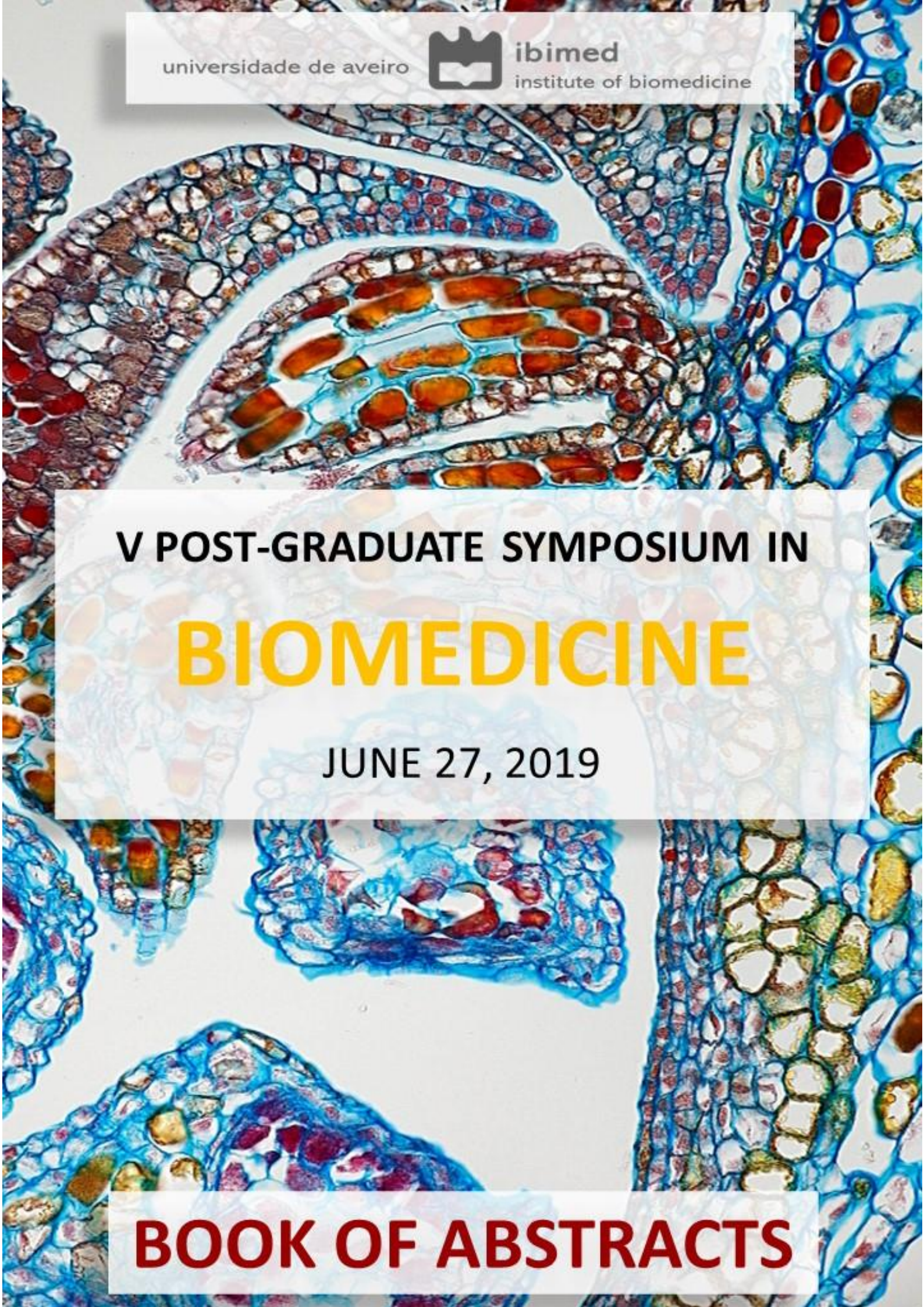
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V POST-GRADUATE SYMPOSIUM IN
BIOMEDICINE
JUNE 27, 2019
BOOK OF ABSTRACTS

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01 Welcome Message

The Post-graduate Symposium in Biomedicine is a very important event of iBiMED´s research program. Teaming building through sharing research results and ideas, open discussions about recent achievements and the latest technical research advancements and concepts, brainstorming without being afraid of failure or criticism, are values embedded in our post-graduation symposium that we wish to see flourish in iBiMED. This year we will have our first PhD program retreat to further strengthen team spirit, reflect about our science and the way we are organized and working together. It will also provide multiple opportunities to improve our research culture, identify and correct aspects of our working habits that can be improved and learn important strategies of career development planning. The annual retreat will complement the post-graduation symposium as one of the high moments of our academic year.

We have consolidated iBiMED´s research program over the last 4 years. A major part of this endeavor and achievements is due to the efforts of our post-graduation students and young investigators. The quality of our research and our national and international networking is increasing steadily and we are growing in a sustainable manner, making contributions to the national and international recognition of our University´s Health Sciences Program. The new Academic Clinical Centre that we have announced last year will soon be a reality. A virtuous partnership with our associated members, namely the Aveiro (CHBV), Sta Maria da Feira (CHEDV), Gaia/Espinho (CHVNG/E) hospital Centers and Health Centers of the Aveiro and Aveiro-Northern regions will allow us to create better working conditions that will allow us to do more and better biomedical research and start implementing a new program on clinical research, which we hope will open new job opportunities to young researchers.

On behalf of iBiMED I am deeply grateful to all of you for your hard work and for helping preparing the 2018 international evaluation exercise carried out by FCT last winter, in particular for having created a positive feeling of our reality during the visit of the international evaluation panel. A special thank you to the organizing committee of the 2019 post-graduation symposium and wishes of a successful and productive post-graduation symposium.

Manuel Santos

iBiMED´s Director

02 Organizing Committee

Daniela Patrício
PhD Student, iBiMED – University of Aveiro

Joana Santiago
PhD Student, iBiMED – University of Aveiro

Mariana Marques
PhD Student, iBiMED – University of Aveiro

Marisa Pereira
PhD Student, iBiMED – University of Aveiro

Paulo Antas
PhD Student, iBiMED – University of Aveiro

Sara Dias
PhD Student, iBiMED – University of Aveiro

Tânia Martins
PhD Student, iBiMED – University of Aveiro

03 Scientific Committee

Prof. Alda Marques
Lab3R, ESSUA and iBiMED – University of Aveiro

Prof. Maria Lourdes Pereira
Dep. Medical Sciences and CICECO – University of Aveiro

Prof. Sandra Rebelo
iBiMED – University of Aveiro

Prof. Teresa Herdeiro
Dep. Medical Sciences and iBiMED – University of Aveiro

04 Venue

The **V Post-Graduate Symposium in Biomedicine** will be hold at the lecture theatre **30A.2.5**, in the Department of Medical Sciences, University of Aveiro

Agra do Crasto, Ed. 30, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

Tel: +351 234 247 242

Site: https://www.ua.pt/dcm/ | https://www.ua.pt/ibimed/

GPS coordinates: latitude: 40° 62’ 36.4692’’
longitude: -8° 65’ 75.7257’’

05 Programme

8:45	Registration
9:00	**Opening Session** Professor Manuel Santos, Director of iBiMED Vice-reitor Luís Castro, University of Aveiro
9:30 – 10:15	**Session 1 | Chair: Catarina Almeida, PhD**
9:30	**Plenary talk 1** **The role of communication in cardiovascular diseases** Henrique Girão, PhD, *iCBR – Univ. of Coimbra*
10:15	**Flash talk 1** **Role of ER stress on plasmacytoid dendritic cells innate activation** Daniela Barros, PhD, *iBiMED – Univ. of Aveiro*
10:30	**Flash talk 2** **Endothelial Damage is related to sedentary time in patients with resistant hypertension** Susana Lopes, MSc, *iBiMED – Univ. of Aveiro*
10:45 – 11:15	Coffee-break
11:15 – 12:00	**Session 2 | Chair: Ana Margarida Sousa, PhD**
11:15	**Flash talk 3** **Saliva's Microbiota as a biomarker of Chronic Obstructive Pulmonary Disease** Sara Dias, MSc, *iBiMED and ESSUA – Univ. of Aveiro*
11:30	**Flash talk 4** **Cellular Proteostasis During Influenza A Virus Infection – Viral Friend or Foe?** Mariana Marques, MSc, iBiMED – *Univ. of Aveiro*
11:45	**Flash talk 5** **Understanding the role of tRNA-modifying enzymes on proteostasis and human disease** Marisa Pereira, MSc, iBiMED – *Univ. of Aveiro*

12:00 – 12:45 **POSTER SESSION I** | Odd numbers

12:45 – 13:45 Lunch

13:45 – 14:30 **POSTER SESSION II** | Pair numbers

14:30 – 16:00 Session 3 | Chair: Ramiro Almeida, PhD

14:30 **Plenary Talk 2**

Neuroendocrine strategies to delay aging: from the bench to lifestyle

Professora Cláudia Cavadas, *CNC – Univ. of Coimbra*

15:15 **Flash talk 6**

Protein aggregation patterns distinguish the response to endocrine treatment of breast cancer cells

Inês Direito**,** MSc, iBiMED – *Univ. of Aveiro*

15:30 **Flash talk 7**

New sensors and chemical chaperones for protein aggregation

Raquel Nunes Silva, PhD, QOPNA & LAQV-REQUIMTE, Dep. Chemistry and iBiMED – *Univ. of Aveiro*

15:45 **Flash talk 8**

Effects of the SET7/9 methyltransferase inhibitor (R)-PFI-2 on mammary epithelial cells

Fátima Monteiro, MSc, iBiMED – *Univ. of Aveiro*

16:00 – 16:30 Coffee-break

16:30 – 17:15 Session 6 | Chair: Rui Martinho, PhD

16:30 **Plenary talk 3**

How to captivate your audience and get your message across

Bruno Mendo

17:00 – 17:15 Closing Session and Awards

06 Invited Speakers

Henrique Girão

Coimbra Institute for Clinical and Biomedical Research (iCBR)

Henrique Girão (HG) is Investigator at the Faculty of Medicine of University of Coimbra (FMUC), Deputy Director for Research and Development, Vice-chairperson of the Cardiovascular Council, Leader of the Group "Ubiquitin-dependent Proteolysis and Intercellular Communication", Director of the Laboratory of Biostructural Imaging. HG also coordinates the PhD programme in Health Sciences and the Master Course of Biomedical Research, and integrates the Board of Directors of the Inter-University Doctoral Programme in Ageing Chronic Diseases. HG is specialized in cellular and molecular mechanisms involved in the regulation of protein degradation and intercellular communication, necessary for the maintenance of cell homeostasis. In particular, HG has been interested in understanding how disturbance of proteolysis, namely non-canonical functions of ubiquitin in signalling lysosomal degradation, and intercellular communication, mediated by gap junctions and exosomes, contribute to cardiovascular disorders.

Cláudia Cavadas

Center for Neuroscience and Cell Biology (CNC), University of Coimbra
Neuroendocrinology and Aging Group

Filipa P. Cláudia Cavadas (CC) is PharmD, Master in Cell Biology, and PhD in Pharmacology, University of Coimbra. During her PhD, Claudia Cavadas spent 3 years at the University of Lausanne and CHUV, Switzerland. CC is Assistant professor, with tenure, at the Faculty of Pharmacy of the University of Coimbra and Coordinates "Neuroendocrinology and Aging group" at CNC - Center for Neuroscience and Cell Biology of the University of Coimbra. CC has been leading more then 15 funded projects and is co-author of around 70 international publications in relevant journals. CC was the elected President of the Portuguese Society of Pharmacology and coordinator of the Science Communication Office at CNC. CC is Evaluator of National and International Grants & Fellowships. Since March 2019, Cláudia Cavadas is director of the Institute for Interdisciplinary Research & Vice rector of the University of Coimbra.

Bruno Mendo

Co-creator of the SDEF project and method (Italy), International Trainer of NLP and Hypnotherapist.

How to captivate your audience and get your message across

07 Oral Communications

01

Role of ER stress on plasmacytoid dendritic cells innate activation

D. Barros, B.H. Ferreira, C. Silva, V. Camosseto, E. Gatti, C.R. Almeida, P. Pierre

02

Endothelial Damage is related to sedentary time in patients with resistant hypertension

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03

Saliva's Microbiota as a biomarker of Chronic Obstructive Pulmonary Disease

Sara Dias, Filipa Machado, Carla Valente, Lilia Andrade, Alda Marques and Ana Sousa

04

Cellular Proteostasis During Influenza A Virus Infection—Viral Friend or Foe?

