods

We calculated BAind and BAcardiac, adapting published methods in human blood samples from 20 to 100 y.o., based on the expression levels of age-related genes in white blood cells and on the amount of cardiac-enriched and muscle-specific microRNAs in plasma, respectively. Subsequently, both indices were assessed related to biometric (descriptors of donor´s physical condition) and biochemical (linked to cardiac damage and mortality) data within the same population.

Results and Conclusions

BAind and BAcardiac of each donor exhibited significantly stronger association with CA (RHO=0.57 and 0.37, respectively) compared to individual genes/miRNAs, suggesting their combined effects enhance the ability to explain age. In addition, both indexes correlated among them (RHO=0.43), indicating the expected relationship between the heart and the individual ageing rates. BAind explained some of health states (weight, IMC and others) and even responded to an anti-ageing intervention (exercise) in centenarian donors. However, BAcardiac only displayed associations with specific biochemical parameters like calcium and creatinine concentration. In conclusion, we have created BA indices that primarily describe individual and cardiac-related physiological conditions. Further studies will focus on validating them as indicators of age-related cardiac risk.

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Developing living myocardial tissue slices as model for studying cardioprotection after ischemic injury

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Background – Despite scientific advances in disease modelling, cardiac regenerative medicine lacks models that recapitulate the adult heart›s complexity and mature phenotype. Hence, the availability of novel technologies would greatly benefit researchers investigating cardiac diseases. We use adult pig hearts to produce viable organotypic tissue slices that can be cultured for a prolonged time and can be manipulated to model diseases and interventions. Unlike other non-in vivo models, myocardial tissue slices recapitulate the architecture and complexity of the adult heart while being cost- and time-effective.

Methods – Left ventricular cardiac slices were generated from adult pigs› hearts and kept in culture for up to 4 days. We tested several treatments, including H2O2-induced stress or viral-mediated gene delivery.

Results – We optimized the slicing procedure to acquire 300 um-thick left ventricular slices. The electrical function and viability of the myocardial slices were assessed by performing pseudo-ECG or optical mapping. Slices could be locally paced up to a few days after slicing. Next, the cultured slices were treated with H2O2. After treatment, changes in the expression of stress marker and apoptosis marker genes were quantified. Next, we tested adeno-associated viruses (AAV)-mediated gene transfer efficiency to our model. Four days after the infection, AAV6 and AAV9 serotypes caused robust transduction and transgene expression of the cardiac slices. Finally, we achieved AAV6-mediated delivery of Zinc Finger E-Box Binding Homeobox 2 (ZEB2), a transcription factor involved in endothelial-mesenchymal transition that has been shown to promote cardiac repair after injury stimulating the secretion of pro-angiogenetic factors. Four days after infection, slices were collected for downstream analyses to further elucidate how ZEB2 stimulates cardioprotection in the heart.

Conclusions – Adult left ventricular tissue slices represent a suitable model for studying the pathophysiology of the adult heart and an alternative to in vivo cardiac disease models currently available.

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Macrophages partecipate in doxorubicin-induced cardiac damage

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The functional contribution of inflammatory cells in the setup of heart failure in response to anthracycline, specifically in response to Doxorubicin (Dox) , is recently becoming of growing interest. Therefore, the study aims to evaluate the role of macrophages in cardiac damage in response to doxorubicin. In vivo mice C57BL/6 were treated with one intraperitoneal injection of Dox (20mg/kg) and followed up for 5 days by cardiac ultrasound. Moreover, we tested the impact of Dox in macrophage-depleted mice by using Clodrosome Liposomes to evaluate the development of cardiotoxicity. In vitro murine cardiomyoblasts were directly treated with Dox (D-Dox) or with conditioned medium from cultured murine macrophages treated with Dox (M-Dox) and in both conditions, cell death and mitochondrial phenotype were evaluated.
In response to Dox, macrophages infiltration preceded cardiac damage. The depletion of macrophages in mice prevents cardiac damage suggesting a key role of these cells in promoting cardiotoxicity. To evaluate whether the crosstalk activation between macrophages and cardiac cells in response to Dox, we compared the compared the effects of D-Dox and M-Dox in vitro.
Both effects lead to cell death but were significantly higher in M-Dox treated cells. These events were linked to p53-induced alterations of mitochondria morphology, function and autophagy. We identify a mechanistic role of catecholamines released by Dox-activated macrophages that lead to mitochondrial apoptosis of cardiac cells trough β-AR stimulation. Our data suggesting that crosstalk between macrophages and cardiomyocytes is determinant in cardiac damage in response to Doxorubicin.

