HEART WITH/OUT BORDERS

# CARDIOVASCULAR DEVELOPMENT, DISEASE AND REPAIR INTERNATIONAL CONFERENCE

ATMOSFERA M, PORTO | NOVEMBER 28-29 2014


WWW.HEART-WITHOUT-BORDERS.COM

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FACULTY OF MEDICINE, COIMBRA UNIVERSITY, COIMBRA

LUÍS VOUGA
UNIDADE DE CIRURGIA CARDÍACA DO CHVNG, VILA NOVA DE GAIA

NATÁLIA ANTÓNIO
FACULDADE DE MEDICINA, UNIVERSIDADE DE COIMBRA, COIMBRA

MARGARIDA DIOGO
IST, UNIVERSIDADE TÉCNICA DE LISBOA, LISBOA

PAULA ALVES
ITQB – INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA, UNIVERSIDADE NOVA DE LISBOA, LISBOA

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VASCO GAMA RIBEIRO
SERVIÇO DE CARDIOLOGIA DO CHVNG, VILA NOVA DE GAIA

# INDEX

# WORD OF WELCOME

It is our great pleasure to welcome you to the international conference "**HEART WITH]OUT[ BORDERS– cardiovascular development, disease & repair"**, that will be held in Porto on 28-29th November 2014. This event has been conceived to nurture and promote fundamental and translational cardiovascular biology, which is emerging in the Portuguese scientific community.

The first research teams addressing this area were only established in Portugal over the last six to seven years. "**HEART WITH]OUT[ BORDERS– cardiovascular development, disease & repair"** will bring together our leading researchers from all over the country and have them present and discuss their work with renowned foreign experts. Just as elsewhere worldwide, cardiovascular abnormalities, injury and disease have a major impact on Portuguese health. This event will improve our knowledge of this organ-system and allow us to contribute to developing effective regenerative/repair therapies to be translated into the clinic.

We are inviting you to join us in this event to promote insight and animated discussion in a way that will inspire our young and senior researchers, to discuss their experience and data amongst peers, to share and to learn from their counterparts in the international scientific arena. We are also open to the generous funding of well-established national and international scientific/R&D/business platforms and companies as well as any kind of support they can provide that will enable us to capture the spirit of this enterprise and keep more hearts beating as they should. We thank you all in advance.

This will be a gathering to promote direct contact amongst young and senior researchers in a trans-disciplinary perspective that will clearly reflect our attempt to conciliate the aspirations of three independent scientific societies: the Portuguese Society of Cardiology (SPC), the Portuguese Society of Developmental Biology (SPBD) and the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC) which are co-responsible for the organization of the event. To strengthen the attendance of young researchers, registration bursaries have generously been made available by the SPBD and the SPCE-TC. Noteworthy, owing to that "**HEART WITH]OUT[ BORDERS– cardiovascular development, disease & repair**" will be one the year's main scientific events organized by SPCE-TC, and under the expectation to reunite a majority of this society fellows, a considerable number of bursaries have been offered for SPCE-TC members.

Last and not the least, the project for an art exhibition, i.e. "**HeART Without Borders**", will also be launched on the last day of the conference with the exhibition of selected art-works expressly made for the occasion by three distinguished Portuguese artists. This is a starting momentum in which two pillars of culture, science and art, join efforts for mutual support embodying a movement that will grow over the next months with the reception of art-works for future exhibition and auction. The proceeds are to be shared between the participating artists and a fund for young scientist/s within the scientific scope of the conference - the heart. Therefore, we are looking forward a worldwide engagement of artists that want to become part of this project, conceiving and sharing their work pieces with the **HeART Without Borders**. The exhibition of the art works, to be held at late spring 2015, will strenght the cooperation between these two cultural spheres and can become a new space to share ideas and to build bridges with society.

*Heartiest* greetings from the Organization,

Perpétua Pinto-do-Ó (INEB, ICBAS – U. Porto and Institut Pasteur, Paris, France)
Adelino Leite-Moreira (CHSJ, FMUP – U. Porto)

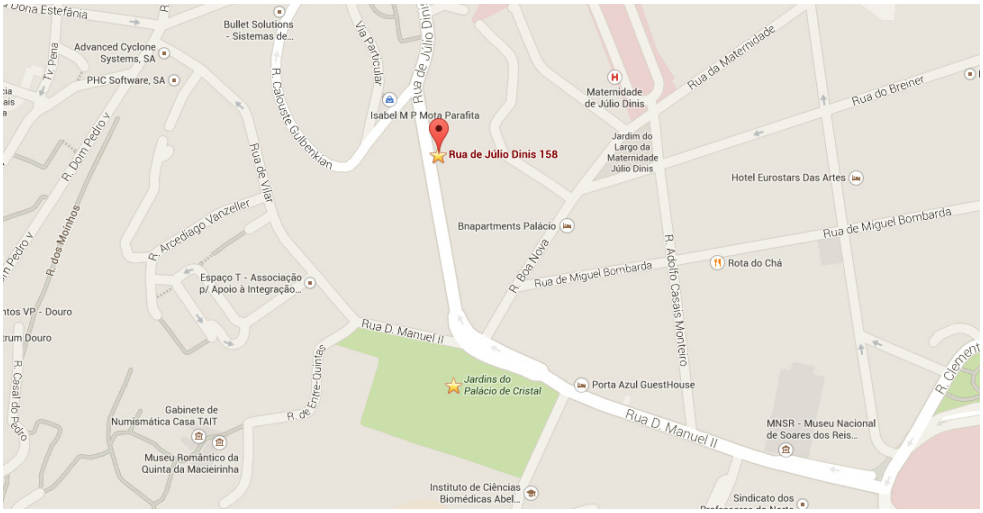
# VENUE

## MEETING VENUE

Atmosfera m

Rua Júlio Dinis, nº 158/160, 5th floor

4050-012 Porto

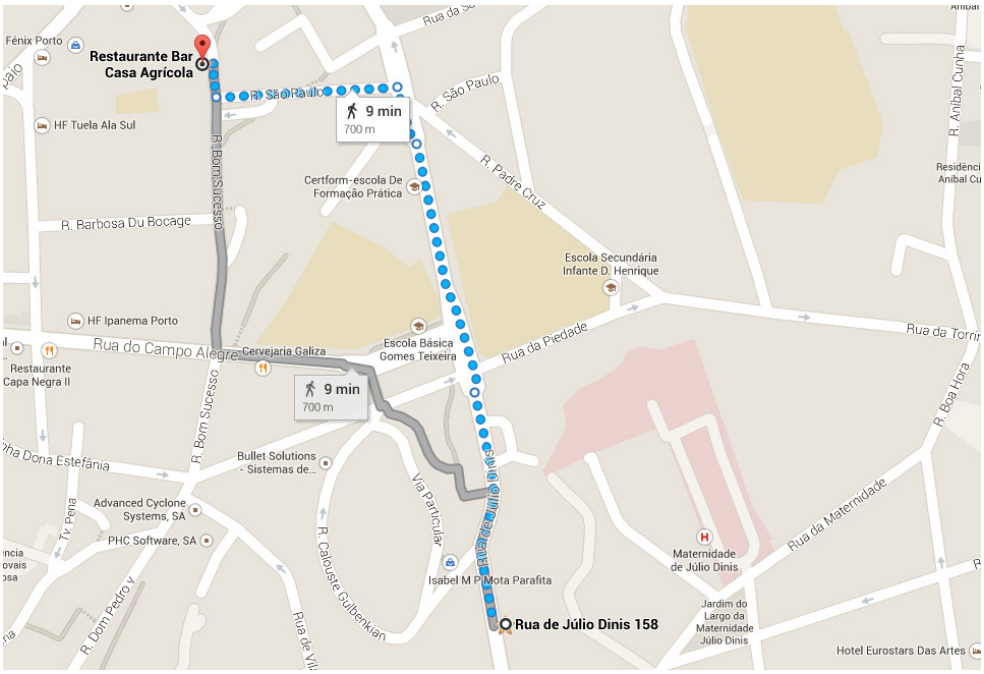



## CONFERENCE DINNER | NOVEMBER 28

Casa Agrícola

Rua do Bom Sucesso, 241

4150-150 Porto



# INFORMATIONS

## REGISTRATION DESK

The Registration desk will open at 13:00 on November 28 and at 8:30 on November 29, at the 5th floor.

## NAME BADGES

For identification and security purposes, participants must wear their name badges when in the venue. The use of the badge is mandatory for the access to the coffee breaks and lunch.

## PRESENTATION INSTRUCTIONS

The plenary lectures should last up to 30 minutes followed by 15 minutes discussion.

Oral presentation should last up to 20 minutes followed by a maximum of 10 minutes for discussion.

A data show and personal computer will be at the presenters' disposal. Technicians will be available to make sure that you have successfully uploaded your presentation. You will be requested to provide your presentation in a USB key.

## POSTER PRESENTATIONS

Posters should have 1.20m high for 0.90m wide, and will be presented on the designated poster areas, in the 5th and 6th floors. Conference staff will be present to provide assistance. Authors should remain next to their poster during their poster sessions.

Poster Session I – Odd number posters

Poster Session II – Even number posters

All posters must be placed on November 28, between 13:00-14:15 and removed on November 29 at 11:30 (after Poster Session II).

## MEALS AND COFFEE BREAKS

Coffee breaks on November 28 afternoon and November 29 morning will be served in the 5th and 6th floors. Coffee break on November 29 afternoon will be served in the 6th floor. Lunch on November 29th will be served in the 5th Floor.

## PARKING

Participants will have access to free parking at the following address Rua Júlio Dinis, number 290 , floor - 4 (next to Repsol's Gas Station). Please pay attention to the sign marking the access to atmosfera m building. Please use only the atmosfera m parking spots. From the car park, you must go to floor 0 and then catch another lift to floor 5.

# PROGRAMME

## FRIDAY, NOVEMBER 28

### OPENING OF THE CONFERENCE

13. 00 – 14. 00 Registration

14. 00 – 14. 15 Welcome session
Perpétua Pinto-do-Ó, Conference chair
Adelino Leite-Moreira, Co-chair

Lino Ferreira, SPCE-TC, President
J. Silva Cardoso, SPC, President
Sólveig Thorsteinsdóttir, SPBD, President

### 1. MAPPING HEART DEVELOPMENT AND DISEASE

**PLENARY LECTURE I**
*Chairs: José Belo, CEDOC & Tatiana P. Resende, INEB*

**14. 15 – 15. 00** **The developing heart**

Sigolène Meilhac, Institut Pasteur, Paris, France

**PLENARY LECTURE II**
*Chairs: Elisabete Martins, CHSJ & José Bragança, U. Algarve*

**15. 00 – 15. 45** **Molecular circuits controlling myocardial remodeling and regeneration**

Thomas Braun, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

**1545 – 16.15** **Will the heart recall "forgotten" paths?**

INEB – Instituto de Engenharia Biomédica; ICBAS, Universidade do Porto, Porto, Portugal and Institut Pasteur, Paris, France

16. 15 – 17. 15 Poster Session I and Coffee Break (sponsored by MILLAR, Inc.)

**PLENARY LECTURE III**
*Chairs: Lino Ferreira, CNC & Margarida Diogo, IST & Solveig Thorsteinsdotti, FC-UL*

**17. 15 – 18. 00** **Derivatives of Human Pluripotent Stem Cells : the new patient?**

Christine Mummery, Leiden University Medical Center, Leiden, The Netherlands

**18. 00 – 18. 30** **Driving the "heartistic" potential of pluripotent stem cells**

José Bragança, Universidade do Algarve, Faro, Portugal

18. 30 – 19. 00 **Signalling pathways in cardiogenesis: role of FHF and SHF derivatives**
José Belo, CEDOC, Universidade Nova Lisboa, Portugal

20. 00 – 23. 00 CONFERENCE DINNER

# SATURDAY, NOVEMBER 29

## 2. NEW AVENUES FOR IMPROVED HEART REPAIR

**PLENARY LECTURE IV**

*Chairs: A. Leite-Moreira, FMUP, CHSJ & Hélder Cruz, ECBio – R&D & Natália António, FMUC*

09. 00 – 09. 45 **MicroRNA function in cardiac disease**
Eva van Rooij, HUBRECHT Institute, Utrecht, The Netherlands

09. 45 – 10. 15 **New insights for acute myocardial infarction prognosis by circulating miRNAs**
Francisco Enguita, IMM, Universidade de Lisboa, Portugal

10. 15 – 11. 30 Poster Session II and Coffee Break (sponsored by ADInstruments – Europea)

11. 30 – 12. 00 **The role of Cx43 in mediating intercellular communication goes beyond gap junctions: exosomes enter the stage!**
Henrique Girão, IBILI, Universidade de Coimbra, Coimbra, Portugal

12. 00 – 12. 30 **Heart disease and current treatment modalities: a review for non-clinicians**
António Fiarresga, Hospital Santa Marta, Lisboa, Portugal

12. 30 – 13. 45 Lunch and Group Photo

**PLENARY LECTURE V**

*Chairs: Diana S. Nascimento, INEB & Henrique Girão, IBILI & Inês Falcão Pires, FMUP*

13. 45 – 14. 30 **The role of epicardium-derived cells in heart homeostasis and repair**
Jose M Pérez-Pomares, Andalusian Centre for Nanomedicine and Biotechnology, University of Málaga, Malaga & CNIC-Madrid, Spain

14. 30 – 15. 00 **Translational Research in Cardiovascular Sciences**
Adelino Leite-Moreira, FMUP-CHSJ, Porto, Portugal

15.00 – 15.30 **Stem cells therapy in Acute Myocardial Infarction: Benefits and challenges**

Lino Gonçalves, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

15.30 – 16.00 Coffee Break

**PLENARY LECTURE VI**

*Chairs: Cláudia Lobato, IST & F. Enguita, IMM*

**16.00 – 1645 The molecular mechanisms of cardiomyocyte proliferation**

Felix B. Engel, Pathologisches Institut, Erlangen University, Germany

1645 – 17.15 **Stem cell-based therapies for cardiac repair: integrating bioprocessing engineering and "omics" tools for better characterized cell products**

Paula Alves, ITQB, Oeiras, Lisboa, Portugal

17.15 – 1745 **Advanced therapies for the heart**

Lino Ferreira, Center of Neuroscience and Cell Biology, University of Coimbra, Portugal

## CONCLUDING REMARKS

1745 – 18.15 **Concluding remarks and best poster award (by the sponsoring entity)**

## OPENING OF THE EXHIBITION

**18.30 – 20.00 LAUNCHING OF THE ART WORK RECEPTION WITH EXHIBITION OF A FEW SPECIAL PIECES (KIND OFFER OF THREE DISTINGUISED PORTUGUESE ARTISTS) AND COCKTAIL**

# INVITED SPEAKERS

# INVITED SPEAKERS

## ADELINO LEITE-MOREIRA

FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO; CENTRO HOSPITALAR SÃO JOÃO, EPE, PORTO, PORTUGAL

Adelino Leite-Moreira is Full Professor of Physiology, Pathophysiology & Cardiothoracic Surgery at the Faculty of Medicine of the University of Porto, being also the Head of the Department of Physiology and Cardiothoracic Surgery and the Coordinator of the Cardiovascular R&D Center of the same institution. He has an appointment as Senior Cardiothoracic Surgeon at the affiliated University Hospital São João in Porto, Portugal. Besides these positions, he also accumulates different management duties at the University. He graduated as a medical doctor in 1989 at FMUP and trained as a research fellow from 1991 to 1994, at the Department of Physiology and Medicine of the University of Antwerp, where he developed his interest in the field of diastolic function and heart failure with preserved ejection fraction. It was in this field that he defended his PhD thesis in 1997. In 2003 he completed his clinical training as cardiothoracic surgeon at Hospital São João. He has special interest in surgical and technical innovation and holds particular expertise in the fields of reconstructive mitral and aortic valves surgery, aortic and aortic root surgery, less invasive surgery, as well as, in total arterial off-pump coronary artery bypass graft surgery.
Besides cardiac surgery, diastolic function and heart failure, his research interests also include right ventricular function and pulmonary hypertension. His double appointment in the fields of Physiology and Cardiothoracic Surgery has been crucial for the translational nature of his research, clearly facilitating the bench-to-bedside and back approach. Over the years, he authored or coauthored more than 200 full-papers in well-recognized international journals and was granted with more than three million € on competitive calls for research. He was also awarded with several major scientific prizes. His future endeavors are mainly focused on continuous promotion of scientific, technological and clinical innovation and excellence.

## ANTÓNIO FIARRESGA

HOSPITAL SANTA MARTA, LISBOA, PORTUGAL

António Fiarresga is an Interventional Cardiologist at Hospital de Santa Marta, CHLC, Lisbon. Is clinical work is centred on coronary and structural percutaneous interventions for the treatment of heart diseases. His group is mainly interested in Cell Therapy and has established a clinical research program focused on acute myocardial infarction patients, which resulted with the creation of the first Portuguese clinical trial in this field. He has also been dedicated to translational research and is currently working on the development of a large animal model for the study of the microcirculation effects of intracoronary delivery of stem cells.

# CHRISTINE MUMMERY

DEPT. ANATOMY AND EMBRYOLOGY, LEIDEN UNIVERSITY MEDICAL CENTRE, THE NETHERLANDS

Christine Mummery is Professor of Developmental Biology and Chair of the Department of Anatomy and Embryology. She trained in Biophysics at London University and after her PhD was Royal Society postdoctoral fellow at the Hubrecht Institute in Utrecht, where she later became staff member and group leader. She became Professor at the Interuniversity Cardiology of the Netherlands (ICIN) at the University Medical Centre Utrecht in 2002. In 2007, she was a joint Harvard Stem Cell Institute/ Radcliffe fellow at Harvard and Mas General Hospital at the time human induced pluripotent stem cells were being developed and was later the first to derive iPSC lines from patients in the Netherlands. Her primary research focus is currently the development and use of stem cells in cardiovascular development and disease. In 2008 she became Professor of Developmental Biology at the LUMC and Head of Department in 2009. She serves on the Ethical Councils of the Ministry of Health, providing specialized advice on human embryos and stem cell clinical trials. She is an elected member of the Royal Netherlands Academy of Arts and Sciences (KNAW), editor/ editorial board member of journals that include Stem Cell Research, Cell Stem Cell, Stem Cells, and elected board member of ISSCR and president of the International Society of Differentiation. In addition she is on the boards of the KNAW, ZonMW (Dutch Medical Research Council) and ICIN.

LINK: http://www.stemcellsportal.com/content/dr-christine-mummerys-lab

# EVA VAN ROOIJ

HUBRECHT INSTITUTE, UTRECHT, THE NETHERLANDS

Eva van Rooij attended University Hospital Maastricht in the Netherlands where she received a Ph.D. at the department of Cardiology. She then went on to complete postdoctoral training in Molecular Biology at UT Southwestern Medical Center in the lab of Dr. Eric Olson where she served as lead scientist in the studies that linked microRNAs to cardiovascular disease. Her work subsequently became the foundation of miRagen Therapeutics, Inc., a company focused on the development of microRNA therapeutics. In the last years Eva van Rooij served as miRagen's Senior Director of Biology and co-founder. During this time period she oversaw all the pre-clinical studies for the company's microRNA programs. In 2013 she started her own laboratory in the academia at the Hubrecht Institute (Utrecht) to further unveil the molecular signaling pathways that are relevant for cardiovascular biology.

Link: http://www.hubrecht.eu/research/vanrooij/

# FELIX B. ENGEL

UNIVERSITY HOSPITAL ERLANGEN (ACADEMIA), NEPHROPATHOLOGY, EXPERIMENTAL RENAL AND CARDIOVASCULAR RESEARCH, ERLANGEN, GERMANY

Felix Engel studied biotechnology at the Technical University in Berlin, Germany. During this time, he worked on the characterization of replication origins in Schizosaccharomyces pompe and developed during his diploma thesis, a selection system for ribozymes that are able to catalyze bi-molecular reactions. Afterwards, he became interested in regenerative medicine and joined the group of Rüdiger von Harsdorf at the Max Delbrueck Center for Molecular Medicine in Berlin (Feb 1997 – Oct 2001). During his PhD thesis he established a mammalian myocardial cell-free system demonstrating that the mammalian heart is, in principle, able to regenerate (Circ Res, 1999; Mol Cell Biol, 2003). Subsequently, he moved to Boston where he worked with Mark T. Keating (Harvard Medical School/Children's Hospital, Nov 2001 – Nov 2006) on the induction of dedifferentiation and proliferation of mammalian cardiomyocytes as a fundamental mechanism of heart regeneration (Genes Dev, 2005; PNAS 2006) leading to the upsetting of the scientific dogma that mature mammalian cardiomyocytes cannot divide. He became faculty member at Harvard Medical School and received for his work the Children's Hospital (Boston) Research Day Award, the Charles H. Hood Foundation Child Health Research Award and the prestigious Sofja Kovalevskaja Award. Following his return to Germany in Dec 2006 Felix Engel went on working on heart development (Development, 2011), cardiac regeneration (Cardiovasc Res, 2010), cardiac remodelling (Basic Res Cardiol, 2013) and tissue engineering (Biomaterials, 2012) at the Max-Planck-Institute for Heart and Lung Research (Academia), Bad Nauheim, Germany. Since Oct 2012 he is an Associate Professor at the University Hospital Erlangen (Academia) in the Department of Nephropathology where he has successfully established the group Experimental Renal and Cardiovascular Research (e.g. Coordinator of the Consortium "CYDER: Cell Cycle in Disease and Regeneration"; PNAS, 2013; FASEB J 2014) (Universitätsstrasse 22, 91056 Erlangen, Germany).

# FRANCISCO J. ENGUITA

INSTITUTO DE MEDICINA MOLECULAR, FACULDADE DE MEDICINA, UNIVERSIDADE DE LISBOA, LISBOA, PORTUGAL

Francisco Enguita is an assistant Professor and Principal Investigator at IMM. His lab is interested in non-coding RNAs and human disease, with special focus on the role on miRNAs and lincRNAs in aging and aging-related diseases, and the functions of non-coding RNAs in heart physiology. Formerly: 2009-2012 – Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal. 2001-2009 – Universidade NOVA de Lisboa, Lisbon, Portugal. 1995-1998 – Genetics and Microbiology, Department of Ecology, Universidad de León, Spain. Oct 1998 – PhD in Microbiology and Genetics at Universidad de Salamanca, Spain. 1991-1992 – Ms.C. in Microbiology, Universidad de Granada, Spain. 1986-Jun 1991 – Bs.C. in Pharmaceutical Sciences, Universidad de Granada, Spain.

LINK: http://fellowsnet.embo.org/your-profile/userprofile/fenguita.html

# HENRIQUE GIRÃO

INSTITUTE OF BIOMEDICAL IMAGING AND LIFE SCIENCES (IBILI), FACULTY OF MEDICINE, UNIVERSITY OF COIMBRA, PORTUGAL

Henrique Girão is the Coordinator of the group "Molecular Mechanisms of Disease" of the IBILI and Director of the Laboratory of Biostructural Imaging of the Faculty of Medicine – University of Coimbra. His broad scientific interest resides on the understanding of the mechanisms of ubiquitin-dependent lysosomal degradation of membrane proteins, either by endocytosis or autophagy, with a particular focus on the gap junction protein Cx43, H Girão has developed much of his recent work using the heart as a model-system, where gap junction intercelullar communication plays a vital role to ensure the correct and efficient electrical impulse propagation. Selected key words:Gap junctions, Ubiquitin, Connexin43, autophagy, endocytosis) He is also the Director of the Pole of the University of Coimbra – RNME. National Network of Electron Microscopy 2012-Present Member of Scientific Council of IBILI and a member of Directive Board of the PhD programme in Health Sciences Faculty of Medicine – University of Coimbra and a Member of Board of Directors of the Inter-University Doctoral Programme in Ageing and Chronic Diseases, a Member of the Coordination Board of the Master in Biomedical Research , Faculty of Medicine – University of Coimbra (2012- Present).Formerly: H. Girão graduated in biochemistry in 1995 from the Faculty of Sciences and Technology of University of Coimbra, Portugal and completed a Ph.D. in 2006 at the Faculty Medicine of University of Coimbra, Portugal. He moved to Institut de Biologia Molecular de Barcelona – CSIC in 2006 as a PostDoc Research fellow. In 2008 he assumed a position at IBILI, as a Research fellow under the Portuguese Program Ciencia 2007.Investigador Auxiliar Faculty of Medicine – University of Coimbra 2010.

LINK: http://www.uc.pt/en/fmuc/centrooftalmologia/Research/BiologyofAgeing/BEstaff/HenriqueGirao

# JOSÉ BELO

STEM CELLS AND DEVELOPMENT LABORATORY, CEDOC, NOVA MEDICAL SCHOOL / FACULDADE DE CIÊNCIAS MÉDICAS, UNIVERSIDADE NOVA DE LISBOA, PORTUGAL;
IBB-INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING, CENTRO DE BIOMEDICINA MOLECULAR E ESTRUTURAL, UNIVERSIDADE DO ALGARVE, FARO, PORTUGAL

José António Belo received is BSc. in Biology in 1993 and completed his PhD in 1998 under the supervision of Eddy M. De Robertis at UCLA, USA. From 1999 to 2006 he was a PI at Instituto Gulbenkian de Ciência, Oeiras, where he now holds an external Associated Group leader status. By 1999 he also joined University of Algarve as an Assistant, and later Associate Professor where he was teaching Developmental and Stem Cell Biology at the Department of Biomedical Sciences and Medicine where he implemented, and was the Scientific Coordinator, of the Regenerative Medicine Program and Director of the Masters in Biomedical Sciences, and a Principal Investigator of the Molecular Embryology Group at CBME/IBB. In November 2013 he joined the NOVA Medical School (FCM) at Universidade Nova Lisboa, where he is an Associate Professor, the Coordinator of the "Cellular and Molecular Medicine R&T Area" and a vice-director of CEDOC. He is also the Program Director (PD) of the recently established PhD program "Pro-RegeM – Regenerative Medicine and Mechanisms of Disease", an association between IBB/CBME at UAlg and CEDOC at FCM-UNL. In September 2013 we was appointed the PT Delegate for the Pillar I "Excellent Science" from H2020. His scientific interests and expertise lye in the field of Stem Cell and Developmental Biology, with a strong focus on early axis establishment, L-R asymmetry and cardiogenesis. He has secured funding for several research projects as a PI and has been involved in several others as a collaborator.