Mariana Marques, Ana Raquel Soares and Daniela Ribeiro

05

Understanding the role of tRNA-modifying enzymes on proteostasis and human disease

MA Pereira, DR Ribeiro, AF Maia, MAS Santos, M Mano, AR Soares

06

Protein aggregation patterns distinguish the response to endocrine treatment of breast cancer cells

Direito I., Enes V., Melo T., Amado F., Moura G., Fardilha M., Helguero L.

07

New sensors and chemical chaperones for protein aggregation

R. Nunes da Silva, Hélio M. T. Albuquerque, Marisa Pereira, L. Fontes, M. Q. Alves, Ana Raquel Soares, J. Rocha, A. M. S. Silva, S. Guieu, S. I. Vieira

08

Effects of the SET7/9 methyltransferase inhibitor (R)-PFI-2 on mammary epithelial cells

Fátima Liliana Monteiro, Catarina Ruivo, Inês Batista, Beatriz Martins and Luisa A Helguero

ORAL COMMUNICATION

01

Role of ER stress on plasmacytoid dendritic cells innate activation

D. Barros[1], B.H. Ferreira[1], C. Silva[1], V. Camosseto[2], E. Gatti[1,2], C.R. Almeida[1], P. Pierre[1,2]

[1]iBiMED – Institute for Biomedicine, University of Aveiro, Portugal
[2]CIML – Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, France

Stressful environmental conditions, such as oxidative stress or bacterial toxins can induce accumulation of misfolded proteins and trigger an adaptive cellular response, collectively known as the unfolded protein response (UPR). UPR is characterized by three distinct downstream signaling pathways– IRE1, PERK or ATF6, which synergize to restore ER
homeostasis. In recent years, several studies suggested the existence of a synergy between endoplasmic reticulum (ER) stress and the innate sensing system, however the mechanisms behind this crosstalk remain unclear. Accordingly, in this work, we aim to study the nature of the cross-talk between UPR signaling pathways and innate activation, and better understand how innate immunity, metabolism dysregulation and ER signaling pathways intersect in plasmacytoid dendritic cells (pDCs). For that, a systematic analysis of different UPR signaling pathways using different microbial stimuli was performed. Our results, suggest that triggering ER stress responses in pDCs favors activation of IRE1 and PERK pathways and stimulates cytokine production. We are currently dissecting the molecular mechanisms regulating/underlying the crosstalk between different ER stress signaling pathways and pDCs innate activation, as well as their contribution to promote interferon production. This is expected to provide a better understanding on how ER stress contributes to the pathogenesis of immune disorders and, ultimately, facilitate the development of diagnostic tools and novel therapies that target the UPR pathways.

Acknowledgments: This work was supported by FEDER through POCI (COMPETE2020) and by Fundação para a Ciência e a Tecnologia – references PTDC/IMI-IMU/3615/2014, POCI-01-0145-FEDER-16768; UID/BIM/04501/2013; and Maratona da Saúde.

ORAL COMMUNICATION

02

Endothelial Damage is related to sedentary time in patients with resistant hypertension

Susana Lopes[1], Catarina Garcia[1], Ana C Gonçalves[2], Ilda P Ribeiro[3], J Barbosa de Melo[3], Daniela Figueiredo[5], José Oliveira[6], Jorge Polonia[8], José Mesquita-Bastos[4], Alberto J Alves[7], Fernando Ribeiro[1]

[1]School of Health Sciences and Institute of Biomedicine - iBiMED, University of Aveiro, Aveiro, Portugal
[2]Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, and Center for Neuroscience and Cell Biology and Institute for Biomedical Imaging and Life Sciences (CNC.IBILI), Coimbra, Portugal
[3]Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra
[4]Cardiology Department, Hospital Infante D. Pedro, Centro Hospitalar do Baixo Vouga, Aveiro
[5]School of Health Sciences and CINTESIS@UA, University of Aveiro
[6]Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Porto, Portugal
[7]Sports Sciences, Health Sciences and Human Development – CIDESD, University Institute of Maia
[8]Faculty of Medicine, University of Porto, & Hypertension Unit, ULS Matosinhos

Background: Resistant hypertension (RH) is associated with endothelial damage, high risk of cardiovascular events and mortality. Studies suggested that daily physical activity and exercise may increase endothelial progenitor cells (EPCs), a marker of endothelial repair / regeneration and decrease the levels of circulating CECs, an indicator of vascular damage.
Purpose: To determine if, in patients with RH, physical activity is associated with circulating CECs, EPCs and hematopoietic stem cells (HSCs).
Methods: Thirty-two RH patients were recruited. Outcome measures included clinical data, ambulatory blood pressure, daily physical activity, circulating levels of EPCs, HSCs and CECs, quantified by flow cytometry. Sedentary time, and time spent at light and moderate-to-vigorous physical activity (MVPA) was assessed by accelerometers over 7 days. Correlation analysis was conducted to assess the association between variables; independent t-test was used for testing differences in EPC, CEC and HSC according to the compliance with physical activity recommendations (<150 versus ≥150 minutes/week of MVPA).
Results: Patients (age, 57.3±8.4years) were overweight (body mass index, 29.2±3.9kg/m^2) with a systolic and diastolic blood pressure of 146.6±21.1 and 87.4±12.1mmHg, respectively. Sedentary time (453.2±90.2min/day) was inversely associated with CECs (0.07±0.004%; r=0.354, p=0.04). A higher MVPA (29.8±21.3min/day) was a trend to be associated with HSCs (0.018±0.008%; r=0.335, p=0.06). Patients with higher MVPA (n=18) showed higher HSC (0.020±0.008 vs. 0.015±0.007%, p≤0.05) than patients with <150 minutes/week (n=14).
Conclusions: Sedentary time is associated with endothelial injury/damage in patients with resistant hypertension, a specific group who is a challenge for clinicians as the available treatment options have limited success.

Acknowledgments: This work is financed by FEDER Funds through the Operational Competitiveness Factors Program - COMPETE and by National Funds through FCT - Foundation for Science and Technology within the project "PTDC/DTP-DES/1725/2014" and a PhD grant “SFRH/BD/129454/2017”.

ORAL COMMUNICATION

03

Saliva's Microbiota as a biomarker of Chronic Obstructive Pulmonary Disease

Sara Dias[1,2,3], Filipa Machado[2], Carla Valente[4], Lilia Andrade[4], Alda Marques[2,3] and Ana Sousa[1,3]

[1]Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
[2]Lab3R – Respiratory Research and Rehabilitation Laboratory, School of Health Sciences, University of Aveiro, Aveiro, Portugal
[3]iBiMED – Institute of Biomedicine, University of Aveiro, Aveiro, Portugal
[4]Department of Pneumology of the Hospital Center of Baixo Vouga

Deviations from the normal composition of the airway microbiota have been proposed to influence Chronic Obstructive Pulmonary Disease (COPD) but this has not been thoroughly explored and their clinical implications are still unclear.

We aimed at exploring saliva's microbiota of patients with COPD and its relation with specific clinical parameters.

Experimental design: Sociodemographic, anthropometric, clinical data and saliva samples were collected from patients with COPD and healthy individuals. Saliva's microbiota was characterized by 16S rRNA sequencing and analysed using Qiime2 pipeline.

Results: 38 patients (33♂, 66±8y, FEV_1pp 33±7, GOLD III-26, IV-12) and 38 healthy controls (33♂, 66±9y, FEV_1pp 103±18) participated. Saliva's microbiota was significantly enriched in *Proteobacteria* (with higher representation of *Neisseria* and *Haemophilus*) and less diverse in COPD than in healthy individuals. Furthermore, loss of microbiota's diversity correlated with disease severity.

Microbiota revealed two major clusters: **Patient I**, comprising 25% of the patients and a larger cluster including the remaining individuals. Additionally, two clusters emerged from the second cluster, **Patient II** (enriched in patients), and **Healthy** (10 healthy individuals).

Microbiota diversity was not different between these latter clusters but was significantly decreased in **Patient I**. *Proteobacteria* was particularly abundant in **Patient I** and **II** and depleted in **Healthy**, whereas *Bacteroidetes* showed the opposite trend. **Patient I** was enriched in more severe, symptomatic and older patients.

These findings support a close association between the microbiota and COPD and open new avenues to promote remodelling of patients' dysbiotic microbiotas aiming at symptom improvement and preventing disease decline.

Acknowledgments: This work was funded by Programa Operacional de Competitividade e Internacionalização - COMPETE, through Fundo Europeu de Desenvolvimento Regional - FEDER (POCI-01-0145-FEDER-028806 and POCI-01-0145-FEDER-007628), Fundação para a Ciência e Tecnologia - FCT (PTDC/DTP-PIC/2284/2014 and PTDC/SAU-SER/28806/2017) and under the project UID/BIM/04501/2013 and UID/BIM/04501/2019.SD was supported by Grant SFRH/BD/140908/2018 from FCT.

ORAL COMMUNICATION

04

Cellular Proteostasis During Influenza A Virus Infection—Viral Friend or Foe?

Mariana Marques[1], Ana Raquel Soares[1] and Daniela Ribeiro[1]

[1]Institute of Biomedicine (iBiMED) and Department of Medical Sciences, University of Aveiro

In order to efficiently replicate, viruses require precise interactions with host components and often hijack the host cellular machinery for their own benefit. Several mechanisms involved in protein synthesis and processing are strongly affected by viral infections.

In this study we have analysed the interplay between influenza A virus (IAV), the causative agent for most of the annual respiratory epidemics in humans, and the different cellular mechanisms of proteostasis. Upon analysis of protein aggregation at several steps of the infection cycle, using a wild-type IAV and a mutant virus lacking NS1 (a multifunctional protein of IAV related to the *translational host shutoff* normally induced by the virus), we have shown the formation of aggregates, solely after exit of the viral genome from the nucleus, upon replication. We have performed different analyses in order to understand the origin and composition of these aggregates and have shown, e.g., that they do not correspond to stress granules and that its formation is not induced by the overexpression on individual viral proteins. We have also performed a mass spectrometry analysis of these protein aggregates and our results corroborate the well-known manipulative capacity of this virus to usurp the cellular protein processing machinery and further reveal new insights regarding the IAV capacity to take over the transcriptional and translational host processes.

Acknowledgments: This work was supported by the Portuguese Foundation for Science and Technology (FCT): PTDC/BIA-CEL/31378/2017 (POCI-01-0145-FEDER-031378), POCI-01-0145-FEDER-016630, CEECIND/03747/2017, SFRH/BD/137851/2018, UID/BIM/04501/2013, POCI-01-0145-FEDER-007628 under the scope of the Operational Program "Competitiveness and internationalization", in its FEDER/FNR component. It was also funded by the Comissão da Região Centro CCDRC and FEDER through the integrated project pAGE - CENTRO-01-0145-FEDER-000003. This work was also supported by national funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

ORAL COMMUNICATION

05

Understanding the role of tRNA-modifying enzymes on proteostasis and human disease

MA Pereira[1], DR Ribeiro[1], AF Maia[2], MAS Santos[1], M Mano[3], AR Soares[1]

[1]iBiMED – Institute for Biomedicine, University of Aveiro, Portugal

[2]3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto

[3]UC-Biotec, CNC – Centro de Neurociências e Biologia Celular, Biocant Park, Universidade de Coimbra

Transfer RNAs (tRNAs) undergo a variety of post-transcriptional modifications that can ensure its functionality as molecular adaptors in the translation machinery. These modifications are catalyzed by different classes of tRNA-modifying enzymes during the tRNA maturation process and are essential for translation accuracy and efficiency. Recent works show that mutations in tRNA-modifying enzymes and/or tRNA modifications are present in a wide-range of human diseases, namely neurological and metabolic disorders. However, the associated molecular mechanisms behind tRNA-modifying enzymes, translational decoding and disease onset are still unknown. We hypothesize that deregulation of tRNA-modifying enzymes can negatively affect protein translation efficiency, resulting in protein aggregation and proteotoxic stress generation, which are hallmarks of protein conformational disorders. To test our hypothesis, we identified the human tRNA-modifying enzymes that affect proteostasis by implementing a high content screening and explored the activation of specific biomarkers of proteotoxic stress. Here, we demonstrate that knockdown of particular tRNA-modifying enzymes, especially those that catalyze wobble uridine modifications, affect translation efficiency, leading to an increase in protein aggregation and deregulation of the integrated stress response. Our results provide the identification of relevant tRNA-modifying enzymes for proteostasis and we are now exploring their therapeutic target potential for protein conformational diseases.