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Long-term ketogenic diet increases heart hypertrophy and modulates glycemic control and physical endurance in a sex-dependent manner in aged healthy rats

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Ketones have recently been shown to have beneficial effects on HF and cardiogenic shock. Ketogenic diet (KD) itself has been shown to reduce cardiovascular diseases risks factors with improved glycemic control in diabetes and weight loss in obesity. However, in healthy individuals, long-term KD-induced effects on cardiovascular function and metabolism remain poorly understood or controversial, as well as the potential sex-specific differences.

We aimed to understand if a long-term KD modulates cardiovascular function in a sex-specific way in healthy aged rats.

Aged (23-month-old) male and female Wistar rats fed with standard or KD from 3 month-old were subjected to: 1) glycemia/ketonemia, 2) glucose tolerance and insulin resistance tests; 3) treadmill running test; and 4) echocardiography. Heart fibrosis and mitochondrial function were evaluated through histology and high resolution respirometry of heart permeabilized fibers, respectively.

KD-fed rats had higher blood ketone levels compared to respective controls, but only significantly increased blood glucose levels and the vulnerability to glucose intolerance in male rats Despite similar food intake, caloric intake in male rats fed with KD was in line with their higher body weight. Although not affecting VO2 max levels, KD decreased the RQ value in both male and female rats, suggesting a higher percentage of fat-based metabolism at VO2max timepoint. Total distance run was only decreased by KD in female rats, suggesting a decrease in their physical endurance. The later, however, is not due to differences in heart mitochondrial respiration. Heart weight was (significantly) increased in both KD-fed male and female rats, consistent with a tendential increase in their cardiomyocytes area and fibrosis. Still E/e' was not affected.

In sum, long-term ketogenic diet induced sex-specific alterations in aged rats regarding glycemic and physical endurance related parameters, while it increased cardiac hypertrophy in both male and female rats. Still, further studies are needed.

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Ketones' impact on a rat model of HFpEF

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Heart Failure with Preserved Ejection Fraction (HFpEF) affects 1.1-5.5% of the general population whilst being associated to poor prognosis and hospitalization. This is particularly concerning given that, until very recently, pharmacological options were extremely limited. Previous studies have shown that increasing ketone levels may have beneficial effects, but their impact on HFpEF remains unknown. In this study, we explore ketones increase as a potential therapeutical option for HFpEF.

At 16 weeks old, 30 ZSF1 Lean (Controls) and 30 ZSF1 Obese rats (a well characterized dysmetabolic HFpEF animal model), were randomly assigned to remain on control diet, change to a ketogenic diet (KD) or keep the regular chow whilst having ketone salts (KS) delivered through drinking water. Glucose and B-hydroxybutyrate levels were accompanied throughout the study. Metabolic and functional assessments including oral glucose tolerance test, VO2max, echocardiography and PET/CT were conducted throughout the protocol, culminating with terminal procedures at 23-30 weeks of age.

By 23 weeks of age, baseline hyperglycaemia was reduced by 48% with KD and KS on these diabetic HFpEF rats, while glycaemic tolerance was improved only under the KD. By itself, HFpEF appears to promote 11Acetoacetate uptake similarly to both treatments under control conditions, hinting at a metabolic shift may be occurring on the starving HFpEF hearts. Importantly, KD and KS were shown to significantly reduce HFpEF-associated cardiac fibrosis and hypertrophy. These changes were further studied resorting to isolated cardiomyocytes, where we observed improvements in calcium handling with KS and contractile function with both therapies. Lastly, we found a significant reduction in cardiac complex-II mitochondrial respiration on HFpEF with KS, which might constitute a defence mechanism to oxidative stress.

Our data seem to suggest that increased ketone levels may alleviate or even reverse some of the cardiometabolic impairments associated with the HFpEF phenotype on this rat model.

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