LINK: http://cedoc.unl.pt/stem-cells-and-development/

# JOSÉ BRAGANÇA

UNIVERSIDADE DO ALGARVE, FARO, PORTUGAL

José Bragança is a Principal Investigator and Assistant Professor (since October 2009) at the Department of Biomedical Sciences and Medicine, IBB-CBME, University of Algarve – Portugal. He is currently investigating genes and regulatory pathways that control pluripotent stem cells (ESC – Embryonic Stem Cells, and iPS – induced pluripotent cells) self-renewal and differentiation, in particular their early commitment to cardiac cell lineages. The long-term aim of his group work is to understand the molecular mechanisms involved in pluripotency and differentiation processes occurring in mouse and human cells in order to achieve cell-types specification in vitro for therapeutic purposes.
Formerly: Assistant Researcher (2008-2009) Animal Cell Technology laboratory, ITQB/IBET – Portugal and a Post-doctoral Research Fellow (1999-2007) Department of Cardiovascular Medicine, University of Oxford – UK. PhD in Biochemistry and Molecular Biology from the University of Paris XI – France (1998).

LINK: http://www.cbme.ualg.pt/jbraganca_cbme.html

# JOSE M PEREZ-POMARES

DEPARTMENT OF ANIMAL BIOLOGY, FACULTY OF SCIENCES, UNIVERSITY OF MÁLAGA, MÁLAGA, SPAIN; ANDALUSIAN CENTRE FOR NANOMEDICINE AND BIOTECNOLOGY (BIONAND), MÁLAGA, SPAIN

He graduated in Biology in 1995 (University of Malaga) and was enrolled in a PhD program that allowed him to visit different cardiovascular developmental biology labs in the USA and Germany. J.M. Pérez-Pomares defended his Ph.D. thesis on January 2000 (University of Malaga, Summa Cum Laude). Then, a post-doctoral training period of two years followed (Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC, USA). After being offered an Assistant Professor position at the University of Málaga, he returned to Spain. He was promoted to Associate Professor in 2010 and currently is PI on grants from the Spanish Ministry of Economy (National Programme for Basic Research & National Cooperative Research Network on Cell Therapy), the Andalusian Government and the EU Frammework Programme 7. J.M. Pérez-Pomares is author of more than 45 peer reviewed papers in the field of cardiac developmental biology (JCR h index=24) and a renowned expert in epicardial and coronary blood vessel biology. J.M. Pérez-Pomares is member of the Nucleus (Board) of the European Society of Cardiology Working Group on 'Development, Anatomy and Pathology', and lecturer in different Masters and PhD programmes in Málaga and Madrid ('Cell & Molecular Biology'; 'Evolutionary Biology'; 'Molecular Biomedicine'). J.M. Pérez-Pomares has just been awarded a Principal Investigator position at the Andalusian Centre for Nanomedicine and Biotechnology (BIONAND), and is an affiliate fellow ('Visiting Scientist') of the well renowned Fundación Centro Nacional de Investigaciones Cardiovasculares, CNIC (Madrid).

## LINO GONÇALVES

COIMBRA UNIVERSITY HOSPITAL CENTER & FACULTY OF MEDICINE UNIVERSITY OF COIMBRA

Lino Gonçalves is Head of the Department of Cardiology CHUC-HG, Coimbra University Hospital Center (Dec 2013 – Present) and an Associate Professor at the Faculty of Medicine, Coimbra University. Clinical Activities and Research Interests – Translational Research: Director of the Basic Research Unit, since 1996. His main research interests reside on angiogenesis, stem cells and the role of connexins in cardiovascular pathology. Clinical Research – Interventional cardiologist since 1992. Main clinical-research interests are coronary artery disease and interventional cardiology. He has also served on several scientific societies and professional organizations, e.g. Portuguese Society of Cardiology Officer: Vice-President of the Portuguese Society of Cardiology (2001 – 2005); (2011 – 2013); Board Member of the PCI Working Group of the Portuguese Society of Cardiology (2007-2010) and President of the XXXIII Portuguese Congress of Cardiology (2012) AND, Director of the National Center for the Collection of Data in Cardiology (2003 – 2006); (2011 – 1013).Among others, Lino Gonçalves is currently: Co-Director from the Harvard-Portugal Program (2011 – 2014); a Coimbra University Medical School's Scientific Council Member (2012 -); a Coimbra University Medical School's Advanced Studies Committee Member (2012 -), a Coimbra University Medical School's PhD Programme in Health Sciences Committee Member (2012 -) and Coordinator of the cardiovascular research strategic area of the Faculty of Medicine, University of Coimbra (2013 – Present).

## LINO FERREIRA

CENTER OF NEUROSCIENCE AND CELL BIOLOGY, UNIVERSITY OF COIMBRA, PORTUGAL

Lino Ferreira holds a Ph.D. in Biotechnology from the University of Coimbra (Portugal). He did postdoctoral work at MIT (USA) in the laboratory of Professor Robert Langer in the areas of human embryonic stem cells, micro- and nanotechnologies. He joined the Center of Neurosciences and Cell Biology (CNC, University of Coimbra) in October 2007. He has published more than 70 peer reviewed papers and has 18 issued or pending patents– 8 of which have been licensed to companies in the biomedical industry. He is the director of the Biomaterials and Stem Cell-Based Therapeutics research group and the CNC coordinator of the MIT-Portugal Program. In 2012 he was awarded with a prestigious European Research Council starting grant. The research group aims at generating fundamental and translational knowledge in the intersection of biomaterials with stem cells.

LINK: http://biomaterials.biocant.pt/wp/

## PAULA M. ALVES

ITQB-UNL/IBET, LISBOA, PORTUGAL

Paula Marques Alves is the CEO of IBET (2012-) and coordinates the Animal Cell Technology Unit of IBET/ITQB-New University of Lisbon (2007-). She is invited Associate Professor at the FCT-New University of Lisbon (2011-). She did her PhD in Biochemical Engineering in 2001 at ITQB. Since 1995, Paula Alves has focused her research on understanding cell metabolism to improve the efficiency of bioprocesses and at developing new tools and technologies for pre-clinical research. Through her career she has been using Animal Cell Technology for R&D in particular for: (i) production of complex biopharmaceuticals such as viral vectors, vaccines and recombinant proteins, (ii) development of 3D culture systems for toxicity testing – using primary cultures and stem cells – and for expansion and differentiation of stem cells in bioreactors for cell therapy applications and (iii) application of systems biology approaches to organize the complexity of the processes involved in the research described in (i) and (ii). In addition, Paula M Alves is currently Vice-President of the ESACT-European Society for Animal Cell Technology and member of the Horizon 2020 Advisory Group for Societal Challenge 1 – Health, Demographic Change and Wellbeing. She serves numerous scientific committees in international conferences and is reviewer for several international committees (BSRC/BRIC-UK, NSF-USA, Welcome Trust/India, ANR-France, ETMR-Scotland, A-Star, EU-FP7).

LINK: http://www.itqb.unl.pt/labs/animal-cell-technology

# PERPÉTUA PINTO-DO-Ó

INEB – INSTITUTO DE ENGENHARIA BIOMÉDICA; ICBAS, UNIVERSIDADE DO PORTO, PORTO, PORTUGAL AND INSTITUT PASTEUR, PARIS, FRANCE

Perpétua Pinto-do-Ó received her Diploma in Biology in 1992 and after a three years research period in Pharmacology (Faculty of Medicine, Porto University/Department for Molecular Medicine, Karolinska Institute, Stockholm), she was in enrolled (1995) in the Gulbenkian PhD Program in Biology and Medicine (Oeiras, Portugal). This was the opportunity to get in touch with the emergent field of stem cell biology in which she got her PhD at Umeå University, Sweden (1996 – 2002). From 2003 to 2006 Perpétua was a postdoctoral fellow at the Infection and Immunity Division at the IBMC (University of Porto). In December 2007 P. Pinto-do-Ó joined INEB (as a Research fellow under the Portuguese Program Ciência 2007) where she currently coordinates the Stem-cell Microenvironments in Repair/Regeneration Team. Her investigation addresses the mechanistic comprehension of how stem/progenitor cells contribute and/or may be directed to repair/regenerate the adult organism. The Team has so far been focused on identifying mechanisms/signals towards the conception of more efficient strategies for restoring function of the failing heart. A comprehensive approach which includes the investigation (i) of the changing ECM and that (ii) of the dynamics of proliferating cellular populations in the cardiac microenvironment in regenerative (embryonic and neonatal heart) vs. repair (adult heart injury models) conditions has been implemented. Furthermore, expertise in several developmental biology models, e.g. chick, mouse and the in vitro embryonic stem cells (ESC) system, is presently enabling the exploration of molecular mechanisms at the crossroads of hematopoietic-vascular and cardiac specification. The final aim is to isolate signals amenable to reshape cell-fate/tissue microenvironment in at least three close, yet distinct, organ-systems. Since 2012, Pinto-do-Ó is also an Adjunct Professor at Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto.

# SIGOLÈNE MEILHAC

INSTITUT PASTEUR, DEPARTMENT OF DEVELOPMENTAL AND STEM CELL BIOLOGY, 75015 PARIS, FRANCE; CNRS URA2578, 75015 PARIS, FRANCE

Sigolène Meilhac attented the Ecole Normale Supérieure of Paris and received a Ph.D. in the lab of Pr M. Buckingham at the Institut Pasteur (Paris) where she demonstrated the early segregation of the first and second myocardial lineages by retrospective clonal analysis. She then went on to complete postdoctoral training at the Gurdon Institute (Cambridge, UK) on the lineage of the extra-embryonic endoderm. In 2009, she set up an interdisciplinary team to study the mechanisms underlying the polarity and growth of the myocardium during heart morphogenesis. She is now junior group leader in the Department of Developmental and Stem Cell Biology of the Institut Pasteur.

LINK: http://www.pasteur.fr/en/research/developmental-and-stem-cells-biology/units-groups

# THOMAS BRAUN

THOMAS BRAUN, MAX-PLANCK-INSTITUTE FOR HEART AND LUNG RESEARCH, BAD NAUHEIM, GERMANY

Thomas Braun is Director at the Max-Planck-Institute for Heart and Lung Reseach, Bad Nauheim, Germany and Professor of Medicine at the Justus-Liebig-University Giessen, Germany. He studied medicine and philosophy at the Universities of Göttingen and Hamburg, where he obtained his MD and MD PhD. After postdoctoral training in Hamburg and in Boston in the lab of Rudolf Jaenisch at the Whitehead Institute, MIT he became group leader at the Technical University of Braunschweig in 1992 before he moved on to an associate professor position at the University of Würzburg in 1996. Work at this time period focused mainly on the analysis of crucial transcription factors determining the fate of muscle cells. After that he was appointed full professor and chair of Physiological Chemistry at the University of Halle-Wittenberg. In 2004 he was recruited by the Max-Planck-Society as founding director of the newly established Max-Planck-Institute for Heart and Lung Research in Bad Nauheim. Since 2004 he is also Professor of Medicine at the University of Giessen, Germany. His primary research currently focuses on the mechanisms driving skeletal and cardiac muscle development, regeneration and remodeling. He serves on various committees and advisory boards in Germany and abroad. He is an elected member of the German National Academy of Science, Leopoldina and the Academy of Europe and is editorial board member of several journals. Furthermore he is in the steering board of several national and international research consortia and spokesperson of the International Max-Planck-School for Heart and Lung Research. In 2014 he was elected president of the German Stem Cell Network.

LINK: http://www.mpi-hlr.de/index.php?id=16&L=1

# ORAL COMMUNICATIONS

ORAL COMMUNICATIONS

# The developing heart

SIGOLÈNE M. MEILHAC[1, 2]

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In the heart, the shape and architecture of the cardiac chambers are critical for its contractile function. How this is established during development remains poorly understood. A question is to understand how different aspects of myocardial cell behaviour, such as cell polarity, proliferation and growth, are coordinated to shape the heart. We used clonal analysis to monitor the growth properties of the mouse myocardium. This indicated that growth of the myocardium is exponential and that myocardial growth is not only regulated in terms of rate but also in terms of direction. We found that oriented growth of the myocardium correlates with the expansion of cardiac chambers at embryonic stages. We developed interdisciplinary tools to show in 3D that, orientation of cell divisions underlies oriented tissue growth, such that cell divisions are locally coordinated. The short range of cell alignments points to a role of cell interactions in coordinating myocardial cells. Fat cadherins, which are involved in heterophilic cell adhesion, are good candidates to mediate local cell coordination. In the fly, fat regulates tissue polarity, as well as tissue growth via the Hippo pathway. We have investigated the role of Fat4 in the heart, using knockout mice and primary cultures of cells. We show that Fat4 is dispensable for the oriented growth of the embryonic myocardium but is required to restrict growth rate at birth. Such knowledge of the developmental mechanisms of heart growth opens perspectives for developing therapeutic strategies of heart repair.

# Molecular circuits controlling myocardial remodeling and regeneration

THOMAS BRAUN

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Dedifferentiation is a common phenomenon among plants but has only been found rarely in vertebrates where it is mostly associated with regenerative responses such as formation of blastemae in amphibians to initiate replacement of lost body parts. Relatively little attention has been paid to dedifferentiation processes in mammals although a decline of differentiated functions and acquisition of immature, “embryonic” properties is seen in various disease processes. Dedifferentiation of parenchymal cells in mammals might serve multiple purposes including generation of progenitor-like cells and protection of cells from hypoxia by reduction of ATP consumption. We found that heart regeneration in newts, which is accompanied by extensive cardiomyocyte dedifferentiation and proliferation, depends on infiltration with macrophages. Organ culture of damaged newt hearts before but not after macrophage infiltration prevented heart regeneration. Similarly, ablation of macrophages in vivo inhibited cardiomyocyte proliferation.

In mammals, we discovered that an inflammatory cytokine of the interleukin 6 family, oncostatin M (OSM), initiates dedifferentiation of cardiomyocytes both *in vitro* and *in vivo*. Activation of the OSM signaling pathway promoted survival of cardiomoyocytes under hypoxia and protected the heart from acute myocardial ischemia probably due to induction of an immature metabolic phenotype. In addition, we found that OSM-dependent dedifferentiation of cardiomyocytes initiates release of multiple cytokines. Using a mass spectrometry approach we identified several new cytokines required for efficient homing of macrophages to the damaged myocardium. Genetic inactivation of cytokines induced by OSM prevented accumulation of macrophages in the damaged myocardium, blocked myocardial healing and scar formation leading to impaired cardiac function and heart rupture.

# Will the heart recall “forgotten" paths?

PERPÉTUA PINTO-DO-Ó

INEB – Instituto de Engenharia Biomédica; ICBAS, Universidade do Porto, Porto, Portugal and Institut Pasteur, Paris, France

# Derivatives of Human Pluripotent Stem Cells: the new patient?

CHRISTINE MUMMERY

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Derivation of many different cell types from human pluripotent stem cells (embryonic stem cells or HESCs and induced pluripotent stem cells or hiPS cells) is an area of growing interest both for potential cell therapy and as a platform for drug discovery and toxicity. Most particularly, the recent availability of methods to introduce specific disease mutations into human pluripotent stem cells and/or to derive these cells as hiPS cells by reprogramming from any patient of choice, are creating unprecedented opportunities to create disease models " in a dish" and study ways to treat it or slow down its rate of development. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to their derivatives and mimicking these in defined culture conditions *in vitro* is now essential for moving the field forward. We have used these methods to produce cardiomyocytes and vascular endothelial cells from diseased hESC- and hiPSC and have examined drug responses of hESC-derived cardiomyocytes to a variety of cardiac and non-cardiac drugs and an hiPSC model for vascular disease. In addition, we show that iPSC derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties observed in primary cardiomyocytes despite being relatively immature. Examples will be shown of how vascular diseases are beginning to be modelled in similar ways, stepping towards therapies for aspects of these complex conditions based on treating stem cells and their derivatives as " patients".

# Driving the “heartistic” potential of pluripotent stem cells

JOSÉ BRAGANÇA

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The transcriptional regulator Cited2 is essential for normal mouse heart development and variations/mutations of the human CITED2 gene sequence are associated with human congenital heart disease. In mouse embryonic stem cells (ESC), the acute depletion of Cited2 reduces the self-renewal, proliferation and survival capacity of these cells, whereas Cited2 overexpression sustains pluripotency and proliferation. Intriguingly, a minute population of Cited2-null mouse ESC has been characterized and showed to be able to self-renew in the presence of leukaemia inhibitory factor, but displayed an impairment for several differentiation processes, including cardiac cell commitment. We have now studied the consequences of Cited2 acute depletion or overexpression in ESC differentiation towards cardiac cell lineages, and determined that Cited2 is necessary for early cardiogenic commitment of ESC, and it enhances cardiac progenitor cells specification and cardiomyocyte terminal differentiation.

# Signalling pathways in cardiogenesis: role of FHF and SHF derivatives

JOSÉ ANTÓNIO BELO

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The making of a heart results from the interplay of a serious players, namely, molecules, tissues and morphogenetic movements. Recent studies indicate that in mice, the heart starts to be formed at gastrulation with the formation of the heart primordium or first heart field (FHF) that will mainly give rise to the left ventricle (LV). Later on in development, another region can be identified, the secondary heart field (SHF) that will mainly contribute to the right ventricle (RV) and outflow tract (OFT) [7]. The heart primordium region fuses at the midline of the embryo to form a primitive heart tube. At this early phase, the heart loops to the right side of the embryo under the control of the signals that regulate the left–right axis (L/R).

*Cerl2* knockout (*Cerl2-/-*) mice display L/R axis randomized and a significant mortality rate within a few hours after birth, mostly due to cardiac defects.

We uncovered that the increased left ventricular muscle observed in *Cerl2-/-* mice is caused by a high cardiomyocyte mitotic index in the compact myocardium which is mainly associated with increased *Ccnd1* expression levels in the left ventricle at E13.5. Importantly, we observed an increase of phosphorylated Smad2 (pSmad2) levels in embryonic (E13) and neonatal hearts indicating a prolonged TGFßs/Nodal-signaling activation.

In mice, *Ccbe1* is expressed in cardiac progenitor cells at the level of the SHF and later also in the pericardium lining the heart

According to our recent observations, *Ccbe1* loss-of-function in mice leads to a heart phenotype that can be correlated with this earlier SHF expression.

The comparative studies of such molecules will help to shed light in the complex process of hear formation involving both SHF and SHF lineage descendents, having important implications for the study and understanding of human cardiac genetic disorders.

# MicroRNA function in cardiac disease

EVA VAN ROOIJ

Hubrecht Institute, the Netherlands

Chronic and acute stress to the heart results in a pathological remodeling response accompanied by hypertrophy, fibrosis, myocyte apoptosis and eventual death from pump failure and arrhythmias. We have identified signature expression patterns of microRNAs associated with different forms of heart disease. Gain- and loss-of-function studies have revealed profound and unexpected functions for these microRNAs in numerous facets of cardiac biology, providing glimpses of new regulatory mechanisms and potential therapeutic targets for heart disease. Disease-inducing cardiac microRNAs can be persistently regulated in vivo through systemic delivery of anti-miRs. The therapeutic opportunities for manipulating microRNA biology in the setting of heart disease will be discussed.

# New insights for acute myocardial infarction prognosis by circulating miRNAs

FRANCISCO J. ENGUITA

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Despite of the great improvements in the diagnostic and therapeutic fields, morbidity and mortality in patients with acute myocardial infarction remains very high. Risk stratification of patients with coronary artery disease has been the subject of intense research in recent years. microRNAs are involved in cardiac development as well as in several pathological cardiac conditions. In the last few years they have been associated with several heart disorders, including coronary heart disease. The recent possibility to quantify them in the peripheral blood paved the way to study their diagnostic and prognostic value.

Recent studies showed that miR-122, a typically hepatic miRNA, increases in the bloodstream during ischemic cardiogenic shock and is up-regulated in the infarcted myocardium. We aimed to determine the potential of circulating miR-122 and its out-of-proportion increase (as defined by the ratio between miR-122 and cardiac-enriched miRNAs) to serve as biomarkers for early prognostic stratification of ST-segment elevation acute myocardial infarction (STEMI) patients.

A prospective observational study of STEMI patients submitted to primary angioplasty with the determination of serum levels of several cardiac-related miRNAs was performed. Kaplan Meier and multivariate Cox regression survival analysis were used to evaluate their association with clinical outcome evaluated by three prognostic end points: all-cause mortality, death or myocardial infarction and any adverse cardiovascular event. Our results indicated that in a population of 142 STEMI patients (75% male, 62 ± 12 years), 9 patients died, 6 had reinfarction and 26 patients suffered an adverse cardiovascular event during a median follow-up of 20.8 months. Event-free survival was significantly worse in patients with high miR-122/133b ratio with almost nine times higher risk of death or myocardial infarction and four times higher risk of adverse cardiovascular events. In addition, miR-122/133b ratio and left ventricular ejection fraction were the only independent prognostic predictors in multivariate Cox regression analyses. In consequence, the out-of-proportion increase of circulating miR-122 as reflected by a high miR-122/133b ratio may be useful for the early identification of patients at risk of adverse prognosis after STEMI.

# The role of Cx43 in mediating intercellular communication goes beyond gap junctions: exosomes enter the stage!

ANA SOARES, TANIA MARQUES, TERESA RODRIGUES, JOAO FERREIRA, MONICA ZUZARTE, SANDRA I. ANJO, BRUNO MANADAS, PAULO PEREIRA, HENRIQUE GIRAO[1]

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A correct and efficient communication between the different types of cells that constitute the heart is essential to provide structure, ensure regulated heart contraction, and, ultimately, efficient blood pump. Intercellular communication between heart cells can occur either directly, via gap junctions (GJ), between adjacent cells, or over long distances through extracellular vesicles. For example, GJ intercellular communication between cardiomyocytes, mediated by connexins (Cx)-containing channels, is vital to ensure the correct and rapid anisotropic electrical impulse propagation, required for a synchronized and coordinated heart beating. Besides direct cell-cell communication, cells can exchange information through extracellular vesicles (EV), released under basal or stress conditions. According to their size, composition and subcellular origin, EVs can be divided in apoptotic bodies, microvesicles and exosomes. Exosomes, that include vesicles with a diameter of 50-130 nm, are formed when multivesicular bodies fuse with the plasma membrane and release their vesicular content into extracellular medium, including cell culture supernatants and body fluids. For these reasons, exosomes have been considered as intercellular messengers that mediate both local and systemic cell communication, through the transference of biological information between donor and acceptor cells. Although it is well established that exosomes can modulate the response of acceptor cells, the mechanisms that govern exosomal uptake are not fully understood and constitute a matter of intense research. Up until now, evidences support a model where exosomal uptake into cells depends on protein interaction between exosomes and target cells. In this study, we hypothesized that Cx43 constitutes a new molecular partner and mediator of the interaction of exosomes with the plasma membrane of cells and, in analogy with cells, Cx43 mediates the communication between exosomes and the cells. Altogether our results, using various complementary approaches, including Westernblot, immunofluorescence microscopy, immunogold labelling and transmission electro microscopy, mass spectroscopy and luciferin-luciferase luminescence assays, demonstrate that Cx43 is present in exosomes released by cardiomyocytes, in the form of a hexameric channel and, more importantly, that Cx43 in exosomes is able to modulate exosomal interaction and/or communication with the acceptor cells. In conclusion, our data ascribes a novel unanticipated biological role to Cx43 in mediating the transference of information between exosomes and acceptor cell.

# Heart disease and current treatment modalities: a review for non-clinicians

ANTÓNIO FIARRESGA

Hospital Santa Marta, Lisboa, Portugal

Translating biotechnology developments to safe, effective and affordable new treatments is not an easy task. The magnitude and complexity of this field do not fit in the scope of any singular area and a multidisciplinary and transversal approach is needed. Clinical and non-clinical researchers must join their efforts, and should use a common language and be in harmony with their different difficulties, capacities and responsibilities. This presentation will focus on important concepts of heart diseases, current treatment modalities, the process of clinical research and unmet clinical needs.

Ventricular remodelling is the key pathophysiological feature of heart disease for which cell therapy could be an expected solution. Acute myocardial infarction, chronic ischemic cardiomyopathy and non-ischemic dilated cardiomyopathy, are the main clinical entities related to the loss of viable myocardial tissue and cardiac function impairment.

Current therapies are particularly focused in limiting myocardial infarct size, ischemia or injury, improving symptoms, modulating some known neuro-hormonal responses and in preventing sudden death.

Practicing-change clinical trials are the ones showing improvements in hard clinical endpoints over the current standard of care. However, smaller studies usually do not rely solely in clinical endpoints but instead they look for changes in measurable biological variables related to the disease process - the surrogate endpoints. The most used surrogate endpoints in cardiac research are left ventricular dimensions and left ventricular ejection fraction, assessed by different imaging modalities. Several others non-imaging surrogate endpoints are being useful in cell therapy clinical trials.

Cardiovascular disease is still the most common cause of death and a major cause of disability, lost productivity and increased health costs worldwide.

Current treatment strategies allow for the survival of many patients, although is not possible to preserve or restore heart function in a significant proportion of them. Stem cell-based therapy opens exciting new possibilities, and hopefully will provide for breakthrough clinical solutions. Myocardial regeneration is best fitted to the biomechanical model of heart failure and can allow of further improvements in morbidity and survival associated with heart disease.

# The role of epicardium-derived cells in heart homeostasis and repair

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Myocardial infarction (MI) is a prevalent cardiovascular disease affecting millions of people worldwide. While increasing efforts are being made to find new therapies to regenerate the infarcted heart, our knowledge of the cellular and molecular physiological mechanisms that repair the injured myocardium remains poor. This is paradoxical, because any experimental cell-based treatment of the injured heart will eventually interact with spontaneous cardiac reparative phenomena. Therefore, understanding such events is necessary to critically analyze the results of clinical trials using the infusion of cells to fix the damaged heart.

Mechanisms of repair in the post-infarcted heart include a progressive fibrosis that severely affects cardiac performance, eventually leading to cardiac failure. Fibrotic progression is, however, a common cardiac response to different pathologic stimuli, and can occur in the absence of the sustained ischemia that characterizes MI. Recent reports suggest that cardiac fibrosis, which is triggered by the activation of cardiac fibroblasts, differs between distinct heart diseases.