Acknowledgments: This research was funded by the Portuguese Foundation for Science and Technology (FCT), POCH, FEDER, and COMPETE2020, through the grants SFRH/BPD/77528/2011, SFRH/BD/135655/2018, PTDC/BIM-MEC/1719/2014 and UID/BIM/04501/2013.

ORAL COMMUNICATION

06

Protein aggregation patterns distinguish the response to endocrine treatment of breast cancer cells

Direito I. [1], Enes V. [1], Melo T.[2], Amado F.[2], Moura G.[1], Fardilha M.[1], Helguero L.[1]

[1]iBiMED - Institute for Biomedicine, Univesity of Aveiro, Aveiro, Portugal
[2]QOPNA – Organic Chemistry and Natural Products Unit, University of Aveiro, Portugal

Introduction: Development of resistance to cancer therapy, remains the clinical major challenge. Alterations in the protein quality control (PQC) network are associated with breast cancer (BC) progression and development of endocrine therapy (ET) resistance. Protein aggregation results from metabolic and environmental stress and can be enhanced by ET. Our preliminary results show that PQC mechanisms are increased in endocrine-resistant cells and contribute towards the clearance of aggregated proteins, suggesting that identification of proteins prone to aggregation could be used to monitor patient response to therapy.

Aim: To identify which proteins are prone to aggregation in BC cells that are sensitive and resistant to ET.

Materials and Methods: ET sensitive (MCF7S) or resistant (MCF7R) BC cells were treated with 17β-estradiol(10nM), 4-hydroxytamoxifen (TAM;500nM) or fulvestrant (ICI;250nM) for 24h. Mass Spectrometry (MS) and bioinformatic analysis of proteins isolated from whole cell lysates and respective insoluble fractions (IF) were performed.

Results: MCF7S cells tended to accumulate more proteins in the IF in response to ET. A total of 1093 validated proteins were recovered from MS data. Seventy-four of these proteins were found to be more prone to aggregation in MCF7S after TAM or ICI treatment, with 13 being common to both treatments. Four proteins were related to protein aggregation-related-neurodegenerative-diseases. GO enrichment analysis of IF showed significant representation of the biological process metabolism and RNA processing; while nucleoplasm or membrane-bounded-organelle were enriched for cellular component.

Conclusions: Unique proteins are significantly more aggregated in sensitive cells after ET and may inform about tumour sensitivity to therapy.

Acknowledgments: Dr. Julia Gee for kindly providing MCF7S and MCF7R cell lines; SFRH/BD/123821/2016; UID/BIM/04501/2019; POCI-01-0145-FEDER-007628; pAGE (CENTRO-01-0145-FEDER-000003); LiM facility of iBiMED (POCI-01-0145-FEDER-022122); BDP/UI51/5388/2017; QOPNA (FCT UID/QUI/00062/2019); RNEM (LISBOA-01-0145-FEDER-402-022125).

ORAL COMMUNICATION

07

New sensors and chemical chaperones for protein aggregation

R. Nunes da Silva[a,b], Hélio M. T. Albuquerque[a], Marisa Pereira[b], L. Fontes[a], M. Q. Alves[b], Ana Raquel Soares[b], J. Rocha[c], A. M. S. Silva[a], S. Guieu[a,c], S. I. Vieira[b]

[1]QOPNA & LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal
[2]iBiMED, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal
[3]CICECO Aveiro-Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

Protein aggregation is a biological process in which misfolded proteins aggregate and accumulate in intra- or extracellular media. Protein aggregation is intimately linked to the pathogenesis of many neurodegenerative diseases (such as Alzheimer's, Huntington, Parkinson's and prion disease) but also in cancer and cardiovascular pathologies
(e.g. atherosclerosis, heart failure and ischemic heart disease).
Luminogenic materials have attracted much interest recently, especially fluorophores with aggregation-induced emission enhancement (AIEE). Fluorophores have been a reliable tool for biological study, and in particular fluorophores with AIEE properties have been successfully applied to the selective staining of protein aggregates.
On the other hand, in 2015, lanosterol was reported to reverse protein aggregation of crystallin clumps in mouse cataracts, due to its amphiphilic nature, being able to intercalate into and coat hydrophobic areas of large protein aggregates, making these water soluble again. Taking into consideration this discovery, we believe that other
steroids, such as cholesterol (with the appropriate chemical modification), can be good lead candidates to lower several types of protein aggregates.
Herein we report our recent work on the design and synthesis of fluorophores and chemical chaperones, as well as the staining and disaggregation results in different types of in vitro and ex vivo aggregation models (Figure 1).

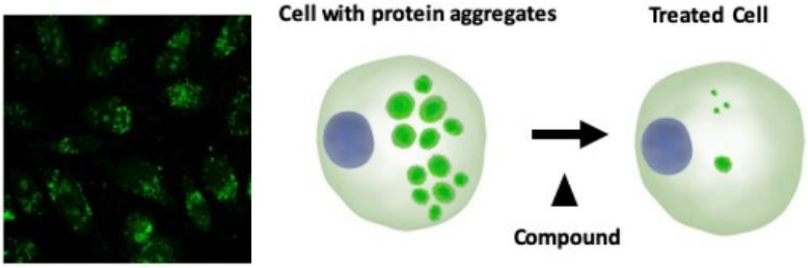

Figure 1. Staining of protein aggregates in cells, as seen using a confocal microscope (left); Schematization of the effect of the chemical chaperones (right).

Acknowledgments: This work was supported by University of Aveiro, FCT/MEC, Centro 2020 and Portugal2020, the COMPETE program, and the European Union (FEDER program) via the financial support to the QOPNA research project (FCT UID/QUI/00062/2019), to the IBiMed Research Unit (UID/BIM/04501/2013), to the Portuguese NMR Network, and to the PAGE project "Agregação proteica ao longo da vida" (CENTRO-01-0145-FEDER-000003), including R. Nunes da Silva and H. Albuquerque Post-Doctoral grants (BPD/UI98/6327/2018; BPD/UI98/4861/2017). This work

was also funded by national funds (OE), through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

ORAL COMMUNICATION

08

Effects of the SET7/9 methyltransferase inhibitor (R)-PFI-2 on mammary epithelial cells

Fátima Liliana Monteiro[1], Catarina Ruivo[1], Inês Batista[1], Beatriz Martins[1] and Luisa A Helguero[1]

[1]iBiMED – Institute of Biomedicine, University of Aveiro, Portugal

*lilianamonteiro@ua.pt

Introduction: SETD7 (SET7/9, KMT7) is a lysine methyltransferase that targets histone and nonhistone proteins that regulate cell cycle and cell differentiation. SETD7 role as tumor suppression or oncogenic signaling is cell context specific. Therefore, its potential as a therapeutic target remain under debate. (R)-PFI-2 is a potent and selective inhibitor of SETD7 activity but its exact effects in normal or disease conditions are unclear. Thus, the aim of this work was to study (R)-PFI-2 effect on proliferation and epithelial differentiation throughout mammary epithelial cell (MEC) differentiation.

Materials and methods: We used the HC11 MEC line to obtain three differentiation stages: stem cell like (SC-L), pre-differentiated (PD) and functionally differentiated (DIF). In each of these stages, cell proliferation was measured with cell counting and KI67 expression, while differentiation was assessed by immunofluorescence and immunoblot of E-cadherin and b-catenin.

Results: (R)-PFI-2 stimulated proliferation in the three differentiation stages. Moreover, a slight increase in %KI67+ cells suggested that (R)-PFI-2 stimulates the cell cycle. In SC-L cells, (R)-PFI-2 upregulated E-cadherin and β-catenin levels. Interestingly, in DIF stage, the inhibitor reduced E-cadherin levels, which suggests that (R)-PFI-2 has cell-type specific effects and that it could have a negative effect in differentiated cells.

Conclusion: (R)-PFI-2 stimulates MEC proliferation and displays dissimilar effects on the expression of epithelial markers depending on the differentiation stage. Therefore, careful analysis of the effects exerted by (R)-PFI-2 and whether they are mediated by SETD7 or result from off-target effects should be considered before it is regarded as a therapeutic option.).

Acknowledgments: UID/BIM/04501/2013; UID/BIM/04501/2019; POCI-01-0145-FEDER-007628; pAGE (CENTRO-01-0145-FEDER-000003); LiM (POCI-01-0145-FEDER-022122); SFRH/BD/117818/2016.

08 Poster Communications

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POSTER COMMUNICATION

01

Influenza A virus induces alterations on the pool of host-cell tRNA-modifying enzymes

Alexandre Nunes[1], Mariana Marques[1], Marisa Pereira[1], Diana Ribeiro[1], Daniela Ribeiro[1]* and Ana Soares[1]*

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Viruses, such as the influenza A virus (IAV), the causative agent for most of the annual respiratory epidemics in humans, take control of the host cell machinery and establish precise interactions with cellular components in order to propagate.

IAV has been shown to specifically manipulate the host-cell transfer RNA (tRNA) populations to enable the efficient translation of viral proteins. tRNAs are modified, post-transcriptionally, by tRNA-modifying enzymes (TMEs) to ensure their stability and efficient translation. The majority of these modifications occurs at the wobble position, located at the anticodon loop, although they may also happen in other areas of the tRNA structure.

In this study we aimed to determine whether IAV infection leads to changes in the expression of the genes that code for the TMEs. Our results demonstrated that some genes (ELP1, ELP3, ELP6 and URM1) were highly expressed two hours post-infection while no striking changes were detected in other time points. We also aimed to determine whether the lack of ELP3 would influence the viral particle production by the infected cells. Using ELP3 knockout cells, our preliminary results show that the absence of this TME notably reduces the viral production, suggesting a relevant role for ELP3 on the IAV life-cycle.

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POSTER COMMUNICATION

02

The effects of ZnO Nanoparticles in Spermatogonia Cells

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Zinc Oxide Nanoparticles (ZnO NPs) have been widely used in biomedicine due to their excellent physicochemical properties. They stand out for example in drug delivery systems, imaging, molecular diagnostics and cancer therapy. However, its possible adverse effects on male reproductive health are not well known. The aim of this work is to evaluate the effects of ZnO NPs on a GC-1 spg cell line, which derived from mouse testis spermatogonia. To achieve our main objective several assays including cell viability, ROS detection, Flow Cytometry and Western Blot assays, were performed in order to evaluate cytotoxicity resulting from exposure to the following increasing concentrations of ZnO NPs (0, 5, 10 and 20 µg/ml) for 6 and 12 hours. The toxic effects of the ZnO NPs were observed in a time and dose-dependent manner. The increasing cell death observed with higher concentrations of ZnO NPs at 6hrs and 12 hrs occurs probably as a consequence of increased DNA damage also observed and measured by the levels of γ-H2AX. Higher levels of reactive oxygen species are also observed. Furthermore, alterations in cell cytoskeleton were also noted. These preliminary results demonstrated that ZnO NPs have the potential to cause adverse consequences for spermatogenesis compromising male fertility. Research is underway to better understand the mechanism of action of these nanomaterials.

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POSTER COMMUNICATION

03

Metabolic reprogramming of tumor-associated macrophages in Triple Negative Breast Cancer

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The immunosuppressive tumor microenvironment, actively promoted by anti-inflammatory M2-like macrophages, facilitates tumor spreading and hinders anticancer immunotherapies. In this project, modulation of human tumor-associated macrophages (TAM) via metabolic reprogramming is proposed as a novel strategy to aid treatment of triple negative breast cancer (TNBC), a highly aggressive BC subtype. Specific aims are to: 1) elucidate howTNBC cells affect human macrophages in vitro, 2) assess the ability of metabolic drugs, selected based on different targets (glucose metabolism, lactate transport, fatty acid oxidation), to reprogram these cells towards pro-inflammatory, anti-tumoral M1-like macrophages, and 3) assess TNBC tumorigenic potential upon metabolic drug-mediated TAM reprogramming. By employing state-of-the-art biochemical methods, such as metabolomics and stable isotope tracing of metabolic pathways, this work will greatly advance current knowledge on the metabolism-phenotype axis in TAM and foster the development of novel adjuvant therapies for TNBC.

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POSTER
COMMUNICATION

04

Impact of pulmonary rehabilitation on the airway microbiota of patients with COPD

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Chronic Obstructive Pulmonary Disease (COPD) is the third worldwide leading cause of mortality. Pulmonary Rehabilitation (PR), a comprehensive intervention that comprises exercise training, education and psychosocial support, is the most cost-effective therapy for patients with COPD.

Exercise training increases ventilation and oxygen uptake, which most likely influences airway microbiota. However, whether this has a role on the positive impact of PR on COPD is still unclear. This study aims to contribute for answering this question by following the impact of PR on the microbiota of 25 patients with COPD (19♂, 73±6y, FEV1pp 48±15) over a period of ~9 months (~3 months before PR, 3 months during PR and 3 months after PR) and of 5 patients not submitted to PR (5♂, 75±6y, FEV1pp 48±13) for a period of 6 months. Sociodemographic, anthropometric, clinical data and saliva samples (once a month) were collected. Saliva microbiota was characterised by 16S rRNA sequencing and analysed using QIIME2 pipeline.

A preliminary analysis of 6 patients (6♂, 72±3y, FEV1pp 46±19) showed that, after PR, the microbiota composition did not converge to a similar composition. Instead, samples collected in different time points from the same patient were more similar amongst themselves than among different patients.
Pooled analysis of the 6 patients showed a significant increase in Neisseria genus from Pre-PR to PR, suggesting that PR contributes to microbiota modulation. Whether this change is related to patients' health status improvement will be the focus of future studies.

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POSTER COMMUNICATION

05

Molecular mechanisms underlying the effect of exercise on prostate cancer-induced testis dysfunction

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Prostate cancer (PCa) is a disquieting cause of cancer death worldwide and, in addition to impairing prostate function, also causes testis adaptations. Exercise training (ET) has been associated with a beneficial effect in both prevention and outcomes of PCa. Regarding the effect of ET in testis function there is no consensus in the literature, but a positive effect has been suggested in preventing or counteracting the impairment of testis function caused by several conditions. In this study, we investigated the preventive effect of ET in PCa-induced testicular dysfunction. PCa negatively affected testicular function, causing spermatogenesis arrest. Oxidative stress-induced DNA damage caused by reduced testis blood flow may have contributed to germ cells apoptosis and consequent impairment of spermatogenesis. Increased glycolysis and branched chain aminoacids metabolism were also evident in PCa animals. Despite not preventing PCa-induced oxidative stress, fifty weeks of treadmill training activated DNA repair mechanisms and counteracted some of the metabolic alterations caused by PCa. These findings confirm a negative effect of PCa in testis function and suggest a benefic role of ET in the prevention of PCa-induced testis dysfunction.

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POSTER COMMUNICATION

06

ER stress as a modulator of plasmacytoid dendritic cells activation

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Upon accumulation of misfolded proteins, an unfolded protein response (UPR) is initiated in order to resolve endoplasmic reticulum (ER) stress, favoring ER proteostasis and cell surviving. Interestingly, ER stress can promote the production of type-I interferon (IFN-I) and inflammatory cytokines, linking UPR to the innate immune system through still unclear mechanisms. Plasmacytoid dendritic cells (pDCs) are a population of innate immune cells that release large amounts of IFN-I upon viral sensing, and thus possess a large ER. However, the role of ER stress on pDC function remains unclear. Thereby, we are analyzing the interplay between ER stress mechanisms and pDCs innate activation. For that, we are focusing on the double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) and inositol-requiring enzyme 1 (IRE1), which are important for the sensing of misfolded proteins. A systematic analysis of these pathways has been performed using combinations of inhibitory and activating compounds, together with TLR stimuli. Our results suggest that ER stress recognition by PERK and IRE1 modulate pDCs activation, with induction of IFN-I production. Currently, we are dissecting the molecular mechanisms promoting IFN-I production at these conditions, trying to understand how ER stress acts as a modulator of pDCs innate activation. With this work we expect to contribute for the development of new drugs with application in the treatment of different autoimmune diseases, such as systemic lupus erythematosus.

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POSTER COMMUNICATION

07

Comparative analysis between protein aggregation levels upon infection with different RNA viruses.

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Upon infection, viruses take control of the cellular machinery, affecting many cellular processes such as protein production and processing. The formation of specialized sites for viral replication can involve extensive rearrangement of cellular cytoskeleton and membrane compartments, resulting in the formation of insoluble protein aggregates or inclusions. These aggregates, as well as specific imbalances in protein folding control, may not only be used by viruses as scaffolds for anchoring viral and host proteins required for replication and assembly, but may also arise as part of the antiviral cellular response that recognizes and sequesters viral components.

We have previously shown that influenza A virus (IAV) infection induces protein aggregation, upon viral ribonucleoprotein (vRNPs) release to the cytoplasm following nuclear replication. In this study we aimed to analyse whether this aggregate formation would be a common feature to other RNA viruses and have chosen the vesicular stomatitis virus (VSV), whose genome replicates solely at the cytoplasm. We have performed a comparative analysis of protein aggregation upon infection with IAV, VSV and specific viral mutants at different time points and our analyses have not shown the presence of protein aggregates upon VSV infection. These results indicate that protein aggregation is not a common feature to all RNA viruses and may be specific to IAV.

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POSTER COMMUNICATION

08

Protein mistranslation modulates hyphal initiation in *Candida albicans*

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Translation of mRNA by the ribosome is a high-fidelity biological process whose error rate is globally low, ranging from 10^{-3} to 10^{-4} in eukaryotic cells. However, recent studies show that mistranslation rates in vivo are variable and can increase up to tenfold in response to stress. For instance, production of reactive oxygen species (ROS) by the host in response to pathogens targets the protein quality control machinery by promoting charging of tRNAs with oxidized amino acids. *C. albicans* takes these ambiguities to the extreme by translating the CUG codon as both leucine (3%) and serine (97%), using a tRNA that is charged by both seryl and leucyl-tRNA synthetases. In this study, we show that changes in the environment, including exposure to antimicrobials and macrophages, increases Leu misincorporation levels from 3% to approximately 50% and these hypermistranslators display remarkable morphological plasticity. Germ-tube (GT) assays in non-inducing conditions show that hypermistranslator strains have enhanced hyphal initiation compared to wild-type strains. During hyphal initiation, two independent pathways are involved in downregulation of the major hyphal repressor (Nrg1). Transcriptional downregulation requires the activation of the PKA pathway, whereas Nrg1 protein degradation requires release from farnesol inhibition. Hypermistranslating strains show no alterations in the cAMP-PKA transcription pathway but the pathway involving degradation of the hyphal repressor Nrg1 is altered as hypermistranslating strains produce less farnesol than control strains. Studies are currently underway to understand how mistranslation alters the quorum sensing mechanism of this pathogen in order to decrease the production of farnesol.

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POSTER COMMUNICATION

09

Unraveling the peroxisome-dependent MAVS signaling pathway

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Upon infection of a host-cell, the viral genome is recognized by the cellular machinery, initiating a signaling cascade that culminates with the production of antiviral effectors which prevent important steps in viral propagation. Peroxisomes and mitochondria have been shown to act in concert as important signaling platforms within this mechanism: while the peroxisomal pathway induces the rapid expression of defense factors providing short-term protection, the mitochondrial pathway activates a signaling cascade with delayed kinetics that amplifies and stabilizes the antiviral response. This suggests the existence of two distinct signaling cascades originating from both organelles.

The mitochondrial signaling pathway has been extensively studied and most of its components have already been identified. With this study, we aimed to unveil the components of the peroxisome-dependent pathway, by investigating the possible involvement of proteins that also belong to the mitochondrial pathway. To that end, we used cells that contain MAVS solely at peroxisomes and overexpressed viral proteins that have been shown to inhibit specific steps of the mitochondrial MAVS pathway: UL36 from herpes simplex virus (shown to cleave the polyubiquitin chains of TRAF3 inhibiting the recruitment of TBK1) or NP from lymphocytic choriomeningitis virus (associates with IKKε blocking its ability to phosphorylate IRF3). These cells were virally-stimulated and antiviral signaling was analyzed by RT-qPCR and immunoblot. Our results revealed that UL36 and NP also inhibited the antiviral signaling in these cells, indicating the presence of TRAF3 and IKKε as downstream molecules of MAVS on the peroxisomal-dependent antiviral signaling pathway.

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POSTER COMMUNICATION

10

Pulmonary rehabilitation closer to patients – feasibility and effectiveness study

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Pulmonary Rehabilitation (PR) remains highly inaccessible to patients with chronic respiratory diseases (CRD). We assessed the effects of a minimal-resource community-based PR programme in patients with CRD.

Seventy-seven patients (48 male; 68±11yrs; 57.7±22.2% FEV1%predicted; 80.3±19.6 FVC%predicted) with COPD (n=52), asthma (n=13), asthma-COPD overlap (n=3), interstitial lung disease (n=7), lung transplant due to COPD (n=1) and bronchiectasis (n=1) participated in a 12-week community-based PR programme. The modified Medical Research Council–dyspnoea scale (mMRC), Saint George's Respiratory Questionnaire (SGRQ), quadriceps muscle strength with hand-held dynamometry (QMS), 1-minute sit-to-stand (1-minSTS), six-minute walk test (6MWT), Brief Balance Evaluation System Test (Brief-BESTest) and Hospital Anxiety and Depression Scale (HADS) were collected pre/post PR. Differences were examined using the Student's t-test/Wilcoxon test and effect sizes (ES) were calculated. The number of patients improving above the minimal clinically important difference (MCID) was established, whenever a MCID was available.

Significant improvements were observed (Table 1). The number of patients above the MCID were: 33 in mMRC (1 point), 47 in SGRQ (4 points); 41 in 1min-STS (3 repetitions); 50 in the 6MWT (25m), 18 in the Brief-BESTest (4.9 points) and 32 and 28 in the HADS Anxiety and Depression scores (1.5 points).

Community-based PR programmes are feasible and effective in patients with CRD.

Table 1. Results from community-based pulmonary rehabilitation (n=77).

Measures	Pre-PR	Post-PR	p-value	ES
mMRC	2[1-3]	1[1-2]	<.001	-.32
SGRQ Total	46±19.6	40.3±16.7	<.001	-.31
HADS Anxiety	6.7±3.8	5.8±3.4	.006	-.26
HADS Depression	6.6±4.1	5.8±3.8	.040	-.21
QMS (kgf)	30.1±8.1	33.4±7.6	<.001	.42
1-minSTS (repetitions)	25.2±9.2	29.1±10.3	<.001	.40
6MWT (m)	401±117.7	443.5±120.6	<.001	.36
Brief-BESTest	17.3±4.7	20.2±3.3	<.001	.71

Values are presented as mean±standard deviation or median [interquartile range]. Significant values p<0.05.

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POSTER COMMUNICATION

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The role of the novel LAP1:TRF2 complex in DNA damage response

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The nuclear envelope (NE) is a specialized double membrane that protects the genome and provides an interface for nucleocytoplasmic communication in eukaryotic cells. Lamina-associated polypeptide 1 (LAP1) is a ubiquitously expressed integral membrane protein of the NE, whose precise physiological role remains unclear. A previous in silico study of the LAP1 protein interactome suggested a function in the regulation of DNA damage response and identified telomeric repeat-binding factor 2 (TRF2) as a putative LAP1 interactor. TRF2 is a shelterin complex protein that inhibits the DNA damage signalling cascade in telomeric regions and is also involved in DNA repair outside the telomeres. Therefore, the main objectives of this work are to establish TRF2 as a novel LAP1-interacting protein and to investigate the functional relevance of the LAP1:TRF2 complex during the DNA damage response. Firstly, the direct interaction between LAP1 and TRF2 was validated in vitro. Moreover, the in vivo formation of the LAP1:TRF2 complex was demonstrated under conditions that promote DNA damage and appears to occur preferentially when LAP1 is phosphorylated. Consistent with a putative co-operative role between LAP1 and TRF2, the induction of DNA breaks resulted in an increase in their protein levels. Finally, the analysis of LAP1's subcellular distribution revealed its co-localization with DNA lesions both in the NE and in intranuclear sites. Considering our current results, we hypothesize that LAP1 may be responsible for the transient recruitment of TRF2 to non-telomeric DNA damage sites, possibly supporting TRF2 interaction with other mediators of the DNA repair machinery.

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POSTER COMMUNICATION

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Modulating immune responses by targeting protein aggregation

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Protein aggregation is induced by a wide variety of cellular stresses, including amino acid starvation, virus infection, endoplasmic reticulum stress, lipopolysaccharide (LPS), and oxidative stress. Inflammasomes are intracellular signalling platforms that can sense sterile stressors such as aggregates of uric acid, ROS, and others, and then activate the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Inflammasome activation can also trigger a rapid, pro-inflammatory form of cell death called pyroptosis. Despite the ongoing research on protein aggregation, the role of these aggregates in inflammation is still poorly understood. The goal of this study is was to dissect the role of formation of protein aggregates on the inflammatory response. We used CAL-1 cells, a plasmacytoid dendritic cell line (pDC), and inhibited either autophagy or proteasome, promoting accumulation of p62-based aggregates with different morphology and chemical composition. Upon autophagy inhibition the overall number of small aggregates increased and upon proteasome inhibition a more prominent and large type of aggregate was observed by laser scanning confocal microscopy. Interestingly, proteasome inhibition promoted an increase in IL-1β secretion, and a reduction on cell viability, suggesting inflammasome activation. Proteasome inhibitors are believed to have anti-inflammatory and immunosuppressive effects and, consequently, they could be used to alleviate inflammatory disorders. But interestingly, our studies reveal that in pDC proteasome inhibition could lead to exacerbation of inflammation.