In this work we have genetically traced the cellular components of the cardiac interstitium from the embryonic to the adult life, and tested the cell lineage-specific contribution to cardiac fibrosis in a MI animal model. Our results show that Wilms tumor suppressor (Wt1) positive epicardial-derived mesenchymal cells pioneer the formation of the cardiac interstitium along embryogenesis, followed by the peri- and post-natal incorporation of bone-marrow derived cells (including hematopoietic progenitors and stem cells). Following the activation of an acute inflammatory response, adult epicardial-derived cells robustly differentiate into cardiac fibroblasts, and through the sustained interaction with bone-marrow derivatives, become the predominant post-infarction fibroblast type in the ischemic heart. Interestingly enough, epicardial-derived cells contribution to fibrosis in a pressure overload experimental model is scarce. We conclude that epicardial interstitial derivatives in the adult heart are the main origin of cardiac fibroblasts in the infarcted heart. Moreover, epicardial-derived interstitial cells display a stromal function respect to recruited bone marrow-cells after MI. Finally, we suggest that pro-fibrotic cell lineage-specific responses to heart damage are time- and disease-dependent.

Our results are expected to increase our understanding of MI, and promote the development of new diagnostic and therapeutic pharmacological and/or cellular tools targeting CFs during pathologic cardiac fibrosis, helping to improve the treatment of MI.

# Translational Research in Cardiovascular Sciences

ADELINO LEITE-MOREIRA

Faculdade de Medicina da Universidade do Porto; Centro Hospitalar São João, EPE

Translational research/medicine represents a discipline that increases the efficiency of determining the relevance of new discoveries in the biological sciences to human diseases and helps clinical researchers to identify through direct human observation, alternative hypotheses relevant to human disease. Ultimately, translational research aims to accelerate the rational transfer of new insights and knowledge into clinical practice for improving patients' outcomes and public health.

Translational research can be subdivided in type 1 and type 2. Type 1 (T1: bench-to-bedside) refers to effective translation of the new knowledge, mechanisms, and techniques generated by advances in basic science research into new approaches for prevention, diagnosis, and treatment of disease. Type 2 (T2: translating research into practice) aims to ensure that new treatments and research knowledge actually reach the patients or populations for whom they are intended and are implemented correctly.

In the talk these topics will be further explored and practical examples of translational research in the field of Cardiovascular Medicine and Cardiothoracic Surgery based in the activity and professional experience of the presenter will be given.

# Stem cells therapy in Acute Myocardial Infarction: Benefits and challenges

LINO GONÇALVES

Coimbra University Hospital Center & Faculty of Medicine University of Coimbra

Major advances in the treatment of patients with acute myocardial infarction were achieved in the last decades. This improvement was associated with a significant reduction of in-hospital mortality. However, after this acute episode many of these patients will still develop chronic heart failure due to the loss of viable myocardium and its replacement by a non-contractile scar. Therefore, new techniques to repopulate these areas with viable contractile myocardial cells are needed. Although innate myocardial regeneration was shown to exist in animals and in humans, unfortunately, it is not enough to induce a clinically relevant recovery of the left ventricular function. There are several studies that tried to improve the efficiency of this myocardial regeneration, but they face many challenges and difficulties. The benefits, challenges and difficulties of these regeneration therapies after acute myocardial infarction will be discussed during this lecture.

# Stem cell-based therapies for cardiac repair: integrating bioprocessing engineering and "omics" tools for better characterized cell products

PAULA MARQUES ALVES

ITQB-UNL/iBET, Lisboa, Portugal

Stem cell (SC) transplantation has emerged as an exciting treatment option for patients with heart failure and acute myocardial infarction. Today, strong business opportunities exist for companies looking to pursue SC-based therapies; within the next years upcoming phase 3 studies will provide definitive answers of whether such therapies truly show potency in the clinic [1]. The challenges of developing cell therapies are manifold including the lack of expertise in product development and characterization as well as specialized cell manufacturing which are imperative to bring SC-based products closer to the clinic [2]. Within this context, our work has been focused on production and characterization of challenging SC-based products for application in both autologous and allogeneic cell therapies: i) cardiomyocytes (CM) derived from induced pluripotent SC (iPSC), which are capable to regenerate myocardium in infarcted hearts [3], and adult SCs, namely human mesenchymal and cardiac SC (hMSC and hCSC, respectively), which trigger paracrine mechanisms that activate endogenous SC to promote regeneration [4]. For this purpose, a systematic approach was developed using robust methodologies for both up- and down-stream bioprocessing and high-throughput proteomic and metabolomic tools for product characterization and process optimization.

Our strategy for iPSC-derived CM production consisted in designing an integrated bioprocess by combining expansion, differentiation and cell lineage purification steps in environmentally controlled bioreactors (stirred tank and Wave bioreactors) operating in perfusion. By optimizing different bioprocessing parameters we were able to improve by 1000-fold the cardiomyocyte differentiation yields (up to 60 CMs/input iPSC) and obtain a highly pure population of CM. Cell expansion, differentiation and purification processes were monitored along culture time using fluorescence microscopy, flow cytometry and qRT-PCR analysis. Metabolome and fluxome analysis is being applied along the differentiation process to disclose which metabolic pathways are differentially activated and/or repressed in low versus high yielding bioprocess conditions. Cell characterization and functional analysis confirmed that iPSC-derived CM presented a typical cardiac morphology, high deposition of extracellular matrix such as collagen type I, reproducible electrophysiological profiles and drug responsiveness.

Regarding hMSC and hCSC biomanufacturing, we were able to implement an efficient protocol for cell cultivation using microcarrier-based stirred culture systems. For downstream bioprocessing, our strategy was focused in combining membrane technology and novel chromatographic tools in a robust and scalable manner while compliant with Good Manufacturing Practices. Cell characterization assays showed that hMSC and hCSC retained their identity, differentiation and proliferation capacity as well as potency throughout the entire bioprocess. State-of-the-art mass spectrometry tools (nanoLC-MS) have been applied to obtain a comprehensive characterization of hMSC and hCSC secretome and receptome. hCSC Receptome analysis rendered the identification of numerous plasma membrane proteins and several cell surface markers (e.g. myoferlin), including more than 100 plasma membrane receptors (e.g. epidermal growth factor receptor, frizzled family receptor 6, etc), overall more than 2000 proteins were identified,

including different proteins allied to cardiac function. Secretome data are still under evaluation. Up to now about 500 proteins were identified, and although we are working with highly complex samples, with a broad dynamic range of protein concentrations, we were able to confidently identify the low abundance cytokine IL-8.

The cell manufacturing platforms developed herein, along with the robust proteomic and metabolomic tools implemented for product characterization, provide important insights to streamline the design of novel cell-based therapies for cardiac repair.

REFERENCES:
[1] Sheridan C 2013, Nat Biotechnol 31 (1): 5;
[2] Serra M et al., 2012, Trends in Biotechnol, 30: 350;
[3] Carpenter L et al., Stem Cells Dev 2012, 21:977; [4] Torella D, et al., 2007, Cell Mol Life Sci, 64: 661

# Advanced therapies for the heart

LINO FERREIRA

Center of Neuroscience and Cell Biology, University of Coimbra, Portugal

My presentation will focus in the integration of biomaterials and cell-based therapies for cardiovascular applications. Biodegradable elastomers have emerged as promising materials for their potential to mimic the viscoelastic properties of cardiac tissue and exhibit compliance without damaging the tissue. The synergistic therapeutic effect of biochemical and biophysical cues has not yet been explored using degradable materials given the absence of materials that can simultaneously deliver bioactive cues and maintain mechanical integrity in a dynamic environment such as the beating heart. During my talk I will present a novel biocompatible and mechanically tunable elastomer, poly(glycerol sebacate urethane), suitable for efficient encapsulation and controlled delivery of bioactive macromolecules and with the potential to be applied to cardiac drug delivery. I will also show that this elastomer can be used as cardiac tissue adhesive that can be activated by UV light within few seconds. The tissue adhesive has strong adhesion to cardiac tissue and is not compromised by exposure to blood. This polymeric adhesive provides a hemostatic seal when applied to cardiac wall defects. In the second part of my talk, I will present data regarding the use of advanced scaffolds to preserve the functionality of cardiac cells for the generation of tissue engineered cardiac tissue for drug screening/toxicology assessment. The development of tissue engineered cardiac tissue for drug screening requires the development of scaffolds that can be easily produced and miniaturize, flexible, and preserve the long-term contractility of cardiomyocytes, ideally in the absence of electrical stimulation. Recently, we have developed a scaffold that reproduces aspects of cardiac ECM, is relatively easy to prepare, flexible, and can preserve the contractility of rat fetal cardiomyocytes for high-throughput drug screening applications.

# POSTERS

# POSTERS LIST

01 Early changes in cardiac structure and function induced by a high caloric diet

N. GONÇALVES, E. CORREIA, A. F. SILVA, C. M. MOURA, R. RONCON-ALBUQUERQUE, I. FALCÃO-PIRES, A.F. LEITE-MOREIRA

02 StemCellNet: an interactive platform for network-oriented investigations in stem cell biology

J. P. PINTO, R.K.R. KALATHUR, R.S.R. MACHADO, J.M. XAVIER, J. BRAGANÇA, M.E FUTSCHIK

03 $VEGF_{165}$-bound beads modulate endothelial cell survival and miRNA expression

S. ADAY, M. BESNIER, J. ZOLDAN, A. G. SWENNES, L. CARRETO, J. SAIF, C. EMANUELI, R. LANGER, L. FERREIRA

04 Lysophosphatidic acid enhances survival of human $CD34^+$ cells in ischemic conditions mainly through peroxisome proliferator-activated receptor γ

IVANA KOSTIC, ISABEL FIDALGO-CARVALHO, SEZIN ADAY, HELENA VAZÃO, TIAGO CARVALHEIRO, MÁRIO GRÃOS, ANTÓNIO DUARTE, LINO GONÇALVES, LINA CARVALHO, ARTUR PAIVA, LINO FERREIRA

05 High-content functional screening identifies microRNAs inducing cardiac regeneration

M. MANO, A. EULALIO, M. DAL FERRO, L. ZENTILIN, S. ZACCHIGNA, M. GIACCA

06 Flexible nanofilms coated with aligned piezoelectric microfibers preserve the long-term contractility of cardiomyocytes

GOUVEIA PJ , ROSA S, RICOTTI L, CARVALHO R, MENCIASSI A, NEVES RP, FERREIRA LS

07 Cardiomyocyte dynamics throughout ontogeny

T.P. RESENDE , M. VALENTE, S. MIRANDA, P. PINTO-DO-Ó

08 Role of *Ccbe1* during cardiovascular development in mice

PAULO N. G. PEREIRA, ANA R. PERESTRELO , JOSÉ A. BELO

18 Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model

D. FONTOURA, S. LEITE, A. P. LOURENÇO, I. FALCÃO-PIRES, N. HAMDANI, W. A. LINKE, W. J. PAULUS, A. F. LEITE-MOREIRA

19 Adenosine $A_{2B}$ positive inflammatory infiltrates contribute to right ventricular (RV) failure in rats with monocrotaline-induced pulmonary arterial hypertension (PAH)

B. BRAGANÇA, T. RODRIGUES, F. FERREIRINHA, P. CORREIA-DE-SÁ, A. P. FONTES-SOUSA

20 Measurement of plasma adenosine as a putative biomarker of canine heart failure

M. CORREIA-DE-SÁ, T. MAGALHÃES-CARDOSO, L. LOBO, B. PEDRO, M. FARIA, P. CORREIA-DE-SÁ, A. P. FONTES-SOUSA

21 Reverse remodelling in wistar rats after surgical removal of chronic pressure-overload

D. MIRANDA-SILVA, P. GONCALVES RODRIGUES, C. MOURA, A. GONZALEZ, J. DIEZ, A F. LEITE-MOREIRA, I. FALCAO-PIRES

22 A Role for Neuro-Induced Cardiac Stress in Autoimmunity

V. NUNES-SILVA, DEMENGEOT JOCELYNE

23 Scaling-up the *ex vivo* expansion and fortification of circulating human progenitor cells

M. F. MATA, J. P. LOPES, J. S. CABRAL, C. L. DA SILVA, M. A. COSTA

24 Mapping the cell's membrane signature of the developing mouse heart

M. VALENTE, T.P. RESENDE, A. CUMANO AND P. PINTO-DO-Ó

25 Ubiquitin targets Connexin43 localized at the intercalated discs in ischemic heart

TÂNIA MARTINS-MARQUES, STEVE CATARINO, CARLA MARQUES, PAULO MATAFOME, TERESA RIBEIRO-RODRIGUES, PAULO PEREIRA, HENRIQUE GIRÃO

26 Modulation of Survivin Reverses Cardiac and Pulmonary Remodeling in Monocrotaline-induced Pulmonary Arterial Hypertension

A. F. SILVA , M. J. FERREIRA-PINTO, J. JUSTINO, R. NOGUEIRA-FERREIRA, A. I. PADRÃO, F. CARNEIRO, D. MOREIRA-GONÇALVES, A. LEITE-MOREIRA, T. HENRIQUES-COELHO

## 44 Regenerative Arms Race in Neonatal Cardiac Injury

VASCO SAMPAIO-PINTO, ANA C. SILVA, MARIANA VALENTE, TIAGO L. SANTOS, ANDREIA NUNES, TATIANA P. RESENDE, SÓLVEIG THORSTEINSDÓTTIR, PERPÉTUA PINTO-DO-Ó, DIANA S. NASCIMENTO

## 45 Neuregulin-1 improves right ventricular function and attenuates monocrotaline-induced pulmonary arterial hypertension

P. MENDES-FERREIRA , R. ADÃO, C. MAIA-ROCHA, D. SANTOS-RIBEIRO, B. S. ALVES, G. W. DE KEULENAER, A. F. LEITE-MOREIRA, C. BRÁS-SILVA

## 46 Urocortin-2 improves right ventricular function in pulmonary arterial hypertension

D. SANTOS-RIBEIRO, P. MENDES-FERREIRA, R. ADÃO, C. MAIA-ROCHA, B. S. ALVES, A. F. LEITE-MOREIRA, C. BRÁS-SILVA

## 47 The long-term therapeutic effect of ATMP-compliant human umbilical cord tissue-derived MSCs transplantation upon acute myocardial infarction – A pre-clinical evaluation

T. LAUNDOS, F. VASQUES-NÓVOA, J. M. SANTOS, R. N. BÁRCIA, P. CRUZ, H. CRUZ, D. S. NASCIMENTO, P. PINTO-DO-Ó

## 48 MicroRNA-155 Promotes Sepsis-Associated Cardiac Dysfunction

VASQUES-NÓVOA F., QUINA-RODRIGUES C., CERQUEIRA R., MENDONÇA L., BAGANHA F., MARQUES M.I., LAUNDOS T., SOARES-DOS-REIS R., NASCIMENTO DS., REGUENGA C., PINTO-DO-Ó P., CASTRO-CHAVES P., LEITE-MOREIRA A.F., RONCON-ALBUQUERQUE R.

## 49 Chronic fatigue syndrome, depressive disease and blood pressure!

J. MEIRELES BRANDÃO; F. ROCHA GONÇALVES, RUI COELHO; LÚCIA R. MEIRELES BRANDÃO

# 01 Early changes in cardiac structure and function induced by a high caloric diet

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INTRODUCTION: Obesity cardiomyopathy is nowadays well characterized 1-2, however, the cardiac impact of a Western diet for a short period of time is still unknown. In this study we evaluated the cardiac structure and function of rats fed with a high-fat diet (HFD) for six weeks.

Materials and Methods: Wistar rats (100-150g) were randomly divided and fed with a regular diet (2.9kcal) or with a western-type diet enriched with carbohydrates, fat and salt (54kcal), resulting in 2 groups: RD and HFD. After 6 weeks biventricular function was assessed by echocardiography and hemodynamic and samples obtained for morphohistological, molecular and skinned cardiomyocyte functional evaluation.

RESULTS AND DISCUSSION: Administration of a HFD for 6 weeks resulted in hyperglycaemia, resistance to insulin and glucose intolerance, without changes in body weight (RD 319±9.3; HFD 342±11.1). At the cardiac level, the HFD lead to heart hypertrophy as well as biventricular increased cardiomyocyte cross sectional area (CSA), higher deposition of fibrosis and collagen type II overexpression. Also, the echocardiography data showed in HDF group a decreased ventricular diameter and increased left ventricle (LV) wall thickness and right ventricle (RV) Tei index. These structural resulted in functional abnormalities such as LV increased passive tension, systolic pressure and RV impaired relaxation.

| Structural data | RD | HFD | Functional data | RD | HFD |
|---|---|---|---|---|---|
| Heart/TL (g/cm) | 2.3±0.01 | 2.5±0.06* | LVDs/BSA (mm/cm²) | 118±4.1 | 96±5.3* |
| LV Cardiom. CSA (μm²) | 381±4.92 | 440±7.67* | LVPWs/BSA (mm/cm²) | 72±3.2 | 89±1.2* |
| LV Fibrosis (%) | 0.8±0.13 | 4.7±0.49* | LV Pressure systolic (mmHg) | 108±3.9 | 113 ± 10.4 |
| LV Collagen III (AU) | 1.0±0.05 | 2.0±0.34* | LV Active tension (kN/m²) | 20±1.7 | 25±3.0 |
| RV Cardiom. CSA (μm²) | 278±3.71 | 304±4.27* | LV Passive tension (kN/m²) | 3±0.13 | 7±1.2* |
| RV Fibrosis (%) | 4.9±0.60 | 8.3±0.48* | RV Index TEI | 0.26±0.01 | 0.33±0.01* |
| RV Collagen III (AU) | 1.0±0.07 | 1.4±0.08* | RV Pressure systolic (mmHg) | 22±1.0 | 27±1.1* |
| * vs RD p<0.05 | | | RV τ g ($ms^{-1}$) | 4.4±0.81 | 18.5±2.90* |

TL: tibial length; AU: arbitrary units; LVDs: left ventricle diameter in systole; BSA: body surface area; LVPWs: left ventricular posterior wall; τ g: tau index.

CONCLUSIONS: We have shown that HFD promotes biventricular hypertrophy and extracellular matrix changes and that the early structural alterations triggered by the HFD affect cardiac relaxation and diastolic function, especially in the RV. This study demonstrates that consumption of a Western diet for a short period of time has an early impact in cardiac structure and function even before body weight is significantly altered.

REFERENCES:
1. Van Horn L et al. J Am Diet Assoc. 2008; 108(2):287-331.
2. Lavie CJ, et al. J Am Coll Cardiol. 2009;53:1925-32.

# 02 StemCellNet: an interactive platform for network-oriented investigations in stem cell biology

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INTRODUCTION: Although research efforts have resulted in a considerable amount of stem cell data, their exploration and exploitation is restricted by the lack of dedicated computational resources. Such resources need to be comprehensive and flexible. They need to provide a high coverage of available interactions, while allowing for integration with other data types. Moreover, they should include tools for assessing the relevance of network components in order to help researchers prioritize further investigations.

StemCellNet provides this versatile platform. It is a web server for retrieval, and interactive analysis of molecular networks associated with stem cells and their marker genes; and publicly accessible at **http://stemcellnet.sysbiolab.eu**.

MATERIALS AND METHODS: The StemCellNet web service is built over a database of stem cell related data, which provides the information for creating and analyzing the molecular networks.

The database was created by integrating different types of data. In particular, StemCellNet combines both gene signatures for stemness, as well as stem cell specific interactions from numerous individual publications.

RESULTS AND DISCUSSION: A large number of regulatory and physical interactions (>100 000) were manually extracted from individual studies and integrated into StemCellNet. We integrated several transcriptomic and proteomic datasets that serve as a reference for network analyses. StemCellNet also enables is rapid detection of a stemness signature in networks and for the identification of novel stem cell relevant genes, not only in stem cell biology, but also in other areas of biological and medical research.

It is an easy-to-use platform for network-orientated analyses based on a streamlined workflow consisting of a sequence of three basic steps: *Search—Select—Analyse*.

After inputing a list of gene identifiers a all matches on the database are presented to the user (*Search*). Next the user selects the presented genes which are of interested (*Select*), the data associated with this genes is then used to create a network which can be analyzed using StemCellNet's tools (*Analyse*).

CONCLUSIONS: StemCellNet complements and extends the currently available repertoire of on-line tools for stem cell biology. In contrast to gene-centric resources that return accumulated data for individual genes, StemCellNet enables researchers to assemble networks for a set of genes. Finally, StemCellNet is a unique resource, combining molecular interaction data with a variety of stemness signatures for network-oriented investigations.

REFERENCES:
José P. Pinto, Ravi K.R. Kalathur, Rui S. R. Machado, Joana M. Xavier, José Bragança, and Matthias E. Futschik (2014) StemCellNet: an interactive platform for network-oriented investigations in stem cell biology. Nucleic Acids Research 42:W154-W16

# 03 $VEGF_{165}$-bound beads modulate endothelial cell survival and miRNA expression

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INTRODUCTION: The development of tools to control cell activity are of utmost importance in Regenerative Medicine. For clinical efficacy, it is imperative that stem cells or their progenies survive and engraft into the host tissue. However, a significant number of cells die or are lost within hours after transplantation (typically >70% in few days). Therefore, the development of new strategies to promote cell survival after transplantation is crucial. Herein, we evaluated the effect of VEGF-bound beads on endothelial cells derived from stem cells isolated from umbilical cord blood (UCB) and identified main regulator in the pro-survival activity of immobilized $VEGF_{165}$ in endothelial cells either *in vitro* or *in vivo*.

MATERIALS AND METHODS: $CD34^+$-endothelial cells (ECs) were obtained according to our previous protocol1. The amount of VEGF immobilized onto beads (diameter of 4 μm) was determined by liquid scintillation. The influence of VEGF-conjugated beads on $CD34^+$-ECs was monitored at different levels: VEGFR-2 phosphorylation (ELISA), single cell calcium measurements, cell viability and apoptosis (ATP and Caspase-9 analyses), miRNA analyses (human miRNA microaray kit), formation of sprouts on Matrigel and *in vivo* studies (ability of cells to survive and form a microvasculature).

RESULTS AND DISCUSSION: The conjugated VEGF amount onto beads was between 271.8±44.3 to 4254±50.2 ng per $10^6$ beads depending on the initial conditions. VEGF-bound beads showed prolonged VEGFR-2 phosphorylation and intracellular $Ca^{2+}$ signaling (up to 1 h) relatively to soluble VEGF. Under hypoxia conditions (0.5% $O_2$), the survival of the cells incubated with VEGF-conjugated beads was 1.6 times higher than soluble VEGF group after 24 h. In addition, VEGF-conjugated beads decreased caspase 9 activity ~20% compared to soluble VEGF while the reduction compared to cell control group (cells incubated with blank beads) was >35%. Both total network length and the number of branch points were higher in the presence of VEGF-conjugated beads. After 60 h, the number of branch points decreased more than 50% for all the conditions, while the decrease was just 30% for conjugated-VEGF group. Our results show that the conjugated VEGF modulates the cell activity by decreasing the expression miRNAs (e.g. miR-17, miR-217, miR-222) related to cell apoptosis and senescence while soluble VEGF does not affect the expression of these miRNAs. Importantly, VEGF-bound beads increased cell survival by downregulating the expression of these molecular targets *in vivo*. After 10 days, the cells transplanted in the presence of VEGF-conjugated beads had 35.5% (±284%; n=7) of the initial fluorescent signal while the cells transplanted with blank beads lost the signal after 3 days *in vivo*. Hind-limb ischemia model indicated that conjugated VEGF decreases the severity of ischemia compared to soluble VEGF group.

CONCLUSIONS: Here we show the potential and underlying molecular mechanism of VEGF-bound beads in endothelial cell survival and angiogenesis both *in vitro* and *in vivo*.

REFERENCES:
1. Pedroso, D.C. et al., PLoS One (2011), 6(1): e16114.

# 04 Lysophosphatidic acid enhances survival of human CD34$^+$ cells in ischemic conditions mainly through peroxisome proliferator-activated receptor γ

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INTRODUCTION: Several clinical trials are exploring the therapeutic effect of human CD34$^+$ cells in ischemic diseases including myocardial infarction. Unfortunately, most of the cells die few days after delivery likely decreasing their therapeutic efficacy. Herein we show that CD34$^+$ cells treated with lysophosphatidic acid (LPA) and cultured under hypoxia and serum deprived conditions double their survival relatively to untreated cells. LPA-treated CD34$^+$ cells, but not untreated cells, preserve cardiac function after myocardial infarction.

MATERIALS AND METHODS: The CD34$^+$ cells were isolated from human umbilical cord blood according to protocol published by us[1]. Isolated cells were incubated for 24 h in hypoxia chamber (0.5% $O_2$), in the presence or absence of pharmacological drugs for further assessment of expression of annexin V and PI by FACS. Cell culture supernatants were evaluated using Bio-Plex Human Cytokine 17-Plex Panel Assay. Myocardial infarction in nude rats was induced by permanent ligation of the left anterior descending coronary artery. After recovery animals were monitored and evaluated by echocardiography.

RESULTS AND DISCUSSION: Untreated CD34$^+$ cells show a very poor survival, with only 30.6% of viable cells. In contrast, LPA-treated CD34$^+$ cells (100 μM) have high survival, being ~69% of viable. The pro-survival effect of LPA is concentration and time dependent and it is induced mainly through peroxisome proliferator-activator receptor γ (PPARγ). During its lifetime, LPA induces cell proliferation. LPA-treated cells increased their number approximately 3-fold over the 7 days period while untreated cells did not change their initial number. LPA is not able to maintain undifferentiated state of CD34$^+$ cells as both LPA-treated and untreated cells undergo differentiation into several lineages: dendritic cells, basophils, monocytes, neutrophiles and mast cells. CD34$^+$ cells treated with LPA secrete higher levels of cytokines such as IL-8 (~ 8 fold) and GM-CSF (~ 2 fold). LPA-treated CD34$^+$ cells delivered in fibrin gels into an infarcted animal model, improve cardiac fractional shortening compared to untreated cells (median for fractional shortening of LPA treated cells 7.5 compared to median of 2.6 for non-treated cells).

CONCLUSIONS: We have identified a new pro-survival factor of CD34$^+$ cells that potentiates its therapeutic effect after transplantation in myocardium after infarction.

REFERENCES:
1. Pedroso D. et al., PO, (2011), 6(1), e16114.

# 05 High-content functional screening identifies microRNAs inducing cardiac regeneration

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INTRODUCTION: In mammals, enlargement of the heart during embryonic development is primarily dependent on the increase in cardiomyocyte number. However, shortly after birth, cardiomyocytes stop proliferating. As a consequence of the minimal renewal of cardiomyocytes during adult life, repair of cardiac damage through myocardial regeneration is very limited.

MATERIALS AND METHODS: To systematically identify microRNAs that promote proliferation of rat neonatal cardiomyocytes, we performed a microscopy-based, high-throughput functional screening using a library of microRNA mimics corresponding to all the annotated microRNAs. MicroRNAs selected from this primary screening were re-screened in mouse neonatal cardiomyocytes to identify microRNAs with a conserved functional effect across species.

To identify relevant microRNA targets, we performed global transcriptome analysis of cells overexpressing selected microRNAs, by deep-sequencing, followed by a targeted siRNA screening of the downregulated transcripts (>600). *In vivo* functional analysis of the selected microRNAs was performed using: i) synthetic microRNA mimics complexed with a lipid transfection reagent, injected directly into the heart of neonatal rats, or ii) viral vectors based on the adeno-associated virus serotype 9 (AAV9), injected intraperitoneally in neonatal mice. The therapeutic effects of these microRNAs were assessed in a mouse model of myocardial infarction, induced by permanent ligation of the left anterior descending coronary artery; AAV9 vectors were injected in the peri-infarcted area and heart function was monitored for 60 days, by echocardiography.

RESULTS AND DISCUSSION: Using an unbiased screening approach we identified 40 microRNAs that strongly increase proliferation of both rat and mouse cardiomyocyte by more than 2-fold. Two of these microRNAs (hsa-miR-199a-3p and hsa-miR-590-3p) were selected for further testing and also shown to promote cell cycle re-entry of fully-differentiated adult cardiomyocytes.

Analysis of the targets of these microRNAs identified several genes that block cardiomyocyte proliferation. Individual knockdown of these genes induced cardiomyocyte proliferation, although none to the same extent as the selected microRNAs, indicating that the striking effect of the microRNAs on cardiomyocyte proliferation ensued from the cumulative downregulation of multiple cellular mRNA targets.

In vivo, these microRNAs promote cardiomyocyte proliferation in both neonatal and adult animals. After myocardial infarction in mice, these microRNAs stimulated cardiomyocyte proliferation, cardiac regeneration and preservation of cardiac functional parameters.

CONCLUSIONS: This work demonstrates that cardiomyocyte proliferation can be stimulated by the exogenous administration of microRNAs and, most importantly, that such treatment preserves cardiac function. The identified microRNAs hold great promise for the treatment of cardiac pathologies consequent to cardiomyocyte loss, most notably myocardial infarction and heart failure.

# 06 Flexible nanofilms coated with aligned piezoelectric microfibers preserve the long-term contractility of cardiomyocytes

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INTRODUCTION: New technologies and models that rapidly and accurately predict cellular toxicity towards chemical/pharmaceutical substances represent a major unmet need for the pharmaceutical industry and regulatory authorities. Cardiotoxicity assessment is of paramount importance in the development of new drugs, consequently, a major issue is to generate heart tissue *in vitro* in which cells can be incorporated into a reliable platform for drug screening and toxicology assessment[1]. The goal of this work was to design a new type of scaffold that is able to support the growth and maintain the functionality of cardiac cells and potentially be used for high-throughput drug screening.

MATERIALS AND METHODS: The scaffold integrates a nanofilm containing superparamagnetic iron nanoparticles (MNF) and an aligned matrix of piezoelectric (PIEZO) fibers. The scaffolds were coated firstly with poly (dopamine) (P-Dopa) and afterwards with gelatin (1%), while the respective control in tissue culture polysterene (PS) were only coated with gelatin. Embryonic CM were obtained from Whistar rat embryos (17-18 days of gestation) sacrificed according to the European Union directives. These cells were then seeded in the aforementioned conditions for 12 days and their phenotypic profile was assessed through protein expression markers performed by RT-PCR, western blot and imunofluorescence.

RESULTS AND DISCUSSION: The resulting scaffold is very flexible with a top layer of aligned porous fibers and can be manipulated with tweezers and magnets. The MNF construct has no charge generation, while MNF+PIEZO construct shows clear charge generation when subjected to mechanical deformation.

Biocompatibility assessments show that MNF+PIEZO scaffolds support cell adhesion and proliferation, maintain CM:CF ratio, and show enhanced CM alignment, while comparing with the MNF and PS. On functional evaluations it was seen a constant average rate of beats on cells cultured in PS and MNF scaffolds maintained constant from day 1 to day 12, and a significant increase in cells cultured on MNF+PIEZO scaffolds. Further assessments through WB show a statistically higher ratio active-Cx43/totalCx43 (P±0.05) on MNF and MNF+PIEZO scaffolds than on PS at day 12. Gene analysis of main maturation markers and ion channels showed no significant impact in CM maturation along the 12 days of culture but MNF+PIEZO scaffolds showed higher expression rates on 5 of the main ion channels subunits. Finally, we assessed the potential of the MNF+PIEZO to replicate a toxicological response in the CM similar to that of the native heart. The drug model used was doxorubicin (DOXO). For day 12 of culture we see a fold increase by 2 times in expression of p53 and GADD45 for the MNF+PIEZO thus revealing a stronger activation of apoptotic pathway and higher damage to the DNA in the MNF+Piezo condition, and demonstrating a greater sensitivity towards the toxicological insult, than the remaining conditions.

CONCLUSIONS: We have engineered a cardiac tissue that shows superior performance in terms of contractility, morphology, metabolism and toxicological prediction than the ones obtained in standard polystyrene tissue culture or described in the literature [2].

REFERENCES:
1. Eschenhagen, T. and W.H. Zimmermann, Engineering myocardial tissue. Circ Res, 2005. 97(12): p. 1220-31.
2. Radisic, M. and K.L. Christman, Materials science and tissue engineering: repairing the heart. Mayo Clin Proc, 2013. 88(8): p. 884-98.

# 07 Cardiomyocyte dynamics throughout ontogeny

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INTRODUCTION: Heart failure is associated with massive cardiomyocytes (CM) death due to ischemia and ventricular wall thinning[1-2]. Thus, regenerative strategies broadly involve approaches to promote renewal of pre-existing CM and/or to increase cardiomyogenic precursor cells activity. However, and despite being the major structural cardiac cells, several aspects of CM development and maturation within the myocardium remain elusive. Additionally, detailed knowledge of adult CM morphology is lacking in the field due to the difficulties in analyzing these cells within the tissue and in efficiently isolating them. A better comprehension of the mechanisms controlling CM development and maturation will be very valuable not only for the Developmental Biology community but also for the Regenerative Medicine field. In the present work we have (i) implemented a new protocol for efficient CM isolation and (ii) initiated a detailed analysis of these cells morphology along murine ontogeny.

MATERIALS & METHODS: Hearts were isolated from embryonic, fetal and adult C57BL/6 mice and processed for paraffin or gelatin inclusion for histochemistry or immunofluorescence, respectively. For the morphometric studies, myocardial samples from embryonic, neonatal and adult animals were collected, minced into $2mm^3$ blocks and flash-frozen. For cell isolation, the tissue was incubated overnight at 37ºC with collagenase and processed for staining with specific antibodies. Cell suspensions obtained were analyzed using either inverted microscope fluorescence or imaging flow cytometry (ImageStream).

RESULTS & DISCUSSION: One main difficulty when investigating CM is their accurate identification within the developing organ. This becomes an even harder endeavor when analyzing the adult heart. Additionally, quantitative studies are impeded by the unavailability of mouse specific CM surface markers. To overcome this, we have optimized a protocol[4] that allows parallel isolation of embryonic, neonatal and adult intact CM. Combining this novel protocol with classic immunohistochemistry, we have performed a detailed analysis throughout cardiac morphogenesis including the qualitative detailing of CM morphology *in situ* and *ex vivo* and their quantification in terms of numbers and size. Additionally, we have initiated a morphometric characterization of CM along ontogeny and the results obtained thus far will be presented.

CONCLUSION: The implemented protocol allows the enrichment of CM with preserved cytoskeletal structure in a suspension of heart cells. These cells can be further processed for distinct protocols, enabling us to (1) obtain high quality images of CMs and (2) perform quantitative studies. This protocol can be used in embryonic, neonatal and adult hearts, and unequivocal identification and comparison of CMs populations through ontogeny, which are lacking in the field, can be achieved.

REFERENCES:
1. Laflamme MA, et al. Nat Biotechnol 2007. 25:1015-1024.
2. Whelan RS, et al. Annu Rev Physiol 2010. 72:19-44.
3. Soonpaa MH, et al. Biochim Biophys Acta 2012. 1833(4):799-803.
4. Mollova M et al. Proc Natl Acad Sci U S A. 2013. 110(4):1446-51.

# 08 Role of *Ccbe1* during cardiovascular development in mice

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Collagen and Calcium Binding EGF domains 1 (CCBE1) is intimately related to the development of the lymphatic system [1, 2]. In mice, *Ccbe1* is expressed in the vicinity of the cardinal veins, which is consistent with its function in the lymphatics, but *Ccbe1* expression can be identified much earlier in cardiac progenitor cells and later also in the pericardium lining the heart [3-5]. Whether CCBE1 has a specific role during mammalian cardiac development remained however unknown. According to our data, Ccbe1 loss-of-function in mice leads to thinning of the compact myocardium specifically in the right ventricle (RV). This phenotype becomes evident from embryonic day (E) 13 and the remaining heart chambers have no obvious alterations. Furthermore, the trabecular myocardium from the RV in *Ccbe1* mutants is normal with organized sarcomeric structures characteristic of definite cardiomyocyte differentiation. Expression of *Wt1* and other markers of the epicardial layer are also not affected in the Ccbe1 mutants at E12.5-E13.5, indicating that the (pro)epicardium where *Ccbe1* is expressed earlier is well established. In contrast, quantification of the expression of several myocardial transcription factors revealed that the expression of *Nkx2.5* is significantly decreased in *Ccbe1* mutant hearts at E13.5. In addition, the NKX2.5-target gene responsible for physiologic hypertrophy *Nppb* is also significantly decreased, but *Nppa* is not. Furthermore, analysis of *Ccbe1* expression using the LacZ reporter in *Ccbe1* heterozygous mice showed that expression of *Ccbe1* in the pericardium is transient and asymmetric. Initially at from E10.5 until E11.5, *Ccbe1* expression spreads throughout the pericardium, but from E12.0 *Ccbe1* expression locates in the pericardium adjacent to the RV until E13.0-E13.5 when the pericardial expression ceases. Since *Ccbe1* is asymmetrically expressed in the right side and the thinning of the compact myocardium is specific of the RV in the *Ccbe1* mutant, it is likely that *Ccbe1* from the pericardium plays a role in the establishment of the compact myocardium from the RV. Furthermore, analysis of proliferation in mouse embryonic fibroblasts (MEFs) isolated from *Ccbe1* mutant embryos showed that proliferation is decreased in the mutant MEFs, which is consistent with a role of CCBE1 in proliferation. We are currently investigating if proliferation or other cellular processes are affected in the *Ccbe1* mutant heart and causing the thinning of the compact myocardium.

REFERENCES:
1. Alders, M., et al., Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. Nat Genet, 2009. 41(12): p. 1272-4.
2. Hogan, B.M., et al., Ccbe1 is required for embryonic lymphangiogenesis and venous sprouting. Nat Genet, 2009. 41(4): p. 396-8.
3. Bos, F.L., et al., CCBE1 is essential for mammalian lymphatic vascular development and enhances the lymphangiogenic effect of vascular endothelial growth factor-C in vivo. Circ Res, 2011. 109(5): p. 486-91.
4. Facucho-Oliveira, J., M. Bento, and J.A. Belo, Ccbe1 expression marks the cardiac and lymphatic progenitor lineages during early stages of mouse development. Int J Dev Biol, 2011. 55(10-12): p. 1007-14.
5. Hagerling, R., et al., A novel multistep mechanism for initial lymphangiogenesis in mouse embryos based on ultramicroscopy. EMBO J, 2013. 32(5): p. 629-44.

# 09 From Stem Cells 2 Heart: Elucidating the role of alternative splicing in cardiomyogenesis

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INTRODUCTION: Cardiomyogenesis is a highly complex and precise process involving a series of spatially and temporally ordered molecular events. While genome-wide expression studies using conventional microarray technology have given us first comprehensive pictures of the activity of genes during the heart development, many key molecular details may still be hidden since they did not distinguish between splice isoforms. In fact, these events have recently emerged as significant contributers for stem cell differentiation and tissue formation through the adjustment of protein structure and function to different cell types [1]. Accordingly, we have generated an RNA-seq genome-wide transcriptome profile of murine embryonic stem cell (ESC) differentiation into cardiac progenitors and cardiomyocytes, which has been computationally analyzed in order to identify relevant alternative splicing events during early cardiac development.

MATERIALS AND METHODS: An RNA-seq time-series was used to profile the transcriptome of a double-transgenic murine ESC line (AD2) [2] during their differentiation process. Cell subpopulations were sorted by FACS based on the expression of dsRed and eGFP reporter genes, which in AD2 ESC are under the control of the enhancers of the cardiac markers *Mef2c* and *Nkx2.5*, respectively. The dataset was analyzed in our in-house computer facilities, using the *Tuxedo* tools pipeline [3].

RESULTS AND DISCUSSION: Differential expression analysis evaluated the effect of time on cardiomyogenesis. We have found a total of 4520 differentially expressed gene isoforms over the 3 time points, of which 2044 were differentially expressed between day 0 and day 4, and the remaining 2476 between day 4 and day6. Additionally, we have found 33 genes where the relative contribution of different isoforms for the total expression of the gene is significantly different between day 4 and day 6. Bioinformatic tools, such as clustering and template matching, indicated novel functional and regulatory components in cardiomyogenesis.

CONCLUSIONS: Our large-scale splicing differential analysis confirmed previous results obtained in studies that show extensive alternative splicing in stem cell differentiation [4,5]. Our ongoing analysis will create a testable set of splice variants important for cardiomyogenesis. Future work includes (1) the experimental validation of the candidates and (2) an analysis of the dynamical wiring of the complex molecular networks governing cardiomyogenesis.

REFERENCES:
1. Salomonis N1,Schlieve CR,Pereira L,et al.Alternative splicing regulates mouse embryonic stem cell pluripotency and differentiation.2010. PNAS.107(23):10514-9.
2. Domian IJ1,Chiravuri M,van der Meer P,et al.Generation of functional ventricular heart muscle from mouse ventricular progenitor cells.2009. Science.326(5951):426-9.
3. Trapnell C1,Roberts A,Goff L,et al.Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.2012. Nat Protoc.7(3):562-78.
4. Wang ET1,Sandberg R,Luo S,et al.Alternative isoform regulation in human tissue transcriptomes.2008456(7221):470-6.
5. Pritsker M1,Doniger TT,Kramer LC,et al.Diversification of stem cell molecular repertoire by alternative splicing.2005.PNAS.102(40):14290-5.

# 10 Loss of *Ccbe1* affects cardiac-specification and proliferation in differentiating mouse embryonic stem cells

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In humans, mutation in *CCBE1* has been associated with Hennekam syndrome, a disorder characterized by abnormal lymphatic system development where some patients present as well congenital heart defects, including hypertrophic cardiomyopathy and ventricular septal defects [1]. This indicates that Ccbe1 may also function during heart organogenesis. Despite the increasing evidence of the role of *Ccbe1* in lymphangiogenesis [2-4], its role in cardiac differentiation and development has not been investigated. In this study, we examined *Ccbe1* expression in differentiating mouse embryonic stem cells (ESCs) and in isolated cardiac progenitor populations, and how *Ccbe1* loss-of-function affects cardiac differentiation from mouse ESCs.

Here, we have identified *Ccbe1* expression in differentiating ESCs, especially in second heart field (SHF) cardiac progenitors at day 6 of differentiation. Furthermore, isolation of these populations from transgenic mice at an equivalent time point, showed a similar *Ccbe1* expression profile for the different populations. Therefore, our data shows that *Ccbe1* expression is unequivocally related to the appearance of specific populations of cardiac progenitors both *in vivo* and during ESC differentiation. In addition, differentiation of *Ccbe1* knockdown ESCs lines resulted in a clear decrease in the expression of early cardiac mesoderm markers *Mesp1* and *Isl1* at day 4 of differentiation, indicating that the development of early cardiac precursors is compromised in the absence of *Ccbe1*. Therefore, it is likely that during ESC-derived cardiac differentiation *Ccbe1* induces or acts synergistically with growth factors to promote the formation of cardiac precursor cells.

In addition, we show that also from day 4 onwards the size of the embryoid bodies is severely decreased and that this is caused by decreased proliferation. Furthermore, full-length recombinant CCBE1 protein was shown to partially rescue the proliferation in differentiating *Ccbe1* KD ESCs. This data indicates that indeed the proliferation defect in *Ccbe1* KD ESCs was directly associated with the knockdown of Ccbe1. Collectively, our results indicate that Ccbe1 is essential for the formation of cardiac mesoderm and proliferation of differentiating mouse ESCs. Whether gain-of-function or supplementation with *Ccbe1* in differentiating ESCs would lead to increased commitment to cardiac precursors remains under investigation.

REFERENCES:

1. Alders, M., Hogan, B.M., Gjini, E., Salehi, F., Al-Gazali, L., Hennekam, E.A., Holmberg, E.E., Mannens, M.M., Mulder, M.F., Offerhaus, G.J., et al. (2009). Mutations in CCBE1 cause generalized lymph vessel dysplasia in humans. Nature genetics 41, 1272-1274.
2. Bos, F.L., Caunt, M., Peterson-Maduro, J., Planas-Paz, L., Kowalski, J., Karpanen, T., van Impel, A., Tong, R., Ernst, J.A., Korving, J., et al. (2011). CCBE1 is essential for mammalian lymphatic vascular development and enhances the lymphangiogenic effect of vascular endothelial growth factor-C in vivo. Circ Res 109, 486-491.
3. Hagerling, R., Pollmann, C., Andreas, M., Schmidt, C., Nurmi, H., Adams, R.H., Alitalo, K., Andresen, V., Schulte-Merker, S., and Kiefer, F. (2013). A novel multistep mechanism for initial lymphangiogenesis in mouse embryos based on ultramicroscopy. The EMBO journal 32, 629-644.
4. Hogan, B.M., Bos, F.L., Bussmann, J., Witte, M., Chi, N.C., Duckers, H.J., and Schulte-Merker, S. (2009). Ccbe1 is required for embryonic lymphangiogenesis and venous sprouting. Nature genetics 41, 396-398.

# 11 Cerl2 and the coordination of Nodal signaling during heart development

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The minor perturbation on the establishment of Left-Right (LR) axis results in a variety of heart defects that arise from abnormal looping and remodeling of the primitive heart tube into a multi-chambered organ. Thus, in order to understand the pathogenesis of LR-related diseases, it is necessary to elucidate the complicated process of LR patterning and underlying mechanisms involved.

The process that can be divided in: a) the initial LR break of symmetry, which occurs at the mouse node (or equivalent structures of other vertebrates); b) transfer of the left-right-biased information generated at the node to the lateral plate mesoderm (LPM), leading to LR asymmetric expression of signaling molecules such as the TGF-ß-related proteins Nodal and Lefty on the left side of the embryo; c) and LR asymmetry present in both the structure and placement of the internal organs is prompted by these signaling molecules.

Nodal plays a central role among the signaling molecules expressed at the node. This gene is expressed in the perinodal cells located at the periphery of the mouse node, and at both sides before onset of its expression in the left-LPM. But at early somite stage, its expression becomes L>R asymmetric. Moreover, several evidences have shown that expression of Nodal at the LR organizer is indispensable for the subsequent expression of Nodal in the LPM. Therefore, the suppression of Nodal activity in the perinodal cells thus prevents Nodal expression in the LPM.

Cerl2 is an extracellular protein belonging to the family of TGF-β antagonists Cerberus/DAN that controls the activity of Nodal at the node, and possibly the transmission of the laterality information to the L-LPM. Cerl2 knockout mice show a wide range of laterality defects including randomization of Nodal expression in the LPM. More recently, we demonstrated that Cerl2 activity is essential, first, to prevent the activity of Nodal in the right-LPM, and later to shutdown Nodal activity in the left side of the node and consequently in the left-LPM, in a precise time window. We also addressed that in the absence of Cerl2, the activity of Nodal is prolonged in the node, and consequently its expression is sustained longer in the LPM. In addition, we showed that the unidirectional fluid flow generated by primary cilia present in cells of the ventral node is necessary for the Cerl2 protein dynamic node-localization.

These evidences assign Cerl2 as the key element for LR axis establishment, however, we have identified Cerl2-associated cardiac defects, particularly a large increase in the left ventricular myocardial wall in neonates that cannot be explained by laterality defects. Interestingly, we observed an increase of pSmad2 levels in embryonic (E13) and neonatal hearts indicating a prolonged TGF-β/Nodal-signaling activation.

Here, the new advances on the coordination of Nodal signaling by Cerl2 protein during the heart development will be discussed.

# 12 A novel RNA-based Therapy to Hypertrophic Cardiomyopathy

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INTRODUCTION: Hypertrophic Cardiomyopathy (HCM) is the most common hereditary disease of the heart and a cause of sudden cardiac death in young adults. There are currently more than 1000 HCM-associated mutations identified in 13 different genes that code for proteins of the sarcomere. At present there is no curative treatment for this disease. This work aims to establish a proof-of-principle for a RNA-based therapy and also a CRISPR-Cas9 based therapy at the gene level for HCM. In parallel, the analysis of 18 HCM-associated genes by DNA Next-Generation Sequencing (NGS) for 16 different HCM patients in which none of the most common HCM mutations were identified will allow for the identification of novel HCM-causing mutations.

MATERIAL AND METHODS: Our experimental approach is based on the transfection of mouse atrial cardiomyocytes (HL-1 cells) with "therapeutic" human transcripts designed to anneal to the two introns flanking the murine TNNT2 exon 11 or either the intron upstream, whose homologue in humans contains a cluster of pathogenic mutations associated to HCM. The success of this strategy will be evaluated by the replacement of murine exon 11 by the "therapeutic" exon 11 via double trans-splicing in murine TNNT2 mRNA or via single 3' trans-splicing from the cDNA exon 11 to 16.

The CRISPR-Cas9 system was used to create a specific double strand break (DSB) in the endogenous exon 10 of the murine TNNT2 gene, which by homology directed repair will favour the insertion of a "therapeutic" cDNA sequence (Ex10-Ex18) present in a plasmid donor vector containing two homology arms flanking the site of the DSB.

To identify novel HCM-associated mutations, NGS data was aligned to the human reference genome, variants annotated, identified and classified through the snpEff software.

RESULTS AND DISCUSSION: Two different "therapeutic" constructs were generated, which are under the control of the murine TNNT2 promoter or of the CMV promoter (for each of the two versions of trans-splicing). These constructions were already used to transfect HL-1 cells and test the efficiency of trans-splicing.

The CRISPR-Cas9 cleavage efficiency was already tested on both HL-1 cells and mouse stem cells. From this point, a double transfection with both the CRISPR plasmid and the homology donor vector (HDV) was already successfully performed, proving the efficiency of this assay to insert the "therapeutic" cDNA into a specific region of the murine TNNT2 locus. At present, directed point mutagenesis is being performed on the HDV to reproduce four TNNT2 HCM-associated missense mutations, generating HCM cell lines that will be use to study the repair efficiency of the CRISPR assay at the cellular level.

The variants identified by the snpEff software are being analysed and compared to various tools that predict potential splicing features (from branch point to splicing enhancer/silencer). In the future, we intend to use the CRISPR methodology to induce these variants and test their effect in splicing and, ultimately, in HCM phenotype.

CONCLUSIONS: All together, these approaches will allow us to supply a "therapeutic" human transcript for efficient replacement of mTNNT2 exon 11 or cDNA 11-18 in HL-1 cells and CRISPR-Cas9 induced gene therapy. Moreover, novel mutations associated to HCM may be unravelled.

REFERENCES:
1. White SM, et al, Am J Physiol Heart Circ Physiol. Mar; 286(3):H823-9, 2004
2. Wally, V., et al, Journal of Investigative Dermatology, doi:10.1038/jid.2012.101, 2012
3. Ran, F. A., et al, Cell 154, 1380–1389, 2013

# 13 Hes5 specifies cardiac fate in a time-dependent manner

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INTRODUCTION: Cardiac specification occurs early during embryo development, shortly after the migration of prospective hematopoietic progenitors to the yolk sac [1]. These differentiation events rely on a specific orchestration of distinct signaling pathways in a spatial/temporal manner. Differentiating mouse embryonic stem cells (mESCs) faithfully recapitulate embryonic development contributing with mechanistic insights into early lineage specification [2]. Notch signaling plays an essential role in specifying cardiac lineages [3] but the involved molecular regulatory mechanisms remain elusive. In this study we aimed to identify downstream players of Notch1 implicated at the onset of cardiogenesis.

MATERIALS AND METHODS: AinV/Bry-GFP/NICD1 mESCs were differentiated into D3.75 mesodermal progenitors and cultured with Doxycycline (Dox) to activate Notch1 intracellular domain expression (NICD1). The transcription profile of Notch effectors was analyzed overtime for selection of candidate genes. A candidate, i.e. Hes5, was selected for further characterization. To dissect Hes5 function knockdown (KD) studies were performed, by using shRNA technology, and Hes5 overexpression was achieved transducing AinV/Bry-GFP mESCs with a Dox-inducible cassette. Cultures were examined for cell contraction and cardiac and hematopoietic gene expression was assessed. Chromatin immunoprecipitation combined with sequencing (ChIP-Seq) was performed to identify Hes5's putative targets. Apoptosis and cell proliferation were analyzed after Hes5 induction by AnnexinV and BrdU flow cytometry staining, respectively.