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POSTER COMMUNICATION

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Epididymal organoid to study and modulate sperm maturation

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Reduced sperm motility and poor interaction between sperm cells and the oocyte are the main causes of male infertility. Sperm acquires motility through the journey across the epididymis, a highly specialized channel, divided into four morphologically and functionally distinct regions. Although the importance of the epididymis on sperm maturation is well recognized, its role on sperm physiology is not fully understood. The challenge on epididymal biology research is the ability to mimic the epididymal environment in a laboratory setting with the goal of deepen the knowledge on sperm maturation. Organoids technology have been essential to model organogenesis, organ function, disease, or drug response, including toxicity of a plethora of tissues. On male reproductive system, organoids and organotypic cultures have already been developed for testis, but the epididymis remains almost forgotten.

We aim to develop epididymal and blood-epididymal-barrier (BEB) organoids based on hollow tubes technology. Hollow tubes are obtained by building-up multilayers (different number of layers (100,200,400)) of marine-derived polysaccharides on sacrificial tubular templates using layer-by-layer technology. Bovine epididymal cells will be cultured in the inner and endothelial cells outer side the tube. The ability of the organoid to maturate sperm will be determined by access sperm motility, morphology and proteomic profile. A successful completion of an in vivo 3D epididymis and BEB tool has a wide range of applications. We will be able to study epididymis-sperm relation, unraveling possible targets to modulate sperm function; evaluate the toxicological and inhibitory effect of drugs on the male reproductive system.

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POSTER COMMUNICATION

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Project APIMedOlder - Operationalization of the EU(7)-PIM List for the Portuguese reality!

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Older patients are more prone to drug adverse reactions when exposed to potentially inappropriate medicines (PIMs). EU(7)-PIM-list is a screening tool developed to identify and compare PIMs prescribing patterns for older people across the Europe. To gain insight into PIMs, we are developing a research, the "APIMedOlder project - avoiding potentially inappropriate medication in older patients through a clinical decision support system. A cluster-randomized trial in primary health care". The first part of this project and the goal of this work is the operationalization of the EU(7)-PIM List for the Portuguese reality.

For all medicines included in the EU(7)-PIM List, a search on the medicines database (Infomed) of the Portuguese Authority for Medicines and Health Products (INFARMED), was made during May 2019. For each drug found with Market Authorization the SmPC was analyzed to check the recommendations made for older patients.

Of the 275 active substances included in the EU(7)-PIM List, 202 have Marketing Authorization in Portugal, belonging to 47 therapeutic classes and 29 therapeutics groups. Non-steroids anti-inflammatory and anti-rheumatic products (M01A) and antipsychotics (N05A) are the therapeutic classes with the greatest number of PIMs. The SmPC of about 17% of the active substances found as PIMs does not refer to special precautions for use in the elderly population.

The knowledge about PIMs for older patients available is an important exercise to develop clinical decision tools to help during the prescription of drugs.

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POSTER COMMUNICATION

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Using CRISPR-Cas9 system to knockout ELP3 in human cells

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As they decode the genetic information organized in the mRNA, tRNA molecules are central players of the translation machinery. To guarantee the efficiency of the translation process tRNA molecules are extensively modified by a host of tRNA modifying enzymes. Numerous tRNA modifying enzymes are deregulated in a myriad of human pathologies but their significance in terms of disease context is not fully understood. Among these is the Elongator Acetyltransferase Complex 3 (ELP3). We had previously showed that knocking down ELP3 significantly increases the levels of protein aggregation and interferes in protein synthesis and quality control mechanisms in HeLa cells. For those reasons, we decided to construct a stable cell line using CRISPR-Cas9 to knockout the expression of ELP3. Our results indicate that a HeLa stable cell line was successfully developed as indicated by increased insoluble fraction and ubiquitination levels in comparison to controls. This stable cell line comprises a valuable tool to exploit the relevance of tRNA epitranscriptome associated genes and pathways for disease onset and progress and hopefully unravel novel therapeutic targets.

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POSTER COMMUNICATION

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The role of NAD metabolism in neurons: from differentiation to injury.

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Background:
Nicotinamide adenine dinucleotide (NAD) is critical for energy production and cell metabolism. It acts both as a coenzyme for oxidation-reduction reactions that culminate in ATP synthesis and as a substrate for NAD-consuming enzymes which include sirtuins, ADP-ribose transferases (ARTs), poly (ADP-ribose) polymerases (PARPs) and cADP-ribose synthases. NAD+ biosynthesis in mammals is supported by several precursors including Nicotinamide (Nam), Nicotinic Acid (NA) and Nicotinamide Riboside (NR) and their respective limiting enzymes Nampt, Naprt and Nmrk1/Nmrk2. Some of these have been shown to protect against neuronal damage and are involved in tissue differentiation.
Goals:
Our aims are to elucidate the roles of the NAD biosynthetic enzymes in neurons. For this, we are using SHSY5Y cells as a model of differentiation and rat primary neuronal cultures exposed to glutamate as a model of excitotoxic damage.
Methods:
Briefly, to induce differentiation SHSY5Y cells were exposed to Retinoic Acid for 5 days and to BDNF for 2 days and collected at timepoints 5 and 7. In the excitotoxic model, rat primary neuronal cultures were exposed to 100µM glutamate at several timepoints (from 1 to 24 hours of exposure). Protein and RNA were extracted for Western Blot and RT-PCR respectively.
Results/ Expected Results:
Our preliminary results show a decrease in Nampt protein expression during SHSY5Y differentiation which indicate that other NAD biosynthesis pathways (e.g. through NA and NR) may be activated. In the excitotoxic model, our results indicate that Nampt protein expression is increased, and suggest a role for the mitochondrial NAD metabolism in protection against excitotoxicity.
Conclusions:
It is crucial to understand the role of NAD precursors and enzymes in brain development and protection from damage to unveil their potential as therapeutic targets in neuronal injury and age-related decline.

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POSTER COMMUNICATION

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The role of mitostasis in protein aggregation during ageing

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Protein aggregation is the biological process by which misfolded and aberrant proteins accumulate and clump together. Since the process of protein aggregation is sufficient to activate stress-response and inflammation, and impairing protein synthesis and quality control mechanisms, aggregation is assumed to negatively affect cellular metabolism and behaviour. The regulation of mitochondria function by mitophagy, a process believed to play a major role in cellular homeostasis, declines with ageing, and recent results have associated gradual increase of protein aggregation associated with the process of ageing.

Using cultured human fibroblast cell lines from donors with different ages, we have studied aging related protein aggregation, inducing protein aggregation by modulating its environment, namely nutritional availability, proteasome and mitochondria activity, or presence of oxidative agents, and measuring its effect in cell survival and metabolism. Cell age was found to be a susceptibility factor for aggregation in response to proteasome inhibition and presence of redox agents. These observations appear to be associated with mitostasis. This ongoing work will clarify whether aging related asymptomatic protein aggregation is by itself a pathological process, which could prove to be a new therapeutic target, or if it is a consequence of other pathological situations, in which case it could potentially be used as a disease indicator. The underlying signalling cascades potentially involved will be discussed.

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POSTER COMMUNICATION

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Myocardial (phospho)proteome characterization of AS patients with in(complete) reverse remodeling

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Background: Aortic stenosis (AS) is a serious condition chronically imposing left ventricle (LV) pressure overload, hypertrophy and remodeling. Upon aortic valve replacement, patients undergo reverse remodeling (RR). Still, myocardial recovery is often incomplete and cardiac hypertrophy/dysfunction may prevail and lead to heart failure.

Goal: To characterize phenotypically AS patients with incomplete RR, through myocardial (phospho)proteomics in order to uncover dysregulated biological processes, phosphoproteins and associated kinases.

Methods: Myocardial biopsies were collected during AVR, from patients with complete (RRC) or incomplete (RRI) RR, as determined by LV mass regression. Protein was extracted and digested. Phosphopeptides were enriched and analyzed by nanoHPLC-MS/MS. Quantification was performed with MaxQuant (FDR 1%), gene ontology enrichment analysis with ClueGO/v.2.5.3., and kinase prediction with GPS/3.0.

Results: From >1800 proteins identified, 40 were found dysregulated, with complement C4-b, C3 and angiotensinogen being notably upregulated. GOEA suggested an association between complement activation, TGF-β production, apoptosis and RRI. Regarding the phosphoproteome, from >22000 phosphopeptides identified, 36 were down-regulated (e.g La-related protein, T515/T526) and 72 up-regulated (e.g. CaMKIIδ, S330/S333/T337) in RRI. DYRK1A, DYRK2 and GSK3(α/β) were predicted as the most active kinases in RRC, while CK2, TAF1/1L and the IKK family were associated to RRI.

Conclusion: Myocardial proteomics suggested that complement activation, apoptosis, fibrosis may favor RRI. In turn, the phospho-proteome/kinome suggested that DYRK1A and GSK3(α/β) may protect AS patients from hypertrophy, while higher activity of CK2, may predispose patients to RRI. These kinases emerge as surrogate RRI prognostic markers and therapeutic targets. Validation in a larger cohort is ongoing.

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POSTER COMMUNICATION

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Widespread protein aggregation across the mouse lifespan

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Aging is characterized by a gradual decline in overall organismal fitness across the lifespan, and greatly enhances the risk for chronic diseases, as neuropathological disorders, diabetes, cancer and cardiovascular diseases. One of the hallmarks of aging is the loss of proteostasis, a regulated process responsible for maintaining the cellular proteome. When proteostasis is altered, proteome and proteostasis network disruptions occur, leading to accumulation of protein aggregates, characteristic of several age-related diseases. Previous work performed in Caenorhabditis elegans and zebrafish unveiled asymptomatic protein aggregation (ARPA), characterized by generalized increased accumulation of insoluble proteins through aging in healthy animals. We hypothesize that proteome imbalances occur with aging in mammals and they correlate with tissue aging and susceptibility for the development of age-related disease.

The main goal of this work is to perform a global proteomic characterization of age-dependent protein aggregation in mice. C57BL/6 mice with different ages (6, 13, 18, 24 and 29 months) were used and the detergent-insoluble fractions isolated from total protein extracts of different tissues. Protein profiles were characterized by automated capillary electrophoresis separation using the LabChip GX. Our results suggest that proteome alterations occur during aging in mammals, and a tissue-specific insoluble protein signature across the lifespan is observed. For the cortex, the identification of the proteins more prone to aggregate during aging was performed by mass spectrometry and characterized to establish the functions and biological processes affected by ARPA. This will allow identifying novel aging biomarkers that may be relevant for disease onset and progression.

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POSTER COMMUNICATION

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Exploiting the role of long non-coding RNAs in the direct conversion of fibroblasts into functional cardiomyocytes

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Direct conversion of fibroblasts into a desired cell type is an alternative to the use of induced pluripotent stem cells (iPSCs). This method has been used to generate cardiomyocytes, although it remains somewhat inefficient. Long non-coding RNAs (lncRNAs), key regulators of gene expression, have been shown to mediate reprogramming of fibroblasts into iPSCs and are differentially expressed during different stages of cardiac development. Likewise, lncRNAs might be novel modulators of direct conversion and may contribute to the efficient generation of cells for regenerative purposes. Therefore, we set out to sequence the non-coding genome and identify lncRNA signatures in mature cardiomyocytes. Mice and human fibroblasts will be directly converted into cardiomyocytes using transcription factors, and identified lncRNAs will be modulated, in order to assess whether they improve the direct conversion efficiency of fibroblasts into induced cardiomyocytes (iCMs).

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POSTER COMMUNICATION

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ATR-FTIR spectroscopy detects changes in lipid profile during aging

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The study of the aging process still brings many questions to this scientific community. Changes in lipidome have already been associated with several age-related diseases, however these biomolecules are also involved in many physiological processes that undergo deep changes with age. So, the study of the lipidome of elderly without age-associated diseases may be a useful tool to understand healthy aging. FTIR spectroscopy is an inexpensive, rapid and sensitive technique that allows to screen the metabolome, including the lipid profile, using peripherical fluids, such as blood plasma. Our main goal was to study possible changes in plasma lipid profile with physiological age, in human plasma samples from the Primary Care-based-Cohort of the region of Aveiro, using FTIR spectroscopy.