RESULTS AND DISCUSSION: Expression of Notch target genes was analyzed overtime upon NICD1 activation in D3.75 cells. Hes5 response suggested a direct and time-specific effect, i.e. rapid increase in the first 24hours after which it diminished, and was thus further evaluated as a mediator in cardiac specification. Upon Hes5-KD rare contracting areas were observed while emergence of hematopoietic-like cells was enhanced. These observations were corroborated by decreased mRNA levels of specific cardiac genes (*Isl*-1, *Tbx5*, *Gata4* and *Myh6*) and increased expression of hematopoietic regulators (*Scl* and *Gata1*) suggesting that Hes5 represses the hematopoietic program. Furthermore, Hes5-KD compromised Notch1 preferential cardiac over hematopoietic fate on mesodermal cells. In contrast, Hes5 overexpression led to an increased frequency of contracting foci and higher expression of cardiac progenitor genes. Interestingly, while Hes5 enhanced cardiac fate in a confined temporal window, its sustained activity compromised maturation into cardiomyocytes. Our data further demonstrate that Hes5 plays an instructive rather than a selective role on mesodermal progenitors as neither apoptosis nor cell proliferation was altered. Furthermore, Isl1 and Scl, identified by ChIP-Seq as putative direct targets of Hes5, showed respectively increased and decreased expression upon Hes5 activation.

CONCLUSIONS: Collectively, the herein data unveil a role this far unknown for Hes5 as a mediator of Notch1 in the determination of cardiac fate. Hes5 regulates Isl1 and Scl levels in mesodermal progenitors determining cardiac versus hematopoietic outcomes. Furthermore, cardiac induction is confined to a specific temporal window that requires a short pulse activation of Hes5 to allow cardiac maturation after commitment to the cardiac program.

REFERENCES:
1. Parameswaran M, et al. (1995) Dev Genet; [2] Keller G, (2005) Genes and Dev; [3] Chen VC, et al. (2008) Nat Biotechnol.

# 14 Combining induced pluripotent stem cells and nanofilms to generate human arterial and venous endothelial patches

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INTRODUCTION: Vascular patches made of synthetic polymers (e.g. expanded polytetrafluoroethylene, polyethylene terephthalate and polyurethane) have been clinically used to reconstruct vascular conduits. Unfortunately, these patches have limitations related to thrombus formation and calcium deposition due to blood and tissue incompatibility[1,2]. Tissue-engineered vascular patches could overcome the problems of synthetic vascular patches. Here, we developed a clinical relevant methodology to derive arterial and venous endothelial cells from induced pluripotent stem cells (iPSCs) in chemically defined and serum-free conditions.

RESULTS AND DISCUSSION: Vascular progenitor endothelial cells were differentiated from iPSCs for 10 days and isolated by magnetic activated cell sorting using CD31 marker. Approximately 90% of the isolated cells were positive for CD31 and vascular endothelial receptor II (VEGFR2/KDR). At this stage cells expressed low levels of arterial, venous and lymphatic endothelial markers, suggesting that their sub-phenotype is not yet defined. After four passages in serum-free medium supplemented with vascular endothelial growth factor ($VEGF_{165}$, 50 ng/mL), the cells, characterized by the expression of endothelial markers (CD31: 88.3±4.2%; KDR: 89.3±5.2%; VE-cadherin: 54±6,7%), preferentially expressed arterial markers , such as EphrB2: 26.5±2%(a percentage not far from the 35±7,9% expressed by human umbilical artery endothelial cells) and other arterial related genes such as JAG1, EFNB1, EFNB2, HEY-2, together with low levels of venous markers EFNB4 and COUP-TFII. In contrast, vascular progenitor cells cultured in serum-free medium supplemented with a lower concentration of VEGF165 (10 ng/mL) differentiated preferentially into venous endothelial cells, characterized by increased expression of the venous marker COUP-TFII, and low expression levels of arterial markers in comparison with cells expanded with a higher VEGF concentration. Both iPSC-derived arterial and venous endothelial cells have the capacity to uptake acetylated low-density-lipoprotein (Ac-LDL), to form a capillary like-network and respond to pro-inflammatory stimuli. These cells are biocompatible with polycaprolactone (PCL) nanofilms (NFs), adhere, proliferate and form a monolayer maintaining their endothelial phenotype.

CONCLUSIONS: In conclusion, we report an inductive protocol in serum-free conditions to obtain a population of vascular progenitor cells that can be further maturated into a more arterial or venous-like endothelial phenotype cells that combined with NFs can be useful for regenerative purposes.

REFERENCES:
1. Cho, SW., et al. Vascular patches tissue-engineered with autologous bone marrow-derived cells and decellularized tissue matrices. Biomaterials, 2004.
2. Muto, A., et al. Patches for Carotid Artery Endarterectomy: Current Materials and Prospects. J Vasc Surg., 2009.

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# 15 Myofilament changes in doxorubicin-induced cardiotoxicity

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INTRODUCTION: Cardiomyopathies are an important cause of premature death from heart failure[1]. Several studies demonstrate that administration of doxorubicin, an anthracycline antibiotic used in cancer treatment, results in cardiac toxicity, and may lead to dilated cardiomyopathy[2]. Alterations in titin have been reported in patients with this disease[3]. The present work aims to evaluate the early myocardial changes in an animal model of doxorubicin-induced cardiotoxicity.

MATERIALS AND METHODS: Male New Zealand white rabbits were injected intravenously twice weekly for 8 weeks with doxorubicin (DOX-HF, 1mg/Kg, n=17) or with an equivolumetric dose of saline (Control, n=18). Echocardiographic evaluation was performed 1 week after the end of protocol. Myocardial samples were collected to evaluate functional properties of isolated skinned cardiomyocytes in terms of myofilaments active tension, passive tension and calcium sensitivity (pCa50and nHill). Sirius-red, hematoxylin-eosin and TUNNEL stained samples, were used to quantify myocardial fibrosis, cardiomyocytes cross sectional area and apoptotic nucleus, respectively. Titin isoform expression, phosphorylation and degradation were quantified.

RESULTS AND DISCUSSION: DOX-HF group presented cardiac hypertrophy as evidenced by the increase in heart weight normalized to body weight (2.38±0.09mg/gvs2.17±0.06mg/g) and by the increased right ventricle(RV) and left ventricle(LV) cardiomyocyte cross-sectional area (RV:268±12$\mu m^2$vs235±16$\mu m^2$ and LV:380±20$\mu m^2$vs331±27$\mu m^2$). Concerning, cardiomyocytes function, DOX-HF group, presented increased active tension (214±1.9mN/$mm^2$vs16.5±1.1mN/$mm^2$) without significant differences in passive tension (3.8±0.5mN/$mm^2$vs3.1±0.3mN/$mm^2$). Myofilaments sensibility to $Ca^{2+}$ was not changed by doxorubicin (pCa50:5.7±0.2vs5.8±0.1; nHill:1.9±0.2vs1.8±0.2). DOX-HF group showed a decrease in total titin phosphorylation (49.0±6.1%vs85.2±9.9%), more pronounced in N2B isoform (62.6±9.1%vs31.0±44%), besides the increase in the ratio between the compliant isoform and the stiffer isoform (N2BA:N2B:0.3±0.1vs0.5±0.1). On the other hand, the percentage of apoptotic nuclei was similar between groups (2.1±1.3%vs3.5±1.8%). The extracellular matrix showed marked alterations as confirmed by the significant increase in myocardial interstitial fibrosis in LV from DOX-HF group (12.1±14%vs7.8±1.2%) and a tendency in the RV (12.0±1.6%vs9.0±1.2%).

CONCLUSIONS:This work describes novel and early myocardial effects of doxorubicin-induced cardiotoxicity, including changes at the level of cardiomyocytes and myofilaments (titin). These early changes precede the initial echocardiographic diagnosis of cardiomyopathy, emphasizing the need for an early detection of cardiac damages associated to cancer treatments in order to allow for therapeutic adjustments and prevent the progression of cardiomyopathy.

REFERENCES:
1. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: The complexity of a diverse genetic architecture. Nature reviews. Cardiology. 2013
2. Sterba M et al. Oxidative stress, redox signaling, and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. Antioxidants & redox signaling. 2013. 3-Kruger M et al. Protein kinase g modulates human myocardial passive stiffness by phosphorylation of the titin springs. Circulation research. 2009.

# 16 Highly Electrical Conductive Chitosan/Carbon Scaffolds for Cardiac Tissue Engineering Applications

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INTRODUCTION: In this work carbon nanofibers were used as doping material to develop a highly conductive chitosan-based composite material. We hypothesized that electrically conductive scaffolds would improve cardiomyocyte function by increasing specific cardiac genes expression, even without electrical stimulation. Our strategy was to combine the biocompatibility and biodegradability of chitosan with the electrical properties of carbon. We investigated the following questions: (*i*) Are chitosan/carbon scaffolds adequate materials for cardiac applications, in terms of their mechanical and electrical properties? (*ii*) Is the integrity of chitosan/carbon scaffolds maintained over time in culture? (*iii*) What are the effects of carbon on metabolic activity of cultured cardiomyocytes and the expression of cardiac genes?

MATERIALS AND METHODS: Scaffolds based on chitosan only and chitosan/carbon composites were prepared by precipitation method. The scaffold porosity, pore interconnectivity, pore size and fiber thickness were measured using micro-CT. The dynamic viscoelastic measurements of hydrated scaffolds were performed using Dynamic Mechanical Analysis (DMA). The electrical conductivity was also measured in dry and wet state. The scaffolds were seeded with neonatal rat heart cells and cultured for 7 and 14 days, without electrical stimulation. The expression profiles of specific-cardiac markers were analyzed by real-time quantitative PCR.

RESULTS AND DISCUSSION: The obtained scaffolds were highly porous with fully interconnected pores. Chitosan/carbon scaffolds had elastic modulus of 28.1 ± 3.3 KPa, similar to that measured for rat myocardium, and excellent electrical properties, with conductivity of 0.25 ± 0.09 S/m. The metabolic activity of cells in chitosan/carbon constructs was significantly higher as compared to cells in chitosan scaffolds (Fig. 1A) Carbon/chitosan scaffolds supported cultivation of cardiac cells and enhanced cardiogenic properties without exogenous electrical stimulation. The incorporation of carbon also led to increased expression of cardiac-specific genes involved in muscle contraction and electrical coupling. Gene expression data clearly show upregulation of Tnnc1 and Cx43 on chitosan/carbon/cell constructs after 7 days, and all genes associated with contraction overexpressed after 14 days (Fig. 1B).

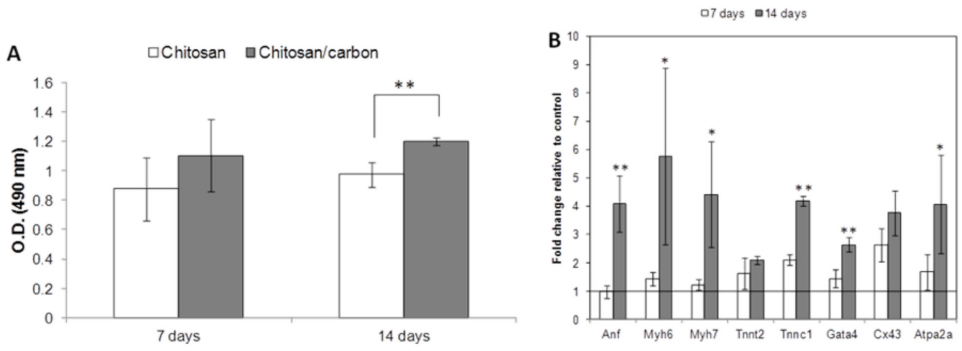


Fig. 1. (A) Metabolic activity of chitosan and chitosan/carbon cell constructs obtained as optical density (O.D.) values from MTS assay after 7 and 14 days of culture; and (B) Gene expression of cardiomyocytes cultured on chitosan/carbon scaffolds for 7 and 14 days vs control samples (chitosan scaffolds cultured using the same conditions).

CONCLUSIONS: We describe electrically conductive composite scaffolds based on chitosan, designed to have mechanical properties similar to those of cardiac muscle, and to provide local increases (by 9 orders of magnitude) in electrical conductivity by addition of carbon nanofibers. The carbon/chitosan scaffolds supported cultivation of cardiac cells and enhanced cardiogenic properties without exogenous electrical stimulation. In the presence of carbon nanofibers, cardiomyocytes were able to adhere and survive for up to 14 days, and elevate their metabolic activity and expression of cardiac genes. Gene expression profiling showed upregulation of Tnnc1 and Cx43 in chitosan/carbon/cell constructs after 7 days of culture, and all measured cardiac-specific genes (Anf, Myh6, Myh7, Tnnt2, Tnnc1, Gata4, Cx43 and Atp2a2) after 14 days of culture.

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# 17 Molecular and functional analysis of DAND5 in human Congenital Heart Disease

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Heart morphogenesis is a complex process involving multiple cell types and precise control developmental mechanisms that can nevertheless fail [1]. Problems in heart morphogenesis lead to CHD, which are the most common form of birth defect (8 in 1000 live births) and the leading non-infectious cause of death in the first year of life [2]. With the advances in medical and surgical management of CHD, the mortality of affected children has decreased, but the reported incidence of nearly 1% has remained unchanged, suggesting that genetic factors could play important roles [3]. The knowledge, from human molecular genetics studies and developmental models in several animal models species, of the heart morphogenesis and the early steps of the left-right axis symmetry determination demonstrate that in most of the cases of laterality disorders, a complex heart malformation is observed, suggesting that the CHD could be due to a laterality defect in the heart morphogenesis [4]. The molecular control of LR axis development is an essential aspect in determining both the structure and function of the heart and has received particular attention being the Nodal molecule, a growth factor from the TGF-ß family, recognized as a key player in this process [5]. Several genes involved in the nodal signaling pathway has already been associated to laterality disorders and CHD in humans, furthermore the recently identified mouse cerberus-like2 (cerl-2), a novel gene asymmetrically expressed on the right side of the mouse node that encodes a secreted protein capable to inhibit Nodal signaling [6], prompt us to the cardiac and laterality disease, since the generated cerl-2 KO mice display a wide range of laterality defects and cardiovascular malformations. Considering the high conservation of genetic pathways regulating cardiac development in mouse and human, the study of the human genes involved in the Nodal pathway will bring us new data in the attempt to define the cellular and molecular mechanisms that underlie cardiac asymmetry on the CHD and the laterality defects in humans. In this study we analyze, by molecular biology techniques, a set of patients presenting CHD and/or laterality defects. Until now we sequenced the *DAND5* (Human cerl-2 gene), *NODAL* and *CFC1* genes in 39 patients. We have found two sequence alterations in the *NODAL* gene; one alteration in the *CFC1* gene and a nucleotide variant in the *DAND5* gene, which leads to an a.a substitution, R152H, predicted to be damaging to the structure of the DAND5 protein. We reproduced this mutation in the mouse Cerl-2 and preliminary results indicated that this alteration cause a derepression of Nodal signaling when compared with the wild-type Cerl-2.

REFERENCES:
1. Ransom J, Srivastava D. The genetic s of cardiac birth defects. Sem in Cell & Dev Biology 18 (2007) 132–139;
2. Hoffman et al, The incidence of congenital heart disease. Journal of the American College of Cardiology (2002); 39:1890 –900.
3. V.Garg, Insights into the genetic basis of congenital heart disease. Cell. Mol. Life Sci. (2006); 63: 1141–1148.
4. Levin M., The embryonic origins of left-right asymmetry. Crit Rev Oral Biol Med (2004); 15(4):197-206.
5. Krista L. C lark, Katherine E. Yutzey, and D.Woodrow Benson. Transcription factors and congenital heart defects. Annual. Rev. Physiol. 2006. 68:97–121
6. Marques, S., et al., The activity of the Nodal antagonist Cerl-2 in the mouse node is required for correct L/R body axis. Genes & Dev (2004); 18: 2342-2347.

# 18 Myocardial titin hypophosphorylation importantly contributes to heart failure with preserved ejection fraction in a rat metabolic risk model

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INTRODUCTION: Among all the patients that suffer heart failure, approximately 50% present heart failure with preserved ejection fraction (HFPEF).[1] Metabolic risk factors, such as obesity and diabetes mellitus, are known to be associated with myocardial diastolic dysfunction (DD) through collagen deposition or titin modification.[2,3] To elucidate the mechanisms underlying myocardial dysfunction in metabolic risk related HFPEF, the present study investigated the importance of collagen deposition and titin modification in obese, diabetic ZSF1 rats.

Materials and Methods: Four groups of rats (Wystar Kyoto, n=11; lean ZSF1, n=11; obese ZSF1, n=11 and obese ZSF1 with high fat diet, n=11) were followed over 20 weeks with repeat metabolic, renal and echocardiographic evaluations and hemodynamically assessed at sacrifice. Myocardial collagen, collagen crosslinking, titin isoforms phosphorylation were also determined. Resting tension (Fpassive)-sarcomere length relations were obtained in small muscle strips before and after KCl-KI treatment, which unanchors titin and allows contributions of titin and extracellular-matrix to Fpassive to be discerned.

RESULTS AND DISCUSSION: At 20 weeks of age the lean ZSF1 group was hypertensive whereas both obese ZSF1 groups were hypertensive and diabetic. Compared to lean ZSF1 rats or WKY rats, obese ZSF1 rats showed many features of high metabolic risk such as visceral obesity evident from elevated perirenal and perigonadal fat, insulin resistance, hyperglycemia and physical inactivity evident from striated muscle wasting. Only the obese ZSF1 groups had developed HFPEF, which was evident from increased lung weight, preserved left ventricular (LV) ejection fraction and LV DD. The underlying myocardial DD was obvious from high muscle strip stiffness, which was largely (±80%) attributable to titin hypophosphorylation. The latter occurred specifically at the S3991 site of the elastic N2Bus segment and at the S12884 site of the PEVK segment.

CONCLUSIONS: Metabolic abnormalities were observed in obese ZSF1 rats after HFPEF development at 20 weeks of age. Titin hypophosphorylation importantly contributed to the underlying myocardial DD.

REFERENCES:

1. 1. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. Eur Heart J. 2011;32:670-679.

2. Zile MR, Gottdiener JS, Hetzel SJ, McMurray JJ, Komajda M, McKelvie R, Baicu CF, Massie BM, Carson PE. Prevalence and significance of alterations in cardiac structure and function in patients with heart failure and a preserved ejection fraction. Circulation. 2011;124:2491-2501.

3. Leite-Moreira AF. Current perspectives in diastolic dysfunction and diastolic heart failure. Heart. 2006;92:712-718.

# 19 Adenosine $A_{2B}$ positive inflammatory infiltrates contribute to right ventricular (RV) failure in rats with monocrotaline-induced pulmonary arterial hypertension (PAH)

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INTRODUCTION: Right heart competence is an important predictor of morbidity and mortality in patients suffering from pulmonary arterial hypertension (PAH). Adenosine is a ubiquitous signaling molecule that is capable of modulating cardiac function through the interaction with four subtypes of P1 purinoceptors ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$). This nucleoside has been implicated in the pathogenesis of PAH by regulating pulmonary vascular tonus. In this context, we aimed at evaluating the role of adenosine on right heart dysfunction associated to PAH.

MATERIALS AND METHODS: PAH was induced in male Wistar rats by a single subcutaneous injection of monocrotaline (60 mg/kg; MCT group) (see e.g. Henriques-Coelho et al., 2004); control animals received the same volume of saline (NaCl 0.9%; CTRL group). Myographic recordings were performed in spontaneously beating atria and in right ventricle (RV) strips paced electrically at 2 Hz-frequency in order to evaluate the cardiac role of $A_1$ and $A_2$ adenosine receptors. Immunolocalization studies were performed by confocal microscopy. Experiments were performed 21 to 25 days after monocrotaline administration.

RESULTS AND DISCUSSION: Activation of the $A_1$ receptor with R-PIA (0.001-1 μM, selective $A_1$ receptor agonist) and NECA (0.01-100 μM, non-selective P1 receptors agonist) produced negative chronotropic and inotropic effects on spontaneously beating atria isolated from both MCT and CTRL animals. Neither of these agonists produced any measurable effect on paced RV strips, despite high expression levels of the $A_1$ receptor were demonstrated in the RV myocardium by confocal microscopy. Selective activation of $A_{2A}$ and $A_{2B}$ receptors respectively with CGS 21680 (0,003-1 μM) and BAY 60-6583 (0,01-10 μM) did not change atrial function. Interestingly, NECA (0.01-100 μM) concentration-dependently increased the contractile force of paced RV strips pretreated with the selective $A_{2B}$ receptor antagonist, PSB 603 (100 nM). This result suggests that activation of $A_{2B}$ receptors in the RV operates a mild negative inotropic effect, which is partially counteracted by a yet undetermined NECA-sensitive P1 receptor. Immunolocalization studies confirmed that RV cardiomyocytes from CTRL and MCT rats express small amounts of $A_{2B}$ receptors. Notably, the RV myocardium of animals treated with MCT present substantial amounts of $A_{2B}$ positive cell infiltrates at interstitial spaces exhibiting reactivity against CD11b (macrophages), CD4 (T lymphocytes) and vimentin (fibroblasts) cell markers.

CONCLUSIONS: Data show that $A_1$-receptor-mediated negative chronotropic and inotropic effects are preserved in spontaneously beating atria of monocrotaline-injected PAH rats. Results also suggest that myocardial infiltration by $A_{2B}$ positive inflammatory cells may, at least partially, contribute to RV failure associated with PAH. This finding expands data showing that activation of $A_{2B}$ receptor on macrophages plays an active role in the pathogenesis of lung fibrosis and PAH (Karmouty-Quintana *et al.*, 2012) and suggests that adenosine signaling through $A_{2B}$ receptors may impact the progression of PAH at multiple tissue levels.

REFERENCES:
1. Henriques-Coelho et al. (2004). Endogenous production of ghrelin and beneficial effects of its exogenous administration in monocrotaline-induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 287: H2885-2890;
2. Karmouty-Quintana et al. (2012) The $A_{2B}$ adenosine receptor modulates pulmonary hypertension associated with interstitial lung disease. FASEB J. 26: 2546-57.

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# 20 Measurement of plasma adenosine as a putative biomarker of canine heart failure

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INTRODUCTION: Heart failure (HF) is a clinical syndrome associated to high morbidity and mortality rates in Human and Veterinary Medicine. Adenosine (ADO) is a retaliatory metabolite involved in the pathophysiology and progression of HF. In humans, plasma ADO is increased in patients with HF (Funaya *et al.*, 1997; Franceschi *et al.*, 2009). The origin of elevated plasma ADO in HF patients is still a matter of debate. Unfortunately, the basal plasma levels of the nucleoside in healthy individuals vary considerably among different studies, making this parameter difficult to use as an accurate measure for medical decision / follow-up. This may be because there is no standardized method to quantify plasma ADO levels (Ramakers *et al.*, 2008). This study was designed to test a standardized technical procedure to collect, isolate and quantify plasma ADO from the canine jugular vein by RP-HPLC-DAD. The sensitivity of determined plasma ADO to discriminate dogs with HF from healthy controls was tested using Receiver Operating Characteristic (ROC) graphs; the area under the ROC curve has been taken as a measure to distinguish between hit rates from false alarm rates of classifiers.

MATERIALS AND METHODS: Fourteen dogs were grouped according to the etiology of HF and categorized using the ISACHC scale (11.8±0.7 yrs old; weight: 21.6±4.0 Kg). Among the HF group, nine dogs had degenerative mitral valve disease (DMVD) and five dogs had dilated cardiomyopathy (DCM). The control group consisted of eighteen healthy dogs (3.3±0.6 yrs old; weight: 17.1±2.7 Kg). Upon blood sample collection, care was taken to avoid rapid *ex vivo* ADO inactivation; this was done by immediate supplementation of every blood sample with a chilled blocking solution containing (i) dipyridamole (20 µM, nucleoside uptake inhibitor), (ii) EHNA (50 µM, ADO deaminase inhibitor), and (iii) EDTA (4.2 mM, $Ca^{2+}$ chelator). After centrifugation, the plasma was stored at -80ºC until ADO determination. Stored samples were deproteinated by ultrafiltration (AMICON® Ultra - 0.5 ml, 50K Filters) and concentrated by lyophilization, before RP-HPLC-DAD analysis.

RESULTS AND DISCUSSION: Plasma ADO levels were higher ($P<0.01$) in dogs with HF as compared to healthy controls (456±101 nM vs 148±24 nM, respectively). We observed a positive correlation between plasma ADO levels and the severity of HF using the ISACHC scale. Plasma ADO levels did not correlate with dog's age. The most significantly difference between HF and control groups was found in dogs with small and medium body weight (<20 Kg) (381±75 nM vs 122±22 nM; $P<0.0001$).

CONCLUSIONS: Here, we described a high sensitive/specific standardized procedure to quantify plasma ADO by RP-HPLC-DAD, which should be followed both in Human and Veterinary Medicine for the sake of accuracy of ADO measurements in blood samples. A high area under the ROC curve (0.829; 95% CI 0.69–0.97; $P<0.05$) is consistent with plasma ADO levels being a highly sensitive biomarker for assessing HF in dogs, which may be useful for medical decision making.

REFERENCES:
1. Franceschi et al. (2009) Heart 95(8): 651-5; Funaya et al. (1997) Circulation 95(6): 1363-5; Ramakers et al. (2008) Curr Drug Metab 9(8): 679-85.

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# 21 Reverse remodelling in wistar rats after surgical removal of chronic pressure-overload

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INTRODUCTION: Incomplete myocardial reverse remodeling (MRR) is a major determinant for patient worse outcome1. We aim to establish and characterize an animal model that mimics the structural and functional changes in MMR after chronic pressure-overload (PO) relief.

METHODS: PO was established in male Wistar rats by ascending aortic constriction. Five weeks later, an echocardiographic evaluation was performed to assess cardiac function. Subsequently, a surgical debanding procedure (Deb) was performed in half of the banding (Ba) and the corresponding control group (Sham), resulting in 2 additional groups: Ba_Deb and Sham_Deb. The cardiac function was monitored weekly by echocardiography and, at the end of the protocol pressure-volume loops were analyzed, active and passive tensions of myofilaments were measured, AGES deposition and collagen I, III PCP, PCPE and Lysis oxidase (LOX) expression was quantified.

RESULTS: Compared to Sham, PO increased LV end-systolic (ESP, Sham vs Ba: 1124±6.7 vs 168.2±6.7mmHg, p=0.001) and end-diastolic pressure (EDP, Sham vs Ba: 5.3±1.1 vs 8.7±0.2mmHg, p<0.001), dP/dtmax (Sham vs Ba: 5627±931 vs 8535±390mmHg/s, p=0.027) and LVmass (Sham vs Ba: 0.78±0.01 vs 1.10±0.05g, p<0.001). Moreover, Ba presented impaired relaxation (τ, Sham vs Ba: 7.8±04 vs 94±1. ms, p=0.046), increased AGES deposition (Sham vs Ba: 5.2±0.5 vs 10.9±2.0, p<0.001) and increase myofilaments passive force (Sham vs Ba: 5.0±0.5 mN/mm2 vs 6.6±0.7 mN/mm2 SL=2.3µm, p<0.05). Despite the presence of diastolic dysfunction (DD), ejection fraction was preserved and myofilaments active force and procollagen I expression were similar between groups. After Deb procedure hemodynamic parameters normalized (Ba_Deb vs Sham_Deb: ESP, 101.3±34 vs 102.2±8.2mmHg; EDP, 44±0.8 vs 4.9±0.7mmHg; dP/dtmax, 7028±565 vs 6977±646mmHg/s; τ, 7.1±0.5 vs 7.7±04 ms). Additionally, LVmass (Ba_Deb vs Sham_Deb: 0.7±0.1 vs 0.7±0.1 g), passive force and extracellular matrix changes, such as AGES deposition, collagen type III and LOX increased expression values normalized.

CONCLUSIONS: A complete regression of LVmass and DD was observed with PO-removal. We successfully characterized an animal model that mimics aortic stenosis and subsequent aortic valve replacement, opening new possibilities for mechanistic and pharmacological studies.

REFERENCES:
1. Gavina C et al. Load independent impairment of reverse remodeling after valve replacement in hypertensive aortic stenosispatients. Int J Cardiol. 2014

# 22 A Role for Neuro-Induced Cardiac Stress in Autoimmunity

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INTRODUCTION: What defines which tissue is targeted in organ-specific autoimmune disorder is still ill defined. Mouse strains show different susceptibility to Experimental Autoimmune Myocarditis (EAM) upon infection, genetic manipulation or cardiac antigen immunization[1]. Balb/C (BA) mice are susceptible to EAM while C57BL/6 mice are not. We hypothesize that beyond TCR repertoire selection, the target tissue may participate to its own demise by responding inadequately to stress. In turn, specific physiological states may be chronically stressing the organ thus explaining strain differences. Imbalanced serotonergic neurotransmission is pathological and results in stress-induced cardiac disturbances2. Balb/C mice constitutively produce lower amount of serotonin in the central nervous system, as consequence of a SNP in the gene encoding tryptophan hydroxylase 2 (*Tph2*), the rate-limiting enzyme in the synthesis of this neurotransmitter3.

METHODS: Using EAM as a disease model, we question whether neurological signals, through their effects on heart function, define disease susceptibility.

RESULTS: We implemented a model of immunization induced EAM in BA mice and confirmed that B6 animals are disease resistant. Strikingly, immunization elicited high anti-myosin specific antibodies (Ab)production, equally in BA and B6 mice. We next questioned whether BA and B6 mice show different heart features at steady state and evidenced occasional calcinosis and increased frequency of neutrophils and T Cells in susceptible animals. We also demonstrate that BA mice are more susceptible than B6 animals to stress induced cardiomyocyte damage, as indicated by higher troponin release upon isoprenaline (ISO) administration. Interestingly this susceptibility is reduced in lymphocyte deficient BA SCID mice. Finally we show that the EAM susceptible strains BA, DBA/2 and C3H-HeN all carry the same non-synonymous SNP in the *Tph2* gene.

DISCUSSION: Upon immunization, the same titers of myosin-specific auto-Ab are generated in both strains, suggesting that EAM susceptibility is not a property of the immune system. Spontaneous calcium deposits occur as a consequence of soft tissue damage, in this case indicating that WT BA mice hearts, but not B6, are exposed to lesions. Different heart resident leukocyte composition together with different cardiomyocyte susceptibility to ISO induced damage, reinforce the difference between the hearts of the two strains. Nevertheless, the lymphopenic model shows that lymphocytes contribute, at least in part, to accentuate BA and B6 steady state cardiac differences. All the susceptible strains tested have the same SNP in the *Tph2* gene, previously associated with congestive heart failure in humans.

CONCLUSIONS: These preliminary data support a scenario by which susceptibility to EAM is pre-conditioned by physiological neuronal stress affecting the heart. Modulating this pathway in both BA and B6 mice, chemically and genetically, before inducing EAM will serve to formally test this model.

REFERENCES:

1. Jennifer M. Myers et al., Autoimmune Myocarditis, Valvulitis, and Cardiomyopathy, Current Protocols in Immunology, 2013. DOI:10.1002/0471142735.im1514s101

2. Andrew G. Ramage et al., 5-Hydroxytryptamine and Cardiovascular Regulation, Trends in Pharmacological Sciences, 2008. DOI:10.1016/j.tips.2008.06.009

3. Xiaodong Zhang et al., Tryptophan Hydroxylase-2Controls Brain Serotonin Synthesis, Science, 2004. DOI: 10.1126/science.1097540

# 23 Scaling-up the *ex vivo* expansion and fortification of circulating human progenitor cells

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INTRODUCTION: The therapeutic properties of Circulating Progenitor Cells (CPCs) (i.e. $CD34^+$ cells, including endothelial progenitor cells -EPCs) have been demonstrated in several pre-clinical and clinical studies, namely for the treatment of ischemic and cardiovascular diseases (1-4). However, the therapeutic application of these cells has been hampered by the difficulty in isolating enough number of circulating $CD34^+$ cells from patient's blood while maintaining their quality for clinical use. To overcome this limitation, different suspension culture systems have been established for the *ex vivo* expansion of these cells, which demonstrated efficiency in promoting neovascularization in ischemic animal models upon transplantation. Of notice, our group had previously established a suspension culture system for human EPCs, with increased quantity and quality (QQ) of the cell product (5). Still, there is the need to scale-up this culture method by moving towards a dynamic and fully controlled bioreactor system in order to meet GMP standards and clinically meaningful cell doses.

MATERIALS AND METHODS: CD34+ cells isolated from mobilized peripheral blood of healthy donors were ex vivo expanded for 7 days in QQ medium in 30 mL Cell Culture Bags and pre and post expansion cells were characterized by flow cytometry (Stem/Progenitor, Myeloid and Lymphoid markers), qPCR (anti- and pro-inflammatory markers, senescence/apoptosis) and their angiogenic potential assessed by *in vitro* tube formation assay.

RESULTS AND DISCUSSION: Our data shows a successful cell expansion (7-fold), while maintaining the stem/progenitor population and increasing the endothelial population. Moreover, cultured cells showed higher tube formation capacity. Adding to this, an up-regulation of the anti-inflammatory gene expression and a down-regulation of pro-inflammatory genes were observed, which preliminarily indicates an increase in angiogenic potential without a parallel increase in the inflammatory profile.

CONCLUSIONS: The QQ expansion method, previously established using tissue culture plates, was successfully scaled-up to cell culture bags, able to meet GMP standards and expanded cells showed higher *in vitro* angiogenic profile compared to uncultured cells. Still, further studies need to be completed to confirm the safety and functionality of the expanded cells (*in vivo* studies).

REFERENCES:
1. Asahara T. Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science. 1997;275(5302):964-6.
2. Assmus B. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation. 2002;106(24):3009-17.
3. Losordo DW, Kibbe MR, Mendelsohn F, Marston W, Driver VR, Sharafuddin M, et al. A randomized, controlled pilot study of autologous CD34+ cell therapy for critical limb ischemia. Circulation Cardiovascular interventions. 2012 Dec;5(6):821-30.
4. Alaiti MA, Ishikawa M, Costa MA. Bone marrow and circulating stem/progenitor cells for regenerative cardiovascular therapy. Translational research : the journal of laboratory and clinical medicine. 2010 Sep;156(3):112-29.
5. Masuda H, Iwasaki H, Kawamoto A, Akimaru H, Ishikawa M, Ii M, et al. Development of serum-free quality and quantity control culture of colony-forming endothelial progenitor cell for vasculogenesis. Stem cells translational medicine. 2012 Feb;1(2):160-71.

# 24 Mapping the cell's membrane signature of the developing mouse heart

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INTRODUCTION: The combined expression of surface molecules defines cellular populations and enables a comprehensive assessment of their well-preserved phenotypic and functional properties following isolation. Cell surface protein signatures have originally been successfully applied in the hematopoietic system to discriminate hierarchical relationships of the distinct blood lineages. Moreover, knowledge on the unique cell signatures allied to the current power of *in situ* analytical methods may provide deeper insight of the cells' dynamics within their native context, *i.e.* the tissue/organ microenvironment.

Despite the indication in recent years of a number of membrane proteins that can aid on identifying cardiomyocytes (*i.e.* SIRPα for humans[1], or VCAM-1 for developing mouse myocytes[2]), the signature of the different cell types and namely that of the stromal repertoire of the heart, remains largely elusive. The same applies to the anatomical distribution and function of such cells, which altogether precludes the definition of a hierarchical relationship for cardiovascular progenitors and their progeny throughout time. To overcome these major bottlenecks of the cardiovascular field, we set out to perform a detailed transcriptional characterization of the distinct cellular subsets engaged in cardiogenesis. Aiming to establish a hierarchy of the cardiac populations, this analysis was performed along heart development.

MATERIALS AND METHODS: To analyze the cell subsets actively engaged in cardiogenesis, a detailed phenotypic evaluation of the heart constituents throughout development was performed by flow cytometry and *in situ* immunostaining. Preferential distribution of the cellular subsets along the different heart structures (atria, auriculo-ventricular junction and ventricles) was also evaluated. The transcriptional profile of identified populations was accessed by multiplex qRT-PCR for critical genes expressed during heart development and maturation by Biomark™ (Fluidigm Corporation).

RESULTS AND DISCUSSION: The frequency of Ki67-expressing cells through embryonic development evidenced a decrease on the heart's proliferative activity. Interestingly, we have identified a panel of surface markers, some of them novel to the cardiac field, which enable discrimination of the proliferative cell fraction, at each time-point and in the 3 heart regions analyzed. Considering the transcriptional profile of the identified cellular subsets, it was also possible to cluster them in 4 main groups.

CONCLUSIONS: The identification of unique cell surface signatures for distinct cardiac subsets constitutes an exceptional tool to further characterize cell populations actively engaged in building the heart. This knowledge will facilitate in future aid to the tracking of cells during cardiovascular repair and/or regeneration. Our results and overall strategy towards the comprehensive mapping of the dynamics of cell subsets in cardiogenesis will be presented.

REFERENCES:
1. Dubois N. NAT BIOTECHNOL. 2011. 29(11): 1011–1018.
2. Pontén A. PLoS ONE. 2013. 8(12): e82403.

# 25 Ubiquitin targets Connexin43 localized at the intercalated discs in ischemic heart

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Efficient electric activation and action potential propagation in the heart largely depends on gap junction (GJ) channels, formed by connexins (Cx) localized at the intercalated discs (IDs)[1]. Therefore, fine-tuning and maintenance of GJ in cardiomyocytes is essential for normal heart function. Several mechanisms have been implicated in the regulation of the amount of Cx43 at the plasma membrane[2]. Results from our lab demonstrated that Nedd4-mediated ubiquitination of Cx43 signals internalization and degradation of GJ[3,4]. However, the pathophysiological relevance of this mechanism has never been addressed before. The main objective of this study was to evaluate the involvement of ubiquitination on GJ remodeling in the ischemic heart. To address this hypothesis we used the rat heart Langendorff model and evaluated the ubiquitination profile of Cx43 and its interaction with Nedd4, after 30 minutes of no-flow ischemia. By confocal microscopy, we show that ischemia induces dephosphorylation of Cx43 and its redistribution to the lateral membranes of the cardiomyocytes. Moreover, we demonstrate that ischemia leads to extensive co-localization of ubiquitin and Nedd4 with Cx43 localized at IDs. Subcellular fractionation and co-immunoprecipitation assays corroborate these results, showing an increased interaction with Nedd4 and ubiquitination of Cx43 localized at IDs. Additionally, by the use of TUBES (tandem ubiquitin binding entities), we were able to demonstrate that in these conditions Cx43 is modified with K63-polyubiquitin chains. Altogether, the results obtained in this study show that ischemia results in the recruitment of Nedd4 to the IDs where it interacts with Cx43. Moreover, we demonstrate that ubiquitination of Cx43 localized at IDs increases in ischemia, suggesting that ubiquitin constitutes a new regulatory signal in GJ remodeling associated with cardiac ischemic events. Given the importance of a fine-tune regulation of the processes required to ensure a correct and efficient GJIC between cardiomyocytes, a better elucidation of the mechanisms involved in Cx43 ubiquitination may open new avenues towards the identification of potential therapeutic targets to preserve GJIC in ischemic heart.

REFERENCES:
1. Rohr S: Role of gap junctions in the propagation of the cardiac action potential. Cardiovasc Res2004, 62:309-322.
2. Duffy HS: The molecular mechanisms of gap junction remodeling. Heart Rhythm 2011, 9:1331-1334.
3. Catarino S, Ramalho JS, Marques C, Pereira P, Girao H: Ubiquitin-mediated internalization of connexin43 is independent of the canonical endocytic tyrosine-sorting signal. Biochem J 2011, 437:255-267.
4. Bejarano E, Girao H, Yuste A, Patel B, Marques C, Spray DC, Pereira P, Cuervo AM: Autophagy modulates dynamics of connexins at the plasma membrane in a ubiquitin-dependent manner. Mol Biol Cell 2012, 23:2156-2169.

# 26 Modulation of Survivin Reverses Cardiac and Pulmonary Remodeling in Monocrotaline-induced Pulmonary Arterial Hypertension

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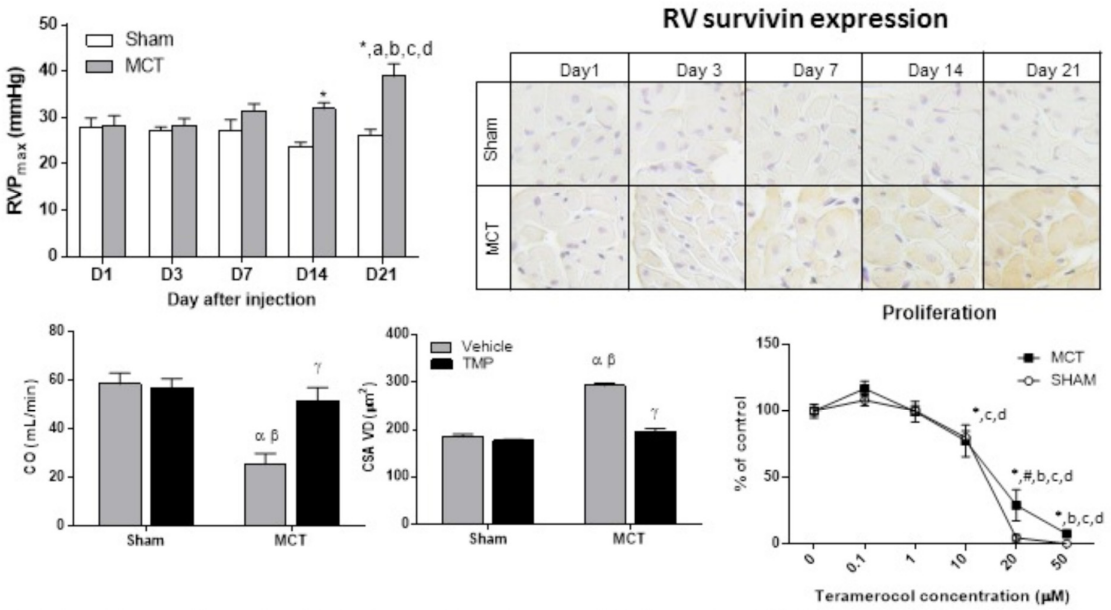
INTRODUCTION: Cardiac and pulmonary expression of the inhibitor of apoptosis survivin [1, 2] and its endogenous inhibitor smac was characterized throughout the progression of monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH). Therapeutic effects of the survivin antagonist terameprocol (TMP)[3] were assessed, both *in vitro* and *in vivo*.

MATERIALS AND METHODS: Protocol I: adult male Wistar rats received a subcutaneous injection of MCT (60 mg/Kg) or equal volume of vehicle. On days 1, 3, 7, 14 and 21 after injection (n=7-12 per group per time-point), right ventricular (RV) pressures were measured, heart and lungs were weighted and collected for histological analysis, immunohistochemistry and western blot. Protocol II: adult male Wistar rats injected with MCT were treated with TMP (166 mg/Kg, ip; MCT-TMP, n=7) or vehicle (MCT-V, n=9) on days 7, 12 and 17 after injection and compared with a SHAM group (n=7). On day 21, cardiac output and RV pressures were measured and heart and lungs were weighted and collected for histological analysis. Protocol III: Primary cultures of pulmonary artery smooth muscle cells (PASMC) were established from sham and MCT-treated rats (day 21) in order to assess the effects of TMP in proliferation and apoptosis.




*p < 0.05 vs. Sham of same day, #p < 0.05 vs. Sham of same TMP dosage ᵃp < 0.05 vs. D14 of same group, ᵇp < 0.05 vs. D7 of same group, ᶜp < 0.05 vs. D3 of same group ᵈp < 0.05 vs. D1 of same group ᵅp < 0.05 vs. Sham+V, ᵝp < 0.05 vs. Sham+TMP and ᵞp<0.05 vs. MCT+V.

FIGURE 1

RESULTS AND DISCUSSION: Survivin upregulation and smac downregulation were present since day 7 after MCT injection and preceded the hemodynamic manifestations of PAH. Increases in RV peak systolic pressure, dP/dtmax and dP/dtmin were only present since day 14. *In vivo*, TMP treatment reduced RV hypertrophy and pulmonary arterial wall thickness, decreased RV peak systolic pressure, dP/dtmax and dP/dtmin and normalized cardiac output. TMP inhibited proliferation and induced apoptosis of PASMC in a dose-dependent manner (Figure1).

CONCLUSIONS: Our findings suggest that survivin has an important role in PAH. TMP could be an effective and highly selective therapeutic strategy for PAH by reversing cardiac and pulmonary remodeling and improving hemodynamic features.

REFERENCES:
1. Levkau, B., et al., Survivin determines cardiac function by controlling total cardiomyocyte number. Circulation, 2008. 117(12): p. 1583-93.
2. McMurtry, M.S., et al., Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. J Clin Invest, 2005. 115(6): p. 1479-91.
3. Chang, C.C., et al., Tetra-O-methyl nordihydroguaiaretic acid induces growth arrest and cellular apoptosis by inhibiting Cdc2 and survivin expression. Proc Natl Acad Sci U S A, 2004. 101(36): p. 13239-44.

# 27 From Stem Cells 2 Heart: Finding novel candidate genes for cardiomyogenesis

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INTRODUCTION: Heart development is an intriguingly complex process, which is tightlyregulated. To understand how this process is controlled and coordinated, it is necessary to study the underlying gene expression and its regulation. While many studies have been carried out in the examination of single genes and their expression patterns, comprehensive analyses of genome-wide expression profiles associated with cardiomyogenesis (i.e. the differentiation of stem cells into cardiomyocytes) are still rare. Combining computational approaches using genome-wide expression data and subsequent experimental validation, we aim to identify a set of novel genes that are involved in the early stages of cardiac differentiation.

MATERIALS AND METHODS: We have carried the first comparative analysis of transcriptomics datasets associated with cardiomyogenesis. To narrow down the list of candidates genes, we searched for genes, which have not been linked to stem cell differentiation or heart development so far. Additional gene expression datasets for *in vivo* and *in vitro* differentiation were examined to confirm the validity of obtained results.

RESULTS AND DISCUSSION: Based on comparative analysis of transcriptomics data and text mining, we selected 14 candidate genes that display similar expression patterns to known early cardiac markers and have yet not been associated with stem cell differentiation or heart development. Notably, several selected genes followed closely the expression profiles of mesodermal markers such as *T/Brachyury* or the early cardiac markers *Mesp1* and *Gata4* suggesting a role in the early decision of cells towards the cardiac lineage. Alternatively, we detected other genes with regulatory potential, whose expression resembles *bona fide* cardiac markers *Mef2c*, *Isl1*, *Nkx2*.5, *Tbx20* and *Tbx5*. We are currently performing qPCR analyses for the set of candidate genes together with early cardiac gene markers (such as *Nkx2*.5 and *Gata4*), markers of the FHF (*Hand1*) and SHF lineages (*Isl1*) and late cardiac markers (*cTnt* and *Myh6*) in order to validate them *in vitro*.

CONCLUSIONS: Combining different data resources and bioinformatics tools, we identified a set of candidate genes, which are expressed in a similar manner as established mesodermal and cardiac markers. We confirmed this selection through *in silico* analysis of several published cardiac transcriptomics datasets as well as a recently generated RNA-seq of mouse ESC differentiated *in vitro*. We believe that our approach helps to discover novel regulatory genes that have remained undiscovered so far, and is an efficient methodology to select candidate genes for further validation using knockdown and overexpression experiments. Finally, we have developed a web-based database called HeartEXpress, which combines various cardiogenesis associated expression datasets and includes tools for data visualization and analysis. HeartEXpress is freely accessible at **http://heartexpress.sysbiolab.eu**.

# 28 High-throughput identification of small molecules that affect human embryonic vascular development

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INTRODUCTION: The development of new technologies and tools for the rapid toxicological profiling of chemical/pharmaceutical substances, at cellular levels, are of great need to reduce and refine the use of animals in research. Here we report a platform combining a microfluidic system with arterial endothelial cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) for the high-throughput screening of compounds that interfere with embryonic vasculature.

MATERIALS AND METHODS: Embryonic arterial endothelial cells (EAECs) were differentiated from hESC or iPSCs and characterized at protein, gene and functional levels. The response of the cells to arterial (20 dyne/cm$^2$) and venous (4 dyne/cm$^2$) shear stress was evaluated on a microfluidic system. To study embryonic vascular toxicity, we exposed EAECs to a library of 1280 pharmacologically active compounds and assessed cell viability after 4 days. We evaluated the hits in a microfluidic system and finally *in vivo*.

RESULTS AND DISCUSSION: We report an inductive differentiation protocol that can give rise to endothelial precursor cells that have the ability to differentiate into arterial ECs. This corresponds to a 2.5-fold enrichment as compared to spontaneous cell differentiation. The cells express arterial genes such as JAG1, ephrin B1 and Hey-2 and the arterial protein EphB2. The cells respond to arterial flow by aligning in the direction of the flow and further up-regulating the expression of arterial genes. The process is likely mediated by heparan sulfate proteoglycan (HSPG), a component of glycocalyx, which is activated by fluidic shear stress. We demonstrate the relevance of our system formed by arterial ECs cultured under flow conditions for toxicology assessment. We show that hPSC-derived ECs have higher sensitivity to terbinafine, an antifungal drug, than somatic human umbilical arterial ECs. Cells cultured under physiologic shear stress have higher sensitivity to terbinafine than cells cultured in static conditions. We further show that our system can be used to identify embryonic endothelial cell inhibitors. From 1280 compounds screened we show that 7-cyclopentyl-5-(4-phenoxy)phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine) induces high EAEC cytotoxicity.

CONCLUSIONS: We report the first platform for the high-throughput screening of drugs against embryonic vasculature under flow.

# 29 Pathological remodeling of cardiac gap junction connexin 43 by chaperon-mediated autophagy

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Gap junction intercellular communication (GJIC), through membrane channels formed by Connexins (Cx), being Cx43 the major GJ protein found in the heart. Cx43 ensures effi-cient electric activation and action potential propagation. Several cardiomyopathies have been associated with an impairment of GJIC, very often due to an increased internaliza-tion and/or degradation of Cx43. We have shown that GJIC can be regulated through macroautophagy-mediated degradation of Cx43-containing GJ. However, when macroau-tophagy is impaired with 3-MA the degradation of Cx43 is only partially inhibited, which suggest that alternative pathways underling Cx43 remodeling. Consistently, a parallel study carried out in our lab demonstrated that Cx43 is a substrate for chaperone-mediated autophagy (CMA), a mechanism whereby soluble cytosolic proteins bearing the KFERQ motif are selectively degraded in the lysosome, via the lysosome-associated membrane protein type 2A (LAMP-2A). In the present study, we aim to elucidate the physiological relevance of such mechanism in pathological conditions, namely in the hy-pertrophic heart. We have found that angiotensin II-induced hypertrophy in cultured car-diomyocytes leads to an increase in the degradation of Cx43 with a concomitant increase in the interaction between Cx43 and CMA-related chaperon Hsc70 and receptor LAMP2A. These observations were consistent with the increase in mRNA levels of Cx43 and alpha skeletal actin, a marker for cardiac hypertrophy. Moreover, hearts from 7-week trans-verse abdominal aortic constriction rats showed higher interaction of Cx43 with CMA adaptors than hearts from sham-operated rats. Also in hypertrophic myocardium samples from patients undergoing surgery we observed that Cx43 interacts with Hsc70 and LAMP2A, suggesting that this mechanisms is relevant in human heart proteolysis and disease. Overall these results support a model in which CMA targets Cx43 for degradation and that this mechanism may be associated with disease.

# 30 Fetal And Adult-Derived ECM As 3D-Culture Systems: Composition, Structure And Cardiac Cell Preferences

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INTRODUCTION: The paradigm of mammalian heart as a post-mitotic organ has been challenged following a set of reports displaying the heart as an organ capable of histo-functional restoration following injury in a period confined to fetal-neonatal transition [1, 2]. Although different mechanisms have been implicated in this process, a full understanding of the transient regenerative capacity of the mammalian heart is still far from reach. Owing to the well-recognized role of extracellular matrix (ECM) in heart morphogenesis and growth, we hypothesize that ECM provides developmental-stage specific cues that govern cell phenotype and thus impact on tissue functional recovery in an injury scenario. Our work aims to unveil how the 3D specific arrangement of fetal (E18) and adult cardiac tissue affects cardiac cell behavior.