We acquired FTIR spectra of 53 plasma samples, and respective triplicates, from healthy individuals (without cognitive impairment (CDR = 0), Diabetes Mellitus, Dyslipidemia, Arterial Hypertension and cardiovascular, oncological and respiratory diseases), and analyzed the 3000-2800 cm-1 spectral region using multivariate statistical analysis. PLS-R showed a positive correlation between spectra and the age of the donors (R = 0.61). In addition, β-coefficients indicate that samples from older donors are characterized by CH3 groups, whereas samples from younger donors are characterized by CH2 groups. These findings may indicate that older individuals have lipids with shorter fatty acids than younger donors. In summary, our preliminary results indicate that there is a specific age-related lipidic spectroscopic profile in human plasma and that FTIR spectroscopy can be a valuable tool for the study of the aging process.

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POSTER COMMUNICATION

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Analyzing the expression of lncRNAs in monocytes and osteoclasts of rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a chronic inflammatory immune-mediated rheumatic disease. Bone erosion is one of the hallmarks of RA pathophysiology and is associated with disease severity. Bone erosions in RA patients result from an imbalanced bone metabolism due, in part, to excessive osteoclastogenesis (the process by which precursor cells of the monocyte lineage differentiate into osteoclasts) and osteoclastic activity. A variable expression of long non-coding RNAs (lncRNAs) has been observed during mouse osteoclastogenesis, suggesting a physiologic role for lncRNAs in the process. In RA patients, lncRNAs expression has been shown to be altered in cellular types critical for its pathophysiology, like peripheral blood mononuclear leukocytes and activated fibroblast-like synoviocytes, in comparison to healthy controls. However, no study has yet examined lncRNAs expression in monocytes and osteoclasts of RA patients. This work aims to address this question by analyzing the expression of a panel of 8 lncRNAs (GAS5, NEAT1, Meg3, DANCR, HOTAIR, Meg9, H19, and Sox2OT) in monocytes and osteoclasts of RA patients (recruited at the Rheumatology Department, Hospital de Santa Maria, Lisbon Academic Medical Centre, Portugal) and of healthy controls (age and sex matched blood donors) by qRT-PCR. Our preliminary results suggest a tendency for a decreased expression of NEAT1 and DANCR along with an increased expression of GAS5 in monocytes of established RA patients in comparison to those of healthy controls. Our ongoing work will provide a higher number of studied patients, to firmly establish whether these lncRNAs are differently expressed in monocytes and osteoclasts in rheumatoid arthritis.

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POSTER COMMUNICATION

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Humanized yeast models overexpressing aggregation prone proteins

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Proteome integrity and cellular health depend on the balance between protein synthesis, folding, and degradation. Under normal conditions, the cell is continuously challenged with misfolded proteins. These are rapidly recognized by a network of chaperones that can promote either their refolding or, if not possible, their degradation. When the formation of misfolded proteins exceeds the capacity of the proteostasis network its accumulation leads to protein aggregation. The association of protein aggregation with several neurodegenerative diseases including Parkinson's disease suggests that gradual proteome aggregation and collapse of proteostasis network in healthy individuals may accelerate aging and the onset of aging related diseases. How the proteotoxic stress triggered by the accumulation of misfolded and aggregated proteins in the cell accelerates aging and triggers disease is still poorly understood due to its pleiotropic effects on the cell. Previous studies in bacteria and yeast showed that induction of synthetic proteotoxic stress increases mutation rate and leads to the accumulation of compensatory DNA mutations.

The objective of this project is to evaluate if protein aggregation and proteotoxic stress destabilizes the genome and increases mutation rate. For that, we will use humanized yeast expressing aggregation prone proteins specifically, α-synuclein and huntingtin as a model system to determine the effect of proteotoxic stress on genome diversification. Humanized yeast will be evolved in the laboratory using routine experimental evolution and their genome will be sequenced using next generation sequencing (NGS).

Through the combination of experimental evolution of humanized yeast, and NGS will be possible to evaluate the long-term effects of protein aggregation on genome diversification.

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POSTER COMMUNICATION

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Pulmonary rehabilitation on inflammatory mediators and IgA in patients with COPD

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Patients with chronic obstructive pulmonary disease (COPD) have a deregulated immune response and show altered levels of pro/anti-inflammatory cytokines and of secretory IgA (SIgA), a first-line airway defense mechanism in the lungs. Pulmonary rehabilitation (PR) leads to physiological and psychosocial improvements in COPD, but its impact on levels of inflammatory mediators remains unclear. This study aimed to (i) compare the levels of inflammatory mediators (e.g. IL-6 and IL-8) and SIgA in saliva of patients with COPD and healthy donors; (ii) evaluate the impact of PR on these mediators and correlate their levels with clinical data.

Outpatients with COPD were enrolled in a PR programme twice a week during 3 months.

Clinical data (e.g., sociodemographic, anthropometrics, muscle strength-handgrip and exercise tolerance-6-minutes walk test [6MWT]) were collected 3 months before, immediately before, after and 3 months after the PR programme. Eleven age and sex matched healthy subjects were evaluated once. Saliva samples were collected every month and stored at -80°C. Inflammatory mediators and SIgA were measured through Sandwich ELISA method.

Twenty participants with COPD (73±7y; 70%♂; FEV1%p 46±14) were enrolled. Mean levels of IL-8 were similar between patients with COPD and healthy subjects. No other trend or significant result was observed. IL-6 was not detected in most samples. Interestingly, a trend for an increase of SIgA during PR was observed. Significant but weak correlations between SIgA level and handgrip (r2= 0,2832) during PR and between SIgA level and 6MWT (r2= 0,3352) after PR were found. Overall, our study suggests that PR influences the immune response of patients with COPD.

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POSTER COMMUNICATION

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UPR-related proteins are present in human sperm and are activated after oxidative stress induction

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Background: The unfolded protein response (UPR) is involved in protein quality control and activated in response to cellular stressors. Although in testis the UPR mechanisms are well described, the presence of these mechanisms in spermatozoa is contentious.
Goals: We aimed to investigate the presence of UPR-related proteins in human sperm and the impact of oxidative stress induction in sperm UPR activation.
Results: To identify UPR-related proteins present in human sperm a bioinformatic approach was adopted. We identified 97 UPR-related proteins in human sperm. We identified, for the first time, the presence in human sperm of HSF1, PERK and GADD34 proteins involved in the sensing and response to unfolded proteins. To explore the activation of UPR, sperm was exposed to hydrogen peroxide (H2O2) and motility, vitality and the levels of UPR-related protein levels were assessed. The incubation with H2O2 resulted in a significant decrease of sperm viability and progressive motility. The levels of UPR-related proteins, such as HSPB1, HSPD1 and eIF2α, significantly increased after exposure to H2O2, suggesting the activation of the UPR in human sperm.
Conclusions: Mapping the proteins involved in UPR in human sperm gave us a first insight on the presence of those mechanisms in the male gamete. However, the belief that sperm are devoid of transcription and translation, points to the need to clarify if the stress response pathways are activated in sperm in the same way as described in somatic cells.

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POSTER
COMMUNICATION

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Development and validation of e-Health tools to support clinical decision and patient empowerment in respiratory infections

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As one of the major public health problems worldwide, antibiotic resistance is mostly fostered by inappropriate use of antibiotics. With the near total ubiquity of mobile technologies nowadays, new possibilities have emerged to enhance clinical decision to improve antibiotic prescribing. It is in this framework that the project eHealthResp proposes to create and evaluate e-Health tools to support clinical decision and patient empowerment in upper respiratory infection management: an online course targeted to physicians and pharmacists and a mobile application based on a clinical decision support (CDS) targeted to physicians, pharmacists and patients are being developed.

The study will undertake cluster randomized controlled trials to evaluate the effectiveness of the aforementioned tools, covering all general practitioners and community pharmacists in centre region of Portugal. These studies will gather quantity and quality indicators as response variables within the context of antibiotic prescription, to be statistically analysed on an intention-to-treat basis.

Expected outcomes include a decrease of antibiotic use, an improvement in antibiotic prescription, and also, a comprehensive list of guidelines in designing and implementing feasible and usable tools for CDS systems in a broad scope but particularly for cases involving upper respiratory system.

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POSTER
COMMUNICATION

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Chronic sildenafil therapy in experimental heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFpEF) already accounts for more than 50% of HF cases and there are still no evidence-based therapeutic strategies to treat these patients. Although decreased protein kinase G (PKG) activity was proposed as a potential therapeutic option, results from randomized clinical trials with type-5 phosphodiesterase inhibitors (PDE5i) were discouraging. Whether specific subgroups of HFpEF patients may benefit from PDE5i remains to be defined. Our aim was to test chronic sildenafil (SIL) therapy on an experimental animal model of HFpEF with severe hypertension and metabolic syndrome. Young male ZSF1 obese rats were randomly assigned to receive SIL 100 mg.Kg-1.d-1 dissolved in drinking water (ZSF1 Ob SIL, n=8), or placebo (ZSF1 Ob PL, n=8). A group of Wistar-Kyoto rats served as control (WKY, n=8). Four weeks later animals underwent peak and endurance effort tests, left ventricular (LV) haemodynamic evaluation, aortic ring preparation and myocardial ATP quantification. ZSF1 Ob PL rats showed systemic hypertension and increased aortic and LV end-diastolic stiffness, with preserved ejection fraction compared to WKY. We also observed a decrease of total workload, peak O2 consumption and LV ATP levels. Chronic SIL treatment significantly attenuated hypertension and decreased aortic and LV stiffness, mostly enhancing effort tolerance and restoring LV energetic resources. Our results showed that chronic treatment with SIL effectively attenuated hypertension, preserved LV end-diastolic function and aortic vascular compliance, as well as improved endurance effort test performance. Further research with the implementation of clinical trials with PDE5i should be performed.

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POSTER COMMUNICATION

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Unravelling the molecular mechanisms underlying the lifestyle-associated decline in human fertility

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Background/Objectives: Evidences suggest that exposure to mercury (Hg) is associated with decline in human fertility, but the molecular mechanisms responsible for the decline of the human reproductive outcomes are still unknown. The main objectives of this work are to: i) assess human exposure to Hg in the Aveiro region using non-invasive biological matrices; ii) examine the influence of variables that may contribute to Hg exposure during pregnancy; and iii) study the impact of Hg exposure on the human fertility.

Methods: This study was carried out in eligible women and men hospitalized at Centro Hospitalar do Baixo Vouga, located in Aveiro. A detailed questionnaire regarding sociodemographic, diet, lifestyle and reproductive data was completed by participants. Samples of hair, saliva, urine, blood, semen, placental tissue and umbilical cord were collected in the normal setting of the hospital from participants. Total Hg levels were quantified in biological samples by atomic absorption spectrometry after thermal decomposition of the sample using the Advanced Mercury Analyzer (AMA-254, LECO).

Results: Our preliminary results yielded additional information for conducting Hg risk assessment for the human reproductive health. Also, our study demonstrated Hg accumulation in biological samples from participants living in the Aveiro region.

Conclusions/Recommendations: Further and continuous monitoring of Hg exposure should be required in order to prevent possible adverse effects in human reproduction. Moreover, it is imperative further investigate the molecular effects of Hg exposure on male and female reproductive health.

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POSTER COMMUNICATION

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Gene expression deregulation across the lifespan

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Aging is characterized by an inevitable, time-dependent decline in physiological function. Despite its inevitability, in the last decades, the improvement of health conditions and the technological development have allowed for a steady increase in human life expectancy. Aging is a major risk factor for a wide variety of diseases that range from cardiovascular and neurodegenerative disorders to cancer and type II diabetes, among others, and living in an aged society comes with a cost: aging and age-related diseases represent a societal and economic burden that are neither negligible nor can be neglected.

This work is an exploratory data mining-based study of age-related gene expression alterations in mice, whose workflow comprises the full re-analysis of raw data available in public databases. Our aim is to identify the genes that change their expression the most across the lifespan. We intend to establish gene expression profiles for different mouse tissues at different lifespan time-points and determine tissue-specific age-related fluctuations in the expression of genes. We further aim to explore the functional implications of aging for each tissue by performing pathway/network analysis based on the obtained differential expression profiles.

Our results will be complemented with additional datasets produced in-house and integrated with proteomics data to comprehensively provide insight into the aging phenotype and underlying biological mechanisms.

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POSTER
COMMUNICATION

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Cellular response to Aβ oligomers is triggered upon local treatment of distal axons

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Retrograde transport within axons has been described to be impaired in several neurodegenerative diseases, including Alzheimer's Disease (AD). However, in AD, it is not well understood how local exposure to amyloid-β oligomers (AβO) in axons can trigger a retrograde cellular response and, as a consequence, trans-synaptic signaling. In this study, we want to unravel if and how axonal degeneration and cell death can be triggered by local treatment of distal axons with AβO, as well as how synapses are affected. With this in mind, we cultured E18 rat primary hippocampal neurons in microfluidic chambers that allow a local stimulation of axons and the assessment of its impact in axonal integrity, cell viability and number of pre-synaptic clusters. Our results show that local application of AβO to the axonal compartment of the microfluidic chambers decreases the number of synaptic clusters, followed by an increase in axonal degeneration. These results suggest that a localized stimulus in axons can trigger a retrograde signal to the cell body. Moreover, these results may also clarify how protein aggregates in general trigger a cellular response that spreads from distal axons to the cell body, which are often localized far apart in the central nervous system and might explain the spread of the aggregation-based diseases between different brain regions.

Key words: Alzheimer's Disease, axonal degeneration, synaptic dysfunction, retrograde transport.

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POSTER
COMMUNICATION

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Multifunctional nanoparticles for multimodal therapy of melanoma

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Melanoma is the most aggressive form of skin cancer and one of the most challenging malignancies to treat with a steeply rising incidence and a poor prognosis in advanced stages. Conventional clinical treatment of melanoma often fails in tumour complete eradication, due to the low response rates to single therapies, chemotherapy side effects and recalcitrance to traditional chemotherapy and radiotherapy. Upconversion nanoparticles (UCNPs) have the ability to generate UV or visible emissions under continuous wave near infrared excitations. These nanomaterials are attracting increasing attention due to their unique properties, as for instance narrow emission bands, high penetration depth into tissues, low background signals, large Stokes shifts, high resistance to photobleaching, photostability and long luminescence lifetimes. These characteristics allow their use in several biomedical applications, including targeted drug delivery, photodynamic therapy (PDT), photothermal therapy (PTT). Therefore, in HOTsPOT project we aim the development of multimodal nanoplatforms for the treatment of melanoma skin cancer. Our approach is based on the development of NIR excitable upconversion nanoplatforms that combine in a single platform, targeted generation of i) hyperthermia, ii) reactive oxygen species and iii) antitumor drug delivery, respectively in a context of photothermal therapy (PTT), photodynamic therapy (PDT) and chemotherapy. For that purpose, SrF2:Yb/Er UCNPs and NaYF4:Yb/Er UCNPs were synthesized and coated with a mesoporous silica shell to allow the incorporation of the tumor targeting molecule, the photosensitizer, and the antitumor drug. Preliminary characterization results regarding size (TEM, DLS), surface charge (zeta potential) and composition (EDS and FTIR) are presented.

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POSTER COMMUNICATION

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Minimal clinically important difference for measures of fatigue, cough and sputum

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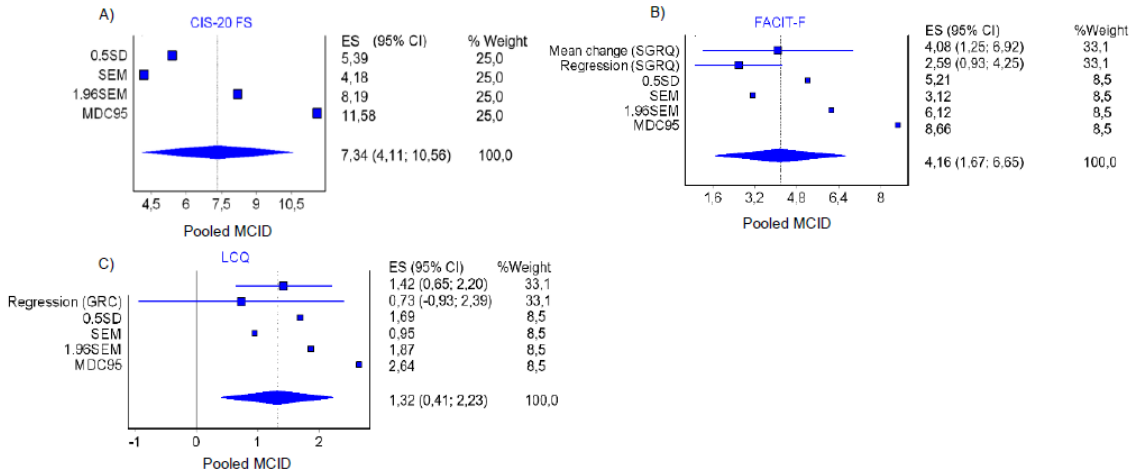
Fatigue, cough and sputum are highly prevalent in patients with chronic obstructive pulmonary disease (COPD). Pulmonary rehabilitation (PR) has shown to be effective in managing these symptoms. However, the interpretation of the magnitude of PR effects is hindered by the lack of cut-off points to identify clinical improvement.

This study established minimal clinically important differences (MCIDs) for the checklist of individual strength – fatigue subscale (CIS-20 FS), functional assessment of cancer therapy – fatigue (FACIT-F), Leicester cough questionnaire (LCQ) and cough and sputum assessment questionnaire (CASA-Q), in patients with COPD following PR.

All measures were assessed pre/post 12 weeks of PR. MCIDs were calculated using anchor- and distribution-based methods. Global rating of change, COPD assessment test and St. George's respiratory questionnaire were used as anchors. Pooled values were obtained using Meta XL with a quality effects model weighting 2/3 for anchor and 1/3 for distribution-based methods.

49 patients with COPD (81.6% male, 69.8±7.4 yrs, FEV1 49.4±19.2%predicted) were included. The pooled MCIDs were: 7.3 for the CIS-20 FS, 4.2 for the FACIT-F, 1.3 for the LCQ, 10 for CASA-Q cough symptoms/ impact and sputum symptoms domains and 7.8 for sputum impact (Fig.1).

MCIDs found in this study can be used by health professionals to interpret PR effects in relieving fatigue, cough and sputum and guide future interventions.



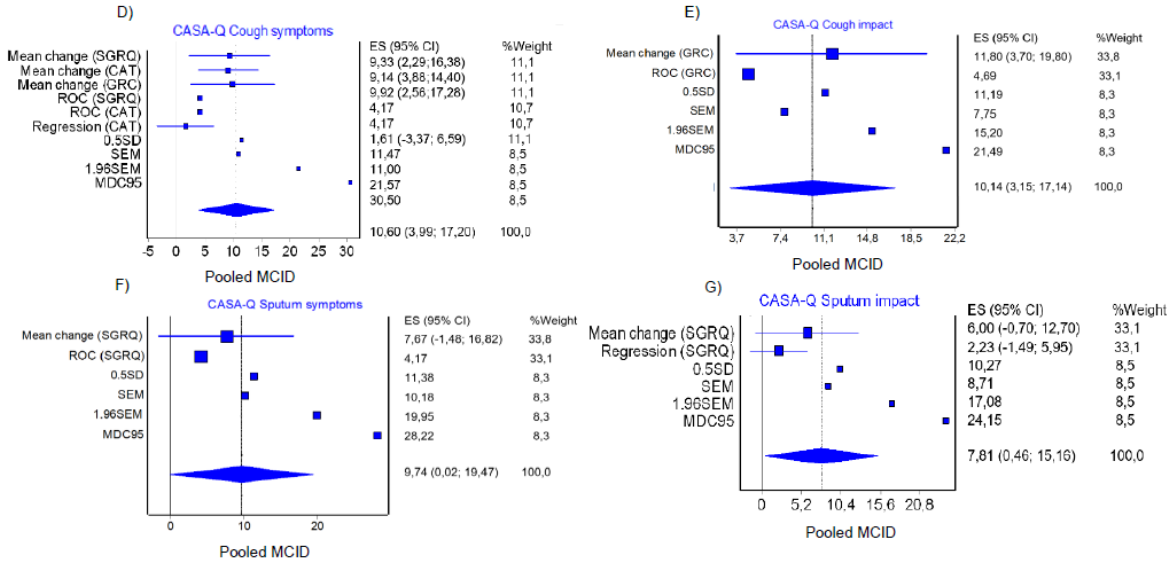


Figure 1: Plot of the pooled MCID for the A) CIS-20 FS; B) FACIT – F; C) LCQ D) CASA-Q cough symptoms; E) CASA-Q cough impact; F) CASAQ sputum symptoms and G) CASA-Q sputum impact. Plots represent the MCID estimates, and when appropriate the estimates include the 95% confidence interval.

Legend: CIS-20 FS – Checklist of individual strength fatigue subscale; FACIT-F – Functional Assessment of Cancer Therapy – Fatigue; LCQ – Leicester cough questionnaire; CASA-Q – Cough ad Sputum Assessment Questionnaire; GRC – Global rating of change; CAT – COPD assessment test; SGRQ – St George's Respiratory Questionnaire; ROC – Receiver operating characteristic curves; SD – standard deviation; SEM – standard error measurement; MDC – minimal detectable change; ES – effect size; MCID - minimal clinically important difference.

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POSTER COMMUNICATION

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Autophagy flux modulates the response of plasmacytoid dendritic cells triggered by TLR activation

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BACKGROUND: Dendritic cells (DCs) are antigen presenting cells that bridge innate with adaptive immunity. Plasmacytoid dendritic cells (pDCs) are a subset specialized in antiviral defense, producing type-I interferons and inflammatory cytokines after viral sensing via Toll-like receptors (TLRs). Infiltration of pDCs in several types of human cancers has been reported, where they are maintained at an immature state that can promote immunosuppression in the tumor microenvironment. Autophagy is a mechanism of lysosomal degradation used for the recycling of cytoplasmic contents such as damaged organelles and protein aggregates, as well as for the elimination of pathogens. This process contributes to the maintenance of intracellular homeostasis and has recently been shown to play different roles in immunity.
GOALS: In this work, we used a human pDC cell line to decipher the interplay between autophagy and pDC activation.
RESULTS/CONCLUSIONS: We studied the impact of macroautophagy inhibition on TLR activation pathways, exposing pDCs to an autophagy inhibitor and analyzing their response after stimulation with TLR7 or TLR9 ligands. The results suggest that the autophagy flux determines the response of human pDCs. These findings can be explored for development of novel immunomodulatory strategies against cancers, infections and to treat autoimmune diseases. Presently, we are dissecting the molecular players regulating this autophagy – TLR signaling crosstalk.

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POSTER COMMUNICATION

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Are translation-related proteins present and functionally active in sperm?

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Spermatozoa are highly differentiated haploid cells originated through a sequence of mitotic and meiotic divisions – the spermatogenesis. During this process, spermatozoa undergo major structural and functional changes at the nuclear and cytoplasmic levels. Because of these changes, the silencing of the gene expression is widely accepted in mammalian spermatozoa. A new perspective emerged when de novo protein synthesis was shown to occurs in sperm cells under capacitation conditions, opening the discussion to established dogma.

The main objective of this work is to characterize and evaluate the translational activity that may occur in mammalian spermatozoa. To achieve this goal a bioinformatic and experimental approach was performed.

Bioinformatic analysis revealed the existence of 315 translation-related proteins present in spermatozoa. Simultaneous analysis of the biological processes and cellular compartments associated with each protein led to the identification of 31 translation exclusive-proteins. Additionally, the PPI network analysis reveals that 315 overlapping proteins are strongly connected and related to each other. Also, the enrichment analysis of PPI network, for biological processes and cellular compartment, evidenced the strong association to processes translation-related process and a preferential location in cytoplasmic and mitochondrial regions.

The SUnSET technique unequivocally established the existence of translational activity through the incorporation of puromycin into the nascent polypeptide chains. A decrease in puromycin incorporation was observed with the use of mitochondrial and cytoplasmic translational inhibitors, which leads us to believe the coexistence of both forms of translation in spermatozoa.

Together, these results evidenced the existence of translational activity in mammalian spermatozoa.