MATERIALS AND METHODS: Fetal hearts and adult left ventricle explants were decellularized by SDS-based protocol. The developed acellular scaffolds were further analyzed by optical and electron microscopy in order to confirm decellularization efficiency and maintenance of key ECM molecules. Cardiac progenitors cells ($Lin^{-}Sca\text{-}1^{+}$ [3]) and neonatal cardiomyocytes were passive seeded onto the scaffolds and cell-viability, -morphology and -phenotype were addressed in the interior of the matrices.

RESULTS AND DISCUSSION: Fetal and adult native cardiac ECM were captured by a novel technology [4] which enables the parallel decellularization of whole-fetal and adult-heart explants thereby ensuring reliable comparison. The produced acellular scaffolds display a complex network, preserving essential ECM components and evidencing intrinsic differences on its composition and rearrangement. Contrary to adult, fetal-derived ECM presents a fine fibrillar organization and a higher content in fibronectin. *In vitro* 3D culture of fetal and adult ECM-scaffolds was established with either $Lin^{-}Sca\text{-}1^{+}$ heart-derived progenitors or neonatal cardiomyocytes. Fetal acellular scaffolds revealed as a more amenable microenvironment for cardiomyocytes and cardiac progenitors, as displayed higher colonization indexes. A first sketch of the colonization mechanism(s) which governs the enhanced fetal colonization will be presented.

CONCLUSIONS: Fetal and adult-derived scaffolds i) display a well-preserved porous structure without cell remnants; ii) retain main constituents of the cardiac ECM and preserve native-differences in regard to the spatial distribution/arrangement of fibronectin; iii) sustain the viability of neonatal cardiomyocytes and $Lin^{-}Sca\text{-}1^{+}$ cells, although fetal bioscaffolds revealed to be a more adequate 3D microenvironment for cardiac cell maintenance.

REFERENCES:
1. E.R. Porrello, et al., Science 331 (2011);
2. B.J. Herdrich, et al., European journal of cardio-thoracic surgery: official journal of the European Association for Cardio-thoracic Surgery 38 (2010);
3. A.G. Freire, et al., Stem Cells and Development 23 (2014); [4] D.S. Nascimento, et al., Journal of Tissue Engineering and Regenerative Medicine. 6 (Issue Supplement s1 - Special Issue: 3rd TERMIS World Congress) (2012).

# 31 Identification of Smurf1 as a novel E3 ligase involved in Cx43 ubiquitination: implications for pulmonary hypertension

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Pulmonary Arterial Hypertension (PAH) is a disorders that affects the arteries of the lungs, that became constricted and subsequently more resistant to blood flow. In this case, the blood pressure in pulmonary arteries increases, leading to right ventricle hypertrophy and eventually heart failure. Although is considered a multifactorial diseases, a dysregulation of bone morphogenetic protein receptors 2 (BMPR2) signaling pathway has been often implicated in PAH [1]. BMPR2 interacts with Smad-family proteins for intracellular signaling. Switching off Smad signaling in the cell is achieved by ubiquitination by Smad Ubiquitination Regulatory Factor-1 (Smurf1) [2], further proteasome degradation of Smad. Indeed, Smurf1 is an ubiquitin ligase that catalyzes mono- or polyubiquitylation of proteins target. The highly synchronized and coordinated heart beating relies an rapid and efficient electrical impulse propagation, through the heart muscle cells via Gap Junction (GJ). Previous studies demonstrated that GJ protein connexin43 (Cx43), the major right ventricle connexin, is decreased in PAH, thus suggesting the involvement of Cx43 degradation in development of the disease. However, the mechanisms underlying such decrease are unknown. Results from our lab demonstrated that Nedd4, a HECT ubiquitin ligase, mediated ubiquitination of Cx43, leads to internalization and degradation of GJ [3]. In this study, we hypothesized that Smurf1, an ubiquitin ligase belonging to the Nedd4 family, could be a novel ubiquitin ligase for Cx43, and that this mechanism could be associated with Cx43 decreased observed in animal model of PAH. Our results showed that Smurf1 interact directly with Cx43 and promoting its ubiquitination. Consistently, we observed that Smurf1 overexpression change turnover of Cx43. Together, these data identify Smurf1 as a novel regulatory player in connexin43 gap junctions life-cycle, and suggested a new player in PAH.

REFERENCES:
1. Galie, N., M. Palazzini, and A. Manes, Pulmonary arterial hypertension: from the kingdom of the near-dead to multiple clinical trial meta-analyses. Eur Heart J. 2010; 31(17): p. 2080-6.
2. Lai, Y.L. and T.C. Law, Chronic hypoxia- and monocrotaline-induced elevation of hypoxia-inducible factor alpha levels and pulmonary hypertension. J Biomed Sci. 2004; 11(3): p. 315-21.
3. Bejarano E, Girao H, Yuste A, Patel B, Marques C, Spray DC, et al. Autophagy modulates dynamics of connexins at the plasma membrane in a ubiquitin-dependent manner. Molecular biology of the cell. 2012;23(11):2156-69. Epub 2012/04/13.

# 32 Combining Hypoxia and Bioreactor Hydrodynamics Boosts iPSC Differentiation Towards Cardiomyocytes

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INTRODUCTION: The production of cardiomyocytes (CMs) from induced pluripotent stem cells (iPSCs) holds great promise for autologous regeneration therapies, patient-specific disease modeling and cardiotoxicity testing [1]. However, the widespread use of these cells is still hampered by the lack of robust bioprocesses for the production of CMs in high purity, consistent quality and relevant quantities [2]. The main goal of this study was to develop scalable platforms for the production of pure and functional iPSC-derived CMs. Our strategy consisted in designing integrated bioprocesses for CM differentiation and purification by culturing iPSCs as 3D aggregates in environmentally controlled bioreactors, where the necessary conditions to control stem cell fate are thoroughly tuned [3].

MATERIALS AND METHODS: Our strategy consisted in designing integrated bioprocesses for CM differentiation and purification by culturing iPSCs as 3D aggregates in environmentally controlled bioreactors. Distinct bioreactor systems (stirred tank and Wave bioreactors) were used and different bioprocess parameters were tested. In particular, we explored the impact of the dissolved oxygen (DO) and hydrodynamic forces promoted by the agitation type/mode on CM differentiation.

RESULTS AND DISCUSSION: Our results showed that DO and agitation type/mode are key parameters in the bioprocessing of iPSCs, affecting cell differentiation towards functional CMs. By combining a hypoxia culture (4% $O_2$ tension) with an intermittent agitation profile in stirred tank bioreactors, we were able to improve about 1000-fold the differentiation yields (up to 43 CM per initial iPSC) and achieve high CM purities (>97%) after 16 days of culture. Moreover, this study shows for the first time that wave-induced agitation enhances further CM differentiation of iPSCs, increasing CM differentiation yields up to 60, while reducing the time needed for differentiation process. In this strategy, an earlier and higher expression of mesoderm and cardiac specific genes was detected when compared to stirred tank bioreactor cultures. In an 11-day differentiation protocol, 2.3±0.1 x$10^6$ CM/mL were obtained with high purity (97%) and quality in 1 L bioreactor run. Morphological and functional characterization analysis confirmed that CMs presented typical cardiac morphology, calcium transients, electrophysiological profiles and drug responsiveness.

CONCLUSIONS: This study shows a robust bioprocess for the production of pure 3D cardiospheres and functional CMs derived from iPSC in a scalable and reproducible manner, paving the way for the implementation of these cells in the clinical and industrial field.

REFERENCES:
1. Oh Y et al., 2012, Heart, 98: 443; [2] Zwi-Dantsis L et al., 2012, Cell Mol Life Sci, 69: 3285; [3] Serra M et al., 2012, Trends in Biotechnol, 30: 350.

# 33 The role of Cx43 in mediating intercellular communication goes beyond gap junctions

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A correct and efficient communication between the different types of cells that constitute the heart is essential to provide structure, ensure regulated heart contraction, and, ultimately, efficient blood pump. Intercellular communication between heart cells can occur either directly, via gap junctions (GJ), between adjacent cells, or over long distances through extracellular vesicles. For example, GJ intercellular communication between cardiomyocytes, mediated by connexins (Cx)-containing channels, is vital to ensure the correct and rapid anisotropic electrical impulse propagation, required for a synchronized and coordinated heart beating. Besides direct cell-cell communication, cells can exchange information through extracellular vesicles (EV), released under basal or stress conditions. According to their size, composition and subcellular origin, EVs can be divided in apoptotic bodies, microvesicles and exosomes. Exosomes, that include vesicles with a diameter of 30-100 nm, are formed when multivesicular bodies fuse with the plasma membrane and release their vesicular content into extracellular medium, including cell culture supernatants and body fluids. For these reasons, exosomes have been considered as intercellular messengers that mediate both local and systemic cell communication, through the transference of biological information between donor and acceptor cells. Although it is well established that exosomes can modulate the response of acceptor cells, the mechanisms that govern exosomal uptake are not fully understood and constitute a matter of intense research. Up until now, evidences support a model where exosomal uptake into cells depends on protein interaction between exosomes and target cells. In this study, we hypothesized that Cx43 constitutes a new molecular partner and mediator of the interaction of exosomes with the plasma membrane of cells and, in analogy, Cx43 mediates the communication between exosomes and the cells. The results obtained in this work, using various complementary approaches, including Westernblot, immunofluorescence microscopy, immunogold labelling and transmission electron microscopy, mass spectroscopy and luciferin-luciferase luminescence assays, demonstrate that Cx43 is present in exosomes released by cardiomyocytes, in the form of a hexameric channel and, more importantly, that Cx43 in exosomes is able to modulate exosomal interaction and/or communication with the acceptor cells. In conclusion, our data ascribes a novel unanticipated biological role to Cx43 in mediating the transference of information between exosomes and cells.

# 34 Cardiac biomarkers in newborns with congenital heart defects

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INTRODUCTION: BNP is released from the ventricular myocardium in response to stretching of the ventricular wall[1]. Troponin I, myoglobin and CK-MB are biomarkers of cardiomyocyte injury widely used in the management of adult patients. The role of these biomarkers in neonates is still not established[2,3]. The purpose of this study was to evaluate the diagnostic and prognostic value of cardiac biomarkers in newborns with congenital heart disease (CHD).

MATERIALS AND METHODS: Cohort consecutive study of 54 newborns, 34 with prenatal diagnosis of CHD admitted at the Neonatal Intensive Care Unit and a control group (n=20) of healthy newborns delivered in the same tertiary hospital. Plasma levels of cardiac biomarkers (BNP, troponin I, myoglobin and CK-MB) were evaluated and echocardiogram performed within the first 24 hours of life. Patients were followed during the first 28 days of life (neonatal period) and accordingly with the outcome categorized as surgical or conservative-treatment group.

RESULTS AND DISCUSSION: Median BNP was higher in patients with CHD than in controls (43.3 vs. 19.8 pg/mL; p=0.001). From the 24 patients with CHD with a surgical indication, 10 underwent cardiac surgery during neonatal period. BNP was higher in patients that had cardiac surgery during the neonatal period (73.7 pg/mL) than in those discharged home without surgery (25.6 pg/mL; p=0.016). A BNP cut-off point of 35.85 pg/mL predicted neonatal surgery (sensitivity 90.0% and specificity 64.3%). Median (P25-75) levels of CK-MB were higher in patients that had cardiac surgery in the neonatal period [7.35 (4.90-1340) ng/mL] than in patients who were discharged home without surgery [4.2 (2.60-5.90) ng/mL; p= 0.032]. Troponin I and myoglobin levels were not significantly different between conservative treatment and surgical groups. Troponin I, myoglobin and CK-MB levels were not significantly different between control and CHD group. BNP and CK-MB levels correlated with the tissue Doppler image (TDI) peak early diastolic velocity of the mitral annulus/late diastolic velocity of the mitral annulus ratio (rho= -0.543, p=0.007; rho=-0480, p=0.018, respectively).

CONCLUSIONS: Newborns with CHD presented higher BNP levels than healthy newborns. BNP and CK-MB levels in the first hours of life have prognostic value for neonatal cardiac surgery and may be indicators of diastolic cardiac function.

REFERENCES:
1. Vijlbrief, D.C., et al., Use of cardiac biomarkers in neonatology. Pediatr Res, 2012. 72(4): p. 337-43.
2. Correale, M., et al., Troponin in newborns and pediatric patients. Cardiovasc Hematol Agents Med Chem, 2009. 7(4): p. 270-8.
3. Trevisanuto, D., et al., Cardiac troponin I, cardiac troponin T and creatine kinase MB concentrations in umbilical cord blood of healthy term neonates. Acta Paediatr, 2003. 92(12): p. 1463-7.

# 35 Impact Of Culture Strategies On Transcriptomic And Metabolomic Profiles Of Human Pluripotent Stem Cells

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INTRODUCTION: Human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), have an enormous potential as source for cell replacement therapies, tissue engineering and *in vitro* toxicology applications. The lack of standardized, robust, safe and cost-effective processes to expand hPSC in relevant quantities and high quality has hindered the feasible application of these cells and their derivatives in industrial and clinical settings [1]. Here, we developed a scalable and well-characterized bioprocess for hPSC expansion under fully-defined conditions, using transcriptomic and metabolomic tools for product and bioprocess characterization.

MATERIALS AND METHODS: In this study, two hESC lines, a feeder-dependent and a feeder-free cell line, were expanded on different xeno-free Synthemax II microcarriers in stirred tank bioreactors, as previously described [2], under fully-defined conditions. In all cultures, cell proliferation and viability were monitored daily and, after cell expansion, the undifferentiated phenotype and pluripotency were evaluated by immunofluorescence microscopy, flow cytometry, RT-qPCR and *in vitro* pluripotency assays. Moreover, the transcriptional and metabolic profiling of hESC cultured in stirred tank bioreactors was analyzed and compared with hESC cultured in 2-dimensional (2D) culture systems.

RESULTS AND DISCUSSION: Our results demonstrated that Synthemax II-polystyrene microcarriers were the most effective beads for the expansion of both hESC lines in stirred tank bioreactors, enabling high fold increase in cell concentration and high viable cell recovery yields after harvesting from microcarriers. Moreover, cells maintained the expression of undifferentiated hESC markers (Oct-4, TRA-1-60, SSEA-4 and SSEA-5) throughout culture time and showed high and similar pluripotency scores (according to PluriTest bioinformatic platform) when compared to 2D cultures, confirming the effectiveness of established bioprocess in maintaining the hESC pluripotency. Genome-wide profiling revealed a transcriptional phenotype convergence between both hESC lines along the expansion process on microcarrier-based stirred culture systems, providing strong evidence on the robustness of the cultivation process to homogenize cellular phenotype.

The transcriptome and metabolome data of hESC expanded in environmentally-controlled stirred-tank bioreactors operated at low $O_2$, showed the up-regulation of the glycolytic machinery along with high ratios of lactate production to glucose consumption, correlated with the down-regulation of the TCA reactions preceding oxidative phosphorylation. In accordance, the expansion in low $O_2$ controlled environment favored an anaerobic glycolysis Warburg-effect like phenotype over 2D cultures, with no evidence of hypoxic stress response. These results offer valuable insights on the metabolic hallmarks of hPSC expansion under fully defined conditions, which can help to guide process design and media optimization towards high hPSC productivities.

CONCLUSIONS: By combining the fully-defined bioprocess system with the characterization tools applied herein, one can aim for more standardized production of cell-based products for regenerative medicine, namely for cardiomyocytes generation, and for high-throughput drug screening.

REFERENCES:
1. Serra M et al., 2012, Trends in Biotechnol, 30:350; [2] Serra M et al., 2010, Journal of Biotechnol, 148(4), 208.

# 36 Exploring proteomics toolbox to unravel molecules and biological pathways involved in cardiac repair

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide. Myocardial infarction (AMI) severely affects patients' heart muscle and microvasculature, critically decreasing the number of functional cardiomyocytes (CM). Stem cell and protein based therapies became promising cardiac repair strategies since it was found that under pathological stress, resident cardiac stem cells (CSC) of the adult myocardium are activated by growth factors (GF) secreted by the surviving CM. Consequently, an auto/paracrine loop is triggered to maintain GF production, which enhances CSC activation and differentiation into new CM, endothelial and smooth muscle cells contributing to the repair of damaged myocardium [1]. Since this repopulation of the myocardium is neither robust nor durable enough to have significant beneficial physiological/anatomical impact in severe and acute myocardial losses, local administration of GF has been shown to be efficient in enhancing CSC activation, improving cardiac output post-AMI through the formation of new vascularized and functional autologous myocardium [2, 3].

Aiming at supporting the development of allogeneic cell-based therapies and providing new insights about molecules/pathways involved in cardiac repair, we investigated hCSCs receptome and secretome profiles.

hCSC cultivated in environmentally controlled stirred tank bioreactors were characterized regarding cell viability, metabolism, phenotype, GF secretion and differentiation potential. A high-throughput proteomics workflow was implemented, enabling identification of low abundance (receptors and GF) and highly hydrophobic proteins (membrane proteins- receptors). Enrichment of plasma membrane proteins was preformed prior to MCX-nanoLC-LTQ-Orbitrap for receptome analysis. For secretome characterization, conditioned medium of hCSC cultures was collected. Samples were run in a SDS-PAGE gel, each lane sliced in several fractions, individually digested and fractionated by nanoLC. The entire run was collected and spotted for further MS analysis (MALDI-TOF/TOF).

hCSC cultured in bioreactors remained phenotypically and functionally similar to cells cultured in standard 2D culture systems. Receptome analyses lead to the identification of more than 2000 proteins/replicate, several hundred with numerous predicted transmembrane domains, from which around 100 were plasma membrane receptors. Cardiovascular system development and function was the top hit of functional analysis by IPA software. Secretome data analysis is on-going, with about 300 human proteins identified up to now.

Proteomics approaches implemented allowed the identification of a wide-ranging list of receptors and secreted factors that are currently being further investigated. Furthermore, to better mimic the ischemia/reperfusion situation we are using the controlled culture system to simulate an AMI *in vitro*, aiming at understanding the regulatory cascades underneath hCSC activation and GF secretion.

REFERENCES:
1. Beltrami AP et al, Cell 2003, 114:763-776; [2] Anversa P et al, Circulation 2006, 113:1451-1463; [3] Ellison GM et al, J Am Coll Cardiol 2011, 58:977-986.

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# 37 The protective effect of starvation in the heart involves an improvement of gap junction intercellular communication

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Heart diseases are the leading cause of death worldwide. Impairment of intercellular communication (IC) between cardiac cells has been often associated with heart dysfunction. IC can occur directly, between adjacent cells, via gap junctions (GJ) or indirectly for long-distances by extracellular vesicles (microvesicles and exosomes). Since exosomes carry molecular information, they have been considered as intercellular messengers that mediate both local and systemic cell communication. GJ, on the other hand, are channels composed of connexins, being Connexin 43 (Cx43) the major ventricular GJ protein that displays a crucial role in IC in the heart. Therefore, changes in Cx43 turnover dynamics have profound impacts in IC and heart function [(1)]. Previous data from our group demonstrated that Cx43 is a substrate for autophagy and this process constitutes a regulatory mechanism associated with IC between cardiac cells [(2)]. Interestingly, various studies have demonstrated that beneficial effects of starvation rely, at least partially, in autophagy activation, thus emerging as a potential cardioprotective therapy [(3)]. Therefore, a better understanding of the mechanisms whereby starvation-induced autophagy protects the heart can provide new and important insight in the field of cardiovascular biology. Since hypothalamus has a pivotal role in energy homeostasis, we pretended to explore whether exosomes released by hypothalamus cells subjected to starvation will improve IC in cardiac cells. Exosomes were isolated by differential centrifugation, protein levels were determined by western blot, the subcellular localization of Cx43 was evaluated by immunofluorescence and cell viability was measured by MTT assay. In this study, we observed that H9c2 rat cardiomyoblast cells that received exosomes from H9c2 cells submitted to short periods of starvation had increased levels of Cx43, suggesting that IC in the heart improve. We have found that these exosomes also increase the levels of p62, Atg7 and LC3 in these cells. Moreover, starvation-derived exosomes did not change the cell viability of H9c2 neither in ischemia nor control conditions. We observed that cardiac cells, either cardiomyoblast (H9c2) or microvascular endothelial (HMEC) cells that received exosomes from hypothalamic neurons (embryonic mouse hypothalamus cell line N42 - mHypoE-N42) submitted to short periods of starvation (30 minutes and 1 hour) had decreased levels of Cx43. However, 2 hours starvation-derived hypothalamic exosomes induced a minor increase in cardiac cells Cx43, p62 and Atg7 levels. Surprisingly, we also observed that starvation leads to an accumulation of cardiac Cx43 levels, being more evident at 72 hours than 48 hours of starvation. Overall, these data suggest that starvation-derived exosomes improve cardiac IC by increasing of Cx43 levels through autophagy activation.

REFERENCES:
1. Su V, Lau AF. Connexins: mechanisms regulating protein levels and intercellular communication. FEBS letters. 2014;588(8):1212-20. Epub 2014/01/25.
2. Bejarano E, Girao H, Yuste A, Patel B, Marques C, Spray DC, et al. Autophagy modulates dynamics of connexins at the plasma membrane in a ubiquitin-dependent manner. Molecular biology of the cell. 2012;23(11):2156-69. Epub 2012/04/13.
3. Ahn J, Kim J. Nutritional status and cardiac autophagy. Diabetes & metabolism journal. 2013;37(1):30-5. Epub 2013/02/27.

# 38 Exosomes produced by cardiomyocytes modulate endothelial dysfunction associated with ischemic heart

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Cardiovascular diseases (CVDs) are the first cause of global morbidity and death in developed countries. The heart is an organ with complex mixture of interactions of cardiac cell that facilitate proper myocardial contractility, sufficient perfusion and balanced myocardial extracellular stiffness. Intercellular communication between heart cells can occur either directly, via gap junctions (GJ) or over long distances through extracellular vesicles (EV). The main populations of released vesicles are classified according to their size, composition and subcellular origin and include apoptotic bodies, microvesicles and exosomes [1]. Exosomes, that include vesicles with a diameter of 30-100nm, are formed when multivesicular bodies fuse with plasma membrane and release their vesicular content into extracellular medium and contain messages in the form of protein, DNA and coding and noncoding RNA molecules, including microRNAs (miRNA) [2]. Several studies have demonstrated that genetic content of exosomes secreted by cardiomyocytes vary in ischemic disease [3,4]. The aim of this study is to evaluate the effect of exosomes released by cardiomyocytes, either in control or ischemic conditions in endothelial cells and their ability to modulate myocardial angiogenesis. In this study we isolated exosomes secreted by rat cardiomyocytes cell line (H9c2) under control and ischemic conditions and each population of isolated exosomes was added to mouse cardiac endothelial cells (MCEC-1). The mRNA profile of each population of exosomes was further determined by Exiqon miRCURY™ LNA system. The angiogenic potential of MCEC-1 after stimulation with the exosomes was evaluated by cell migration assay (scratch wound assays), capillary-like tube formation assay on matrigel and cell sprouting assay. Our results show that exosomes produced by cardiomyocytes subjected to ischemia have a pro-angiogenic effect upon cardiac endothelial cells. Indeed, we demonstrated that, in comparison with controls, ischemic exosomes lead to increased cell migration, cell sprouting and tubulation. Furthermore, this study is of particular relevance in the context of cardiac lesion, namely myocardial infarction, where altered communication between cardiac cells may lead to adverse remodeling of the heart, including angiogenesis. In conclusion, the data gathered in this study, may pave the way towards the development of novel approaches to prevent cardiac dysfunction.

REFERENCES:
1. K. R. Vrijsen, J. P. G. Sluijter, M. W. L. Schuchardt, B. W. M. van Balkom, W. A. Noort , S. A. J. Chamuleau , P. A. F. M. Doevendans , Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. J Cell Mol Med. 2010 May;14(5):1064-70.
2. Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, Chang J, Peng T, Fan GC. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol. 2014 Sep;74:139-50.
3. Giricz Z, Varga ZV, Baranyai T, Sipos P, Pálóczi K, Kittel Á, Buzás EI, Ferdinandy P. Cardioprotection by remote ischemic preconditioning of the rat heart is mediated by extracellular vesicles. J Mol Cell Cardiol. 2014 Mar;68:75-8.
4. Sahoo S, Losordo DW. Exosomes and cardiac repair after myocardial infarction. Circ Res. 2014 Jan 17;114(2):333-44.

# 39 Exosome-mediated cross-talk between cardiomyocytes and macrophages in ischemic heart disease

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Ischemic heart disease is the leading cause of death worldwide, making it a major burden for Health Care Systems and a priority field of both basic and translational research. Ischemia triggers an intense inflammatory response that is essential for cardiac repair, but when exacerbated is also implicated in the extent of cardiac injury and the pathogenesis of left ventricle remodeling and heart failure. Cells constitutively release small vesicles known as exosomes, which have been shown to play an important role in both local and distant cell-cell communication, carrying a biologically functional content to target cells and, ultimately, modulating their behavior (Buzas *et al.*, 2014, Sluijter *et al.*, 2014). Macrophages have recently been recognized as a major cell population of the heart and are known to play a preponderant role as key mediators of the inflammatory response upon cardiac ischemia (Frantz *et al.*, 2014). Therefore, a better understanding of the cross-talk between macrophages and cardiomyocytes via exosomes in ischemia is of the utmost importance.

We characterized macrophages and cardiomyocyte-released exosomes using transmission electron microscopy and western blot for TSG101 and ALIX. We isolated exosomes secreted by steady-state or ischemic cardiomyocytes by diferential centrifugation (Théry *et al.*, 2006), which were then incubated with macrophages for 24h, or vice-versa. Our results show that ischemic cardiomyocyte-derived exosomes reduce macrophages (pro)inflammatory response, as seen by a decrease in nitrite production, expression of inducible nitric oxide synthase (iNOS) and phosphorylation of p38MAPK when compared to macrophages stimulated with steady-state exosomes. On the other hand, cardiomyocytes incubated with ischemic macrophage-derived exosomes display a loss of cell viability when compared with control exosomes-stimulated cardiomyocytes.

Interestingly, these results suggest that exosomes released by ischemic cardiomyocytes might be playing a protective role through down-regulation of the pro-inflammatory abilities of macrophages while ischemic macrophage-released exosomes could be perceived as aggressors in the ischemic event, impairing cardiomyocyte function. A better understanding of such cell-specific biological potential of exosomes could disclose new targets in the battle against ischemic heart disease.