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POSTER COMMUNICATION

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Signalling pathways commonly altered in different models of imbalanced proteostasis

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The healthy ageing process is characterized by loss of physiological integrity and is associated to several hallmarks, including loss of proteostasis and altered intercellular communication. The rate of aging is controlled to some extent by genetic pathways and biochemical processes, and unbalanced proteostasis may affect ageing-associated pathways and vice-versa. A previous bioinformatics analysis performed by D. Neves and R. Silva retrieved 28 genes that were simultaneously associated with proteostasis and ageing, including signaling genes. Aiming to further understand if unbalanced proteostasis had consistent effects in ageing-associated pathways in different cells, age and proteostasis-associated signaling pathways were monitored in two models of unbalanced proteostasis. For that, HeLa and neuroblastoma SH-SY5Y cells were seeded, let to adhere overnight, and then incubated with: a) the proteasome inhibitor MG132 (10 µM for the HeLa cells and 5 µM for the SH-SY5Y cells) and b) 100 nM thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase. Cells were incubated with the compounds for several time points (0, 10, 14 and 18h for the MG132 assays; 0, 2, 16 and 24h for the thapsigargin assays), collected and lysed in SDS, and subjected to SDS-PAGE and Immunoblotting. Age and proteostasis-associated signaling pathways such as the mTOR, ERK, STAT3/5, BAG3, ATF4 were monitored in these cellular models. Herein we report the similarities between the signaling responses of the different cells to different inducers of proteostasis imbalance.

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POSTER COMMUNICATION

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FTIR spectroscopy reveals changes in protein aggregation profile in plasma from middle to old age

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The loss of proteostasis is one of the hallmarks of ageing and is well described in different aging models. Nevertheless, there is a substantial lack of information regarding the pattern of age-related protein aggregation in biofluids. FTIR spectroscopy is a simple and inexpensive method, widely used in biomedical research. It gives a metabolic fingerprint of the sample and is also sensitive to the secondary structure of proteins. Therefore, our goal was to assess the age-related protein aggregation profile in human plasma samples from the pcb-Cohort from the Aveiro region, using ATR-FTIR spectroscopy.

We first tested 127 samples from individuals without cognitive impairment (CDR=0) between 50-93 years. Our PLS-R results showed a positive correlation between FTIR spectra and the age of the donors (R=0.46). Younger samples have increased content of antiparallel β-sheets, associated to the presence of oligomeric structures. To assess the effect of the most common age-related pathologies on the correlation model, we excluded samples of the CDR=0 group with Diabetes mellitus, dyslipidemia, cardiovascular, oncologic, neurologic and respiratory diseases (n=53) and repeated PLS-R analysis. Results showed an improved correlation (R=0.57) with the same spectral profiles, corroborating the age-associated changes in protein aggregation.

At this point, our results show a decrease in protein oligomers from middle to old age. They also support FTIR as a suitable approach for protein conformational studies and to reveal a healthy ageing signature.

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POSTER
COMMUNICATION

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Respiratory muscle strength: a systematic review with equation testing in Portuguese healthy adults

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Respiratory muscle weakness is frequent in chronic respiratory diseases. Several equations exist to predict maximum respiratory pressures but there are no recommendations of which should be used and none was developed for Portugal. This study revised predictive equations of maximum inspiratory (MIP) and expiratory (MEP) pressure for healthy adults and explored their suitability for the Portuguese population. A systematic review was conducted. Studies were eligible if they presented at least 1 equation for MIP or MEP developed for healthy adults. For equation testing, MIP/MEP were collected from healthy adults. Predicted values were computed from the equations and compared with actual values using Wilcoxon tests and Bland-Altman plots. 19 studies were included. 36 MIP and 30 MEP equations were found but only 32 and 25 were possible to test in 229 subjects (62%♂, 101.8±20.5FEV1pp, 66.7±9.7yrs). 4 MIP equations showed no significant differences between actual and predicted values ($p>0.05$, rs=0.32-0.47, R2=9-47%). From these, 3 overestimated (bias=0.19-4.06 cmH2O, men) and 1 underestimated (bias=0.99 cmH2O, women) the actual values (Fig. 1). All MEP equations showed significant differences between actual and predicted values.

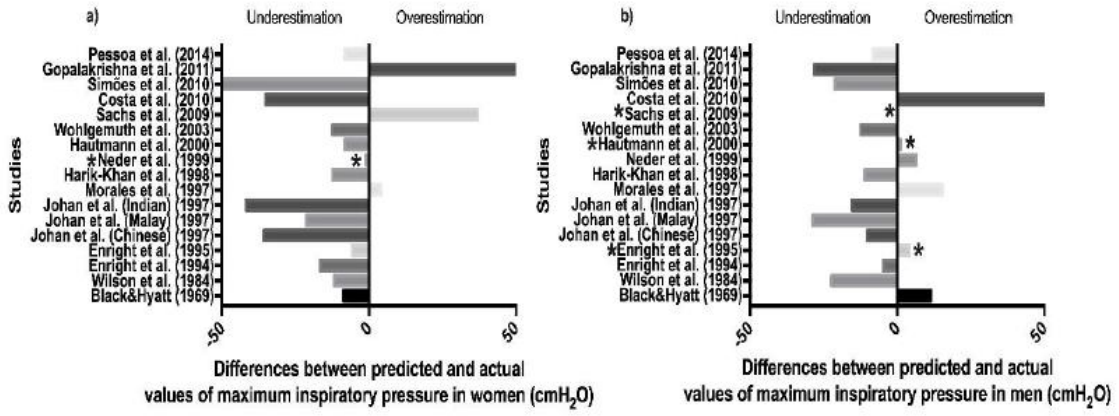

Fig 1. Estimation differences across studies between predicted and actual values of maximum inspiratory pressure in a) women and b) men.

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The genetics of cognitive aging: Genotyping the Minho aging Cohort

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Aging is a complex biological process characterized by a progressive loss of physiological integrity and accompanied by a decline in cognitive capabilities. Understanding the genetic architecture implicated in age-associated cognitive decline will likely provide novel biological insights into why some individual are prone to cognitive deficits. Here, we present some preliminary data regarding the genotyping of a healthy aging cohort showing distinct cognitive profiles (strong and poor cognitive performers). The Minho aging cohort is composed of 1051 community-dwelling individuals and is representative of the general Portuguese population regarding gender, age and education. Participants underwent a thorough neurocognitive and psychological evaluation and MRI scans for brain gray and white mater volumetric measurements were acquired. Individual neurocognitive test were used as quantitative traits and a multi-trait analysis was performed. Additionally, since mood and stress have an impact in cognitive performance, we sought to identify variants mediating these effects by performing a genome-wide interaction study (GWIs). Finally, we explored the use image-derived phenotypes to evaluate whether genotypes modify age-associated structural changes. Our preliminary data suggests that cognitive aging is likely associated to deficits in DNA-repair mechanisms and a deregulated immune response.

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Use of *Drosophila melanogaster* to screen for novel druggable targets for breast cancer therapies

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The association between tumour genomic features, such as homologous recombination (HR) repair, and clinical relevant endpoints in breast cancer is yet not well established. The most recent generation of targeted therapies are inhibitors of PARP1 (PARPi), a protein essential for the initiation of several DNA repair pathways. This therapy is based on the principle that when tumour cells are deficient in a particular DNA repair pathway (e.g., HR deficiency caused by BRCA1/2 mutated genes), this lack of source is compensated by the activation of a second pathway. The tumour is hence dependent on this alternative pathway for survival and inhibition of which will kill malignant cells specifically - synthetic lethality.

Although the use PARP1 inhibitors is promising, tumour resistance is frequently seen and has become a major concern in long-term treatments. We have been using Drosophila melanogaster to identify novel druggable targets that may be used to develop new targeted therapies for breast cancer. We're using a straightforward and unbiased genetic approach, being our hypothesis, that yet unidentified components of the DNA repair/DNA replication/Gene expression machineries are likely to provide novel targeted therapies for breast cancer.

So far, 419 and 383 genes were tested for synthetic lethality, respectively, with dPARP1 and dBRCA2, and several candidate genes were already isolated. A secondary screen is currently being performed to rule out false positive hits. A significant breakthrough would be the identification of genes whose depletion (and inhibition) would specifically induce apoptosis in human cells mutant for BRCA2 or where PARP1 was inhibited.

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POSTER COMMUNICATION

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Proteomic urinary biomarkers for Prostate cancer management: Pilot study

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Prostate cancer (PCa) is the most prevalent cancer among men in developed regions. Despite molecular biomarkers, like PSA, have emerged as crucial tools for PCa diagnosis and prognosis, they have revealed limited sensitivity and specificity. Hence, the development of more accurate biomarkers is mandatory.
For this purpose, urine appears as an attractive source of potential biomarkers especially due to its non-invasive collection. An exploratory research was conducted in urine samples from five PCa patients and five healthy controls in order to determine a proteomic profile of PCa. Gel electrophoresis combined to liquid chromatography-tandem mass spectrometry (GeLC-MS/MS) analysis together with an evaluation of the proteolytic activity and ensued by immunoblotting validation was performed in those samples.
The urine proteome revealed eighteen dysregulated proteins between PCa and controls, some of them associated to ECM remodelling. In agreement, the urinary gelatinolytic activity as well as the activity of some metalloproteinases (MMPs) and their inhibitors (TIMPs) was tendentiously higher in PCa. Along with protein expression analysis, no association between increased MMP2 activity and MMP2 or TIMP2 expression was observed, and the increase in MMP-9/TIMP-1 activity was related to increased MMP9 expression but not with changes in TIMP-1 or TIMP-2 expression. Besides, the expression of two proteins involved in cell-cell adhesion (galectin-3 and vinculin) was evaluated. Only a significant increase of vinculin levels was observed in PCa.
These results, despite preliminary, confirm the potential of urine as a source of PCa biomarkers. Presently, we are validating some other protein targets disclosed by MS analysis.

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COMMUNICATION

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FTIR spectral analysis of serum samples from Alzheimer´s disease cases

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide. Until now, there is no reliable molecular blood biomarkers available and the only molecular approach is based in cerebrospinal levels of Aβ species and Tau forms. The identification of new blood-based biomarkers would improve AD diagnosis, allowing earlier interventions and the improvement of patients' quality of life. Fourier Transformed Infrared spectroscopy is rapid, inexpensive, highly reproducible and only requires small amounts of sample. In this preliminary work, serum spectra of Alzheimer's disease cases and Controls from two independent cohorts were analysed by multivariate analysis. The results obtained indicate a moderate discrimination of AD cases vs controls using FTIR spectroscopy, supporting the potential of this technique in AD diagnosis.

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Screen for novel druggable targets for breast cancer therapies

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Tumor suppressor BRCA2 has recently been reported to regulate RNApolII transcription elongation and prevent the formation of R-loops (PMID 29386125), which are a three-stranded nucleic acid structure composed of a DNA:RNA hybrid. Accumulation of R-loops is implicated in carcinogenesis, among other reasons due to the generation of single-stranded DNA (ssDNA). RPA binds to ssDNA, protecting it from endonucleases and avoiding the generation of abnormal secondary DNA structures. The most recent generation of targeted therapies are inhibitors of PARP1, a protein essential for the initiation of several DNA repair pathways. Although breast cancer cells mutant for BRCA2 are particularly sensitive to PARP1 inhibitors, tumour resistance is frequently seen and this has become a major concern in long-term treatments.

Our aim is to identify novel druggable targets whose inhibition could be used in combination (or alternatively) to PARP1 inhibitors, minimizing the risk of tumor resistance. Our current working hypothesis is that the combinatory inhibition of PARP1 and RPA will specifically enhance apoptosis in breast cancer cells. We are currently testing this hypothesis in three distinct breast cancer cell lines.

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Metabolic networks in age-related diseases

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Aging is a natural physiological process, but its specific causes are not entirely understood at the molecular level. During aging, the levels of the redox cofactor Nicotinamide Adenine Dinucleotide (NAD) decrease. This molecule is essential for energy production by the cell and is also a substrate to a range of enzymes that regulate gene expression and cell survival.

To gain insight into the metabolic networks of age-related disorders, we took a combined bioinformatics and molecular approach. We used text-mining methods to extract protein interaction data from 1500 PubMed abstracts containing keywords related to proteostasis, aging and age-related diseases. Protein networks were obtained with Cytoscape and Gene Ontology analysis was performed with ClueGo. In addition to the expected enrichment in functional categories such as Protein Processing in the Endoplasmic Reticulum or Alzheimer's Disease, one of the overrepresented protein clusters was the IL-17 signalling pathway. This reinforces the role of inflammation in age-related disorders.

As a cellular model, we have used a NAD metabolism inhibitor in SH-SY5Y neuroblastoma cells to mimic the NAD decline during aging. At different time points (8h, 24h, and 48h) we measured cell viability along with the expression levels of NAMPT, the rate-limiting enzyme in NAD biosynthesis. Our results show a 50% decrease in cell viability at 48h and no significant alterations in NAMPT protein levels in all time points. It is possible that other NAD biosynthesis pathways are activated, and further studies intended to elucidate this are underway.

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