REFERENCES:
1. Buzas EI, György B, Nagy G, Falus A, Gay S. Emerging role of extracellular vesicles in inflammatory diseases. Nat Rev Rheumatol. 2014 Jun;10(6):356-64.
2. Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doevendans PA. Microvesicles and exosomes for intracardiac communication. Cardiovasc. Res. 2014 May 1;102(2)302-11.
3. Frantz S, Nahrendorf M. Cardiac macrophages and their role in ischaemic heart disease. Cardiovasc Res. 2014 May 1;102(2):240-8.
4. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006 Apr;Chapter 3:Unit 3.22.

# 40 Connexin 43: a new substrate for chaperone-mediated autophagy

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Gap junctions (GJ) are specialized cell-cell contacts formed by connexins, which provide direct intercellular communication between eukaryotic cells. GJ channels allow the transfer of small metabolites, ions and second messengers between adjacent cells, playing a major role in the heart where mediate electrical and metabolic coupling. Although connexin channels may be regulated directly by the opening or closure of the channel pore, connexin internalization and degradation also plays a pivotal role in the regulation of gap junction intercellular communication. We have previously shown that macroautophagy modulates the degradation of Cx43-containing GJ plaques. The degradation of cellular components by lysosomes can also occur through another and more selective type of autophagy, chaperone-mediated autophagy (CMA), in which a KFERQ sequence in the substrate protein is recognized by a cytosolic chaperone, hsc70, that then delivers them to the lysosomes surface for internalization through a translocation complex formed by LAMP2A. Despite the degradation of Cx43 through the endolysosomal pathway or macroautophagy has already been described its degradation through CMA has never been addressed. In this study we demonstrate that CMA activation in cardiac cells, either by nutrient deprivation or with the CMA activator, 6-AN, leads to an increase in the degradation of Cx43 with a concomitant increase in the interaction between Cx43 and CMA components namely hsc70 and LAMP2A. We also identify a putative KFERQ motif on Cx43 and demonstrate that a mutation in this motif increases the half-life of the protein and modulates with both hsc70 and LAMP2A.

Here we provide evidence showing that Cx43 contains a functional KFERQ motif, interacts with both hsc70 and LAMP2A and is a substrate for CMA.

# 41 Heart development in the mouse: different extracellular matrix niches – different cell types and behaviours?

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INTRODUCTION: During embryonic development, the heart is the first organ to form and to function. The cardiac precursors are specified bilaterally in the anterior epiblast of the early mouse embryo and, through the movements of gastrulation, these precursor populations, become localized in the anterior splanchnic mesoderm. The cells of the first heart field, come together in the midline and form the scaffold of the heart, the primitive heart tube, while the second heart field, characterized by the expression of the transcription factor Islet1, migrate to colonize this early scaffold, and form most of the atria, right ventricle and outflow tract. Meanwhile, regionalized differentiation occurs where endocardial cells line the heart tube internally, while the outer myocardial cells become contractile and electrically coupled. The heart starts beating at E8.5 and at E9.5 cells from the ventral somatopleura, the epicardial precursor cells, migrate to surround the myocardium.

Several extracellular matrix (ECM) molecules are thought to play important roles in the development of the heart. However much less is known about what cell types participate in the deposition of these ECMs and how they are distributed in the heart. Here we focus on the spatiotemporal deposition of fibronectin and laminins during cardiac development.

MATERIALS AND METHODS: E8.5-E18.5 mouse embryos were collected from timed pregnancies, fixed and embedded for cryosectioning. Sections were processed either for in situ hybridization, using *Fn1* (courtesy of RP Andrade), *Lama1*, *Lama2*, *Lama4*, *Lama5*, *Lamb1* and *Lamb2* (courtesy of P Ybot-Gonzales) probes, or for immunohistochemistry, using rabbit polyclonal antibodies against fibronectin and laminin (Sigma), as well as chain-specific antibodies against laminin $\alpha$1 (courtesy of M Durbeej), $\alpha$4 and $\alpha$5 (courtesy of J Miner), $\alpha$2 (Sigma), $\beta$1 (Abcam) and $\gamma$1 (DSHB) chains.

RESULTS AND DISCUSSION: Both Nkx2.5-positive cells in the splanchnic mesoderm (1$^{st}$ heart field) and premigratory Islet1-positive cells, are in close contact with fibronectin- and laminin-rich matrices, the latter which are composed of laminins 111 and 511. When these cells become migratory, they appear to migrate through an area free of fibronectin but enriched in laminin 411. Endocardial cells strongly expresses the *Fn1* gene and a fibronectin matrix comes to separate the endocardium from the myocardium. Also, later on, when the epicardium starts surrounding the developing heart, it expresses *Fn1* and a fibronectin matrix is deposited between those two layers. In contrast, a fibronectin matrix is never seen surrounding cardiomyocytes. Rather, laminin 211 seems to specifically surround cardiomyocytes from E9.5 until birth while the laminin matrix that lines the endocardium and epicardium is primarily composed of laminin 511. Thus different subregions of the developing heart contains different laminin isoforms.

CONCLUSIONS: We conclude that premigratory cardiac precursors interact with fibronectin and laminins 111 and 511 while migrating cardiac precursors do not appear to use fibronectin as a migration substrate. After cardiac tube formation and colonization of the heart by the second heart field, two different matrices can be identified within the heart: (1) the matrices separating the endocardium from the myocardium and the myocardium from the epicardium contain fibronectin and laminin 511 while (2) the matrix surrounding cardiomyocytes lacks fibronectin and contains laminin 211.

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# 42 Adipokines affect the cardiac structure

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INTRODUCTION: Heart failure (HF) represents a major and growing public health problem in the developed countries and several risk factors, such as obesity, are associated with its development. Obesity is defined as a pro-inflammatory state associated with increased secretion of bioactive polypeptides, called adipokines, which regulate the function of numerous organs and tissues. Epicardial adipose tissue segregates adipokines that can act in a paracrine and vasocrine manner on the myocardium, exerting direct effects in fibroblasts and cardiomyocytes. This project aimed at investigating the possible role of adipokines in cell proliferation and collagen production by cardiac fibroblasts and evaluating the crosstalk between epicardial adipose tissue and the myocardium.

METHODS: Experimentally, atria tissue from 25$^{th}$ week old ZSF1 lean (ZSF1 Ln, n=11) and ZSF1 obese (ZSF1 Ob, n=11) rats was used for the isolation of cardiac fibroblasts, as well as for obtaining conditioned medium from pericardial adipose tissue. Fibroblasts were cultured separately with apelin (100 nM) and adiponectin (10µg/ml). After 48h, BrdU assays and Sirius Red staining were performed in order to evaluate the effect of those adipokines in cell proliferation and collagen production, respectively. Moreover, organotypic cultures were obtained from cardiac explants from 7 day-old Wistar rats and incubated with conditioned medium from pericardial adipose tissue of obese and lean groups. After 24h of incubation, fibrosis and cross-section area of hematoxylin-eosin stained cardiomyocytes were assessed.

RESULTS: Incubation with apelin and adiponectin led to a significant increase in fibroblasts' proliferation in both groups ($p=0.0496$). In ZSF1 Ln, apelin induced a significant decrease in collagen secretion, while in ZSF1 Ob it increased collagen secretion and production ($p=0.0054$). Adiponectin significantly reduced collagen synthesis in both groups and decreased collagen secretion in ZSF1 Ln ($p<0.001$). Regarding the organotypic cultures, pericardial adipose tissue secretome from obese rats triggered a significant increase in fibrosis deposition (348%±1.51% vs 4.79±1.53%, $p<0.05$) and in the cross-section area of cardiomyocytes (100.7±18.98 $\mu m^2$ vs 111.25±24.02$\mu m^2$, ($p<0.05$), compared with the secretome from lean animals.

CONCLUSIONS: Adipokines produced by adipose tissue depots from obese animals seem to modulate the cardiac structure, through changes in extracellular matrix components and cardiomyocytes, features typically related to the appearance of diastolic dysfunction.

REFERENCES

Iacobellis, G., D. Corradi, and A.M. Sharma, Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. Nat Clin Pract Cardiovasc Med, 2005. 2(10): p. 536-43.

# 43 Emergence of the First and Second Heart Fields/Lineages – Is Notch Signaling Playing a Part?

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INTRODUCTION: Heart formation involves a complex crosstalk between various signaling pathways in a temporal and context–dependent manner. The molecular events taking place from pre-gastrulation up to formation of cardiomyocytes can be recapitulated *in vitro* by differentiating mouse embryonic stem cells (mESCs). Importantly, by closely following the kinetics of cell fate decisions occurring in the embryo, ESCs facilitate mechanistic studies aimed at the dissection of early lineage specification. Likewise, similarly to heart embryo development, the existence of different subsets of cardiac progenitors *in vitro* seems to mimic the emergence of cardiac lineages/fields which shape the embryonic heart [1]. First (FHF) and second (SHF) heart fields contribute to distinct cardiac territories and have been mostly discriminated by differential expression of specific transcription factors (e.g. Tbx5 vs. Isl1, respectively). A recent finding [2] demonstrated co-localization of the voltage-gated ion channel Hcn4 with Tbx5 in FHF progenitors, enabling isolation of the latter subset based on expression of this surface protein. Notch pathway has been implicated in the determination of a cardiac fate from mesodermal progenitors [3] and our laboratory identified Hes5 as a Notch1 effector during the onset of cardiogenesis. By using the mESCs *in vitro* model-system we hereby propose to investigate the role of Notch in the subsequent determination of the distinct cardiac lineages of the developing heart.

MATERIALS AND METHODS: AinV/Bry-GFP mESCs were transduced with a Doxycycline (Dox)-inducible cassette containing Hes5 coding sequence. mESCs were differentiated into Day (D) 3.75 mesodermal progenitors. Sustained or 24h-pulsed Hes5 expression was induced by addition of Dox at D3.75. Gene and protein expression for FHF and SHF markers were assessed and cultures were examined for cell contraction.

RESULTS AND DISCUSSION: To infer the time of emergence of the distinct cardiac progenitors, detailed gene expression analysis for genes associated with distinct cardiac cell lineages was performed at precise-points time along in vitro differentiation. Hes5 expression was induced by addition of Dox at D3.75 owing to the direct regulation of Isl1 expression in mesodermal progenitors revealed in our previous studies. Our preliminary data indicates a dynamic temporal expression of Tbx5 and Isl1 hinting for the onset of cardiac mesoderm at D3.75 of differentiation. Gene expression analysis at 24, 48 and 96h in sorted D3.75 mesodermal progenitors showed increasing and decreasing levels of Tbx5 and Isl1, respectively suggesting the appearance of a wave of FHF progenitors, *i.e.* Tbx5+. Hes5 activation, either as a 24h-pulse or in a sustained manner, induced Isl1 upregulation overtime, indicating a role in the determination of SHF progenitors. Studies are underway aiming to identify the timing for specific isolation of FHF and SHF lineages based on Hcn4 surface expression.

CONCLUSIONS: Our preliminary data suggest a role for the Notch effector Hes5 in regulating SHF progenitors, and gives further support for that Notch signaling may specify the emergence of different cardiac lineages through different mediators. Future work contemplates the identification of the part distinct Notch effectors might play in the determination of each particular cardiac lineage/field.

REFERENCES

[1] P. Van Vliet et al (2012) Cardiovasc Res; [2] Spater, D et al (2013) Nat Cell Biol; [3] Chen, V.C., et al. (2008) Nat Biotechnol.

# 44 Regenerative Arms Race in Neonatal Cardiac Injury

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Cardiac diseases lead the raking of causes of death worldwide [1]. This is the ground for an increasing number of studies on the characterization of the heart's response to injury and on the development of new therapies to functionally restore the damaged myocardium. Recently, several reports have shown that the mammalian heart, when injured in a short-period after birth, displays the capacity to regenerate, whereas, if injured after post-natal day (P)7, it triggers the formation of a non-functional fibrotic scar [2]. Nonetheless, the signals determining the development of a reparative (adult) or regenerative response (neonate) are largely unknown. The premise of our Team is that the transient regenerative capacity of the heart correlates to specific organ changes during this ontogenic period. The herein work focuses on the dynamics of cardiac fibroblasts and that of the extracellular matrix (ECM) during ontogeny and following injury in the neonate. We demonstrate that fibroblasts, here defined as the $CD31^{-}CD45^{-}Ter119^{-}CD90^{+}$ population, colonize the heart following birth and also that the cell-surface signature of this compartment changes throughout post-natal life. Our results demonstrate that the neonatal heart response to apex resection involves the recruitment of inflammatory cells, fibroblast activation, ECM production and neo-vascularization. High Content Analysis (HCA) revealed that the removed tissue was, at least in part, re-established by the proliferation of resident cardiomyocytes. Moreover, despite formation of scarring tissue (inner core of the injured area) and incomplete histological restoration, resected hearts were functionally competent at 21d post-lesion.

Overall this work describes the microenvironmental alterations, with particular emphasis on fibroblasts and ECM, triggered following neonatal apex resection and that culminate on partial restoration of the organ.

REFERENCES

1. Celermajer DS, CK Chow, E Marijon, NM Anstey and KS Woo (2012). Cardiovascular disease in the developing world: prevalences, patterns, and the potential of early disease detection. J Am Coll Cardiol 60: 1207–1216.

2. Porrello ER, AI Mahmoud, E Simpson, J a Hill, J a Richardson, EN Olson and H a Sadek (2011). Transient regenerative potential of the neonatal mouse heart. Science (80- ) 331: 1078–1080.

# 45 Neuregulin-1 improves right ventricular function and attenuates monocrotaline-induced pulmonary arterial hypertension

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PURPOSE: This study evaluated the effects of NRG-1 treatment in the monocrotaline (MCT)-induced model of pulmonary arterial hypertension (PAH).

MATERIALS AND METHODS: Two weeks after MCT administration, animals were randomly selected to receive NRG-1 (40 ug/kg/d) or vehicle obtaining 4 groups (CTRL, CTRL+NRG, MCT, MCT+NRG). At week 4, echocardiographic and hemodynamic evaluations were performed with subsequent sample collection for further analysis. Isolated skinned cardiomyocytes, morphometrical, histological and molecular analysis were achieved in order to dissect the underlying mechanisms. Pulmonary artery (PA) endothelial function was carried out in all groups.

RESULTS: PAH induction resulted in abnormal PA flow (decreased acceleration/ejection time -PAAT/PAET ratio), and increased right atria area (RAA). RV function was deteriorated with increased systolic and diastolic pressures, and decreased ejection fraction (EF) and cardiac output (CO). RV dilation was clear in animals with PAH. Diastolic impairment in PAH was confirmed in isolated cardiomyocytes where single cell passive tension was higher versus controls.

NRG-1 ameliorated MCT-induced changes. PAAT/PAET and RAA were reverted to control levels. RV pressure rise was attenuated, and EF and CO were improved with treatment. RV dilation was abolished and cardiomyocyte passive tension development was lowered.

Morphometric RV hypertrophy together with changes in cardiomyocyte cross sectional area were evident in the MCT group and were attenuated in MCT+NRG group.

Treating animals with NRG-1 attenuated MCT-induced endothelial dysfunction, decreased lung oedema and PA wall thickness, when compared to the MCT group.

NRG-1, B-type natriuretic peptide, endothelin-1 and hypoxia inducible factor-1$\alpha$ RV expression was increased in MCT group, and was attenuated or normalized with treatment. Phosphorylation of the protein titin, which was lower in MCT animals when compared to CTRL, was recovered with NRG treatment.

CONCLUSION: NRG-1 treatment in MCT-induced PAH leads to improved pulmonary flow, improved RV function, decreased RV and pulmonary remodeling and attenuates the expression of hypertrophy and overload-associated markers.

# 46 Urocortin-2 improves right ventricular function in pulmonary arterial hypertension

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This study aims to explore the pathophysiological and therapeutic effects of UCN-2 treatment in an animal model of RV failure secondary to PAH.

Male Wistar rats randomly received monocrotaline (MCT, 60mg/Kg) or vehicle. After 2 weeks, animals were randomly assigned to receive UCN-2 (5µg/Kg/day) or vehicle. The study resulted in 4 groups: CTRL (n=9), CTRL+UCN-2 (n=9), MCT (n=7) and MCT+UCN-2 (n=10). Hemodynamic studies and sample collection were performed 4 weeks after MCT injection. Only significant results (mean±SEM, $p<0.05$) are given.

Hemodynamic studies revealed that MCT group developed PAH, as shown by increased RV end-systolic pressure (MCT vs CTRL: 60±3 vs 22±1mmHg), end-diastolic pressure (6.0±0.7 vs 3.7±0.3mmHg), RV dilation (end-diastolic volume) (280±14 vs 222±11µL) and decreased cardiac output (35±6 vs 64±3mL/min) and ejection fraction (32±4 vs 75±3%). UCN-2 treatment resulted in attenuation of these changes (48±4mmHg; 4.3±0.3mmHg; 213±12µL; 47±2mL/min and 60±3%, respectively). Moreover, the survival rate for UCN-2 treated rats was higher (76%) than for MCT rats (44%).

PAH rats presented RV hypertrophy as shown by the morphometrical analysis (RV weight/tibia length ratio, MCT vs CTRL: 0.08±0.00 vs 0.04±0.00g/cm) and by histology (cardiomyocyte cross-sectional area: 366±25 vs 255±27µm2). UCN-2 treatment attenuated RV remodeling (0.06±0.00g/cm and 288±26µm2, respectively).

The MCT group presented increased UCN-2 expression (MCT vs CTRL: 2.5±0.9 vs 1.0±0.3AU) and decreased CRHR2 expression (0.5±0.1 vs 1.0±0.1AU) in the RV, that were reversed by UCN-2 treatment (0.2±0.1 and 0.9±0.1AU, respectively). The increased expression of pathology markers in MCT animals, such as BNP (15.3±2.5 vs 1.0±0.1AU), ET-1 (34±04 vs 1.0±0.2AU) and HIF-1$\alpha$ (1.6±0.3 vs 1.0±0.2AU), as well as markers of apoptosis including caspase-3 (3.9±0.6 vs 1.0±0.1AU) and caspase-8 (2.8±0.3 vs 1.0±0.2AU) were attenuated by UCN-2 (6.9±2.1, 1.8±0.6, 1.0±0.1, 2.0±04 and 1.3±0.2AU, respectively).

The protein expression of both ERK and p38 kinases was decreased in MCT animals (MCT vs CTRL: 0.5±0.1 vs 1.0±0.1 and 0.5±0.04 vs 1.0±0.1, respectively) and was reversed by UCN-2 (0.9±0.1 and 1.1±0.2, respectively).

UCN-2 treatment significantly reduced the severity of PAH and RV hypertrophy, as well as the expression of genes associated with overload, hypertrophy, hypoxia and survival. These findings suggest that the UCN-2/CRHR2 pathway has a relevant role on the pathophysiology of PAH and RV failure, representing a potential therapeutic target in these conditions.

# 47 The long-term therapeutic effect of ATMP-compliant human umbilical cord tissue-derived MSCs transplantation upon acute myocardial infarction – A pre-clinical evaluation

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Upon myocardial infarction (MI), a cascade of biochemical and morphologic events known as cardiac remodeling takes place in the heart, leading to the formation of a nonfunctional scar in place of the damaged myocardium. Cell therapies have been explored as a therapeutic alternative to direct cardiac response towards efficient repair and/or myocardial functional improvement. Among the cell types investigated in this context, mesenchymal stromal cells (MSCs) are particularly valuable because of their know paracrine regenerative effects and hypoimmunogenicity, enabling major histocompatibility complex-mismatched allogeneic transplantation. Unlike other MSCs sources, human umbilical cord tissue (hUC)-derived MSCs isolation is non-invasive, granting higher cell yields with short-doubling times, alongside with virtually no ethical concerns. Our previous work has shown that, in a context of acute ischemic heart disease, the transplantation of a well-defined hUC-derived MSCs population (UCX®), obtained using proprietary technology developed by ECBio, preserves cardiac function and attenuates cardiac remodeling after intramyocardial transplantation in a 21 days MI murine model [1]. Recently, envisioning end user safety, a protocol adaptation was established in order to produce UCX® as an advanced therapy medicinal product (ATMP) [2]. Herein we evaluate the long-term therapeutic effect of UCX®-ATMP as a cell therapy to attenuate the aftermath of myocardial infarction on a chronic murine model.

Briefly, UCX®-ATMP isolated from two donors (UC-A and -B) were suspended in a physiological saline solution ($0.2\times10^6$ cells/heart) and delivered via intramyocardial injection to 12 weeks old C57BL/6 mice, immediately after permanent ligation of the left descending coronary artery. A vehicle-injected group was used as control. Twelve weeks post-infarction and UCX®-ATMP delivery, cardiac function was evaluated through transthoracic echocardiography using a Vevo 2100 microultrasound platform coupled with a high resolution 38 MHz microscan transducer. Diastolic arrested hearts were processed for histological analysis and infarct size quantification was assessed, assisted by a semi-automatic tool developed within our team – MIQuant[3].

The first sketch of the UCX®-ATMP long-term cardiac protective effect upon MI will be herein presented.

REFERENCES

1. Nascimento DS, Mosqueira D, Sousa LM, Teixeira M, Filipe M, Resende TP, Araújo AF, Valente M, Almeida J, Martins JP. Human umbilical cord tissue-derived mesenchymal stromal cells attenuate remodeling after myocardial infarction by proangiogenic, antiapoptotic, and endogenous cell-activation mechanisms. Stem Cell Res Ther. BioMed Central Ltd; 2014;5(1):5.

2. Martins JP, Santos JM, de Almeida JM, Filipe MA, de Almeida MVT, Almeida SCP, Agua-Doce A, Varela A, Gilljam M, Stellan B, Pohl S, Dittmar K, Lindenmaier W, Alici E, Graca L, Cruz PE, Cruz HJ, Barcia RN. Towards an advanced therapy medicinal product based on mesenchymal stromal cells isolated from the umbilical cord tissue: quality and safety data. Stem Cell Res Ther. 2014;5(1):9.

3. Nascimento DS, Valente M, Esteves T, de Pina M de F, Guedes JG, Freire A, Quelhas P, Pinto-do-Ó P. MIQuant – Semi-Automation of Infarct Size Assessment in Models of Cardiac Ischemic Injury. PLoS ONE. Public Library of Science; 2011 Sep;6(9):e25045.

# 48 MicroRNA-155 Promotes Sepsis-Associated Cardiac Dysfunction

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INTRODUCTION: Sepsis-associated cardiovascular dysfunction (SACVD) remains a leading cause of death in critically ill patients. MicroRNA-155 targets important transcripts in inflammation and cardiovascular system. The present study assessed the role of miR-155 in SACVD.

MATERIALS AND METHODS: Experimental sepsis was induced using endotoxin injection (LPS) or cecal ligation and puncture (CLP) models. Genetic loss (miR-155 KO) and pharmacological inhibition (LNA-anti-miR; AM) of miR-155 were evaluated. Cardiac function was assessed through echocardiography; mortality analysis was also performed. Quantification of myocardial miR-155, pro-inflammatory cytokine mRNA profile, miR-155 targets, apoptosis, cellular adhesion proteins and activation of pro-inflammatory intracellular signaling pathways were assessed. In another experiment, myocardial miR-155 expression, cellular localization and associations between its levels and other clinical variables were evaluated using post-mortem samples from septic shock (SS) patients.

RESULTS AND DISCUSSION: Myocardial miR-155 levels were increased in experimental and human septic myocardium. This was associated with a decreased ejection fraction (EF) and cardiac output (CO) and increased diastolic LV diameter (LVDd) in septic WT animals. Knockout and anti-miR-155 treated animals and presented with preserved EF, CO and LVDd, compared with WT; this was accompanied by ~50% mortality reduction. Experimental sepsis induced downregulation of SOCS1 and CD47, myocardial cytokine upregulation, increased apoptosis and adhesion protein expression as well as pro-inflammatory kinase and transcription factor activation, all of which were attenuated in KO and AM. In human myocardium, miR-155 expression was positively associated with 24h antemortem period plasma troponin I levels.

CONCLUSIONS: MicroRNA-155 is upregulated in experimental and human septic myocardium. Loss or inhibition of miR-155 attenuated sepsis-induced cardiac dysfunction, blunted pro-inflammatory activation and reduced mortality. This suggests miR-155 as a potential target in human sepsis-associated cardiac dysfunction.

# 49 Chronic fatigue syndrome, depressive disease and blood pressure!

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OBJECTIVE: Evaluation of arterial blood pressure in 109 patients with ages between 20 and 50 years, with CFS criteria and suffering clinical depression – unspecified depressive syndrome (DSU) – with evolution under 3 years.

DESIGN AND METHOD: Of 109 patients, 97 (89%) were women, with a mean age of 32 years, ranging between 20 and 44 years. 82 patients (84,5%) have ages between 20 and 35 years, and the other 15 (15,5%) are over 35 years of age. In 101 (92,6%) patients there were family priors of cardiovascular risk factors (CVRF) of which 36 (32,7%) knew they suffered of hypotension – "very low blood pressure" and 58 (59,8%) had at least two CVRF. 12 (11%) of the 109 patients were men , with a mean age of 38,5 years, ranging between 27 and 50 years, and presented family priors of CVRF, were normotensive and had at least two CVRF.

Rx Thorax, ECG, Echocardiogram and Ambulatory Monitoring of Blood Pressure (AMBP) were performed on all patients and, in the 16 cases (14,7%) of severe hypotension (TA < 88/48 mmHg), encountered in the women's group, with a mean age of 32 years, TILT was performed.

RESULTS: 71 (65,1%) patients had mean values of 95 mmHg in their systolic blood pressure (SBP), ranging between 102 and 88 mmHg, whereas the mean diastolic blood pressure was of 58 mmHg, ranging between 68 and 48 mmHg. 22 (20,2%) patients presented mean SBP values of 110 mmHg, ranging between 122 and 102 mmHg, with a men DBP of 70 mmHg, ranging between 78 and 62 mmHg. All 16 (14,7%) patients with severe hypotension were subjected to a TILT exam, which was positive in 10 (62,5%) cases.

In all other auxiliary diagnosis exams no other relevant alterations were found.

CONCLUSIONS: Hypotension is present in 87 (79,8%) patients and should therefore be considered an important variable, so it's imperative to find a precocious differential diagnosis, considering CFS, depressive syndrome and eventually autonomic dysfunction, when TILT exam is positive.

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