TOLEDO

9-11 NOVEMBER 2022

HOTEL BEATRIZ

SEFAGIA 2022

Sociedad Española de Autofagia

ABTRACTS BOOK´S

SEFAGIA 2022

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ABSTRACTS

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Wednesday, November 9th

16:30-17:30h Session 1

“Physiological Roles of Autophagy”

Chair: **Guillermo Velasco**

Role of autophagy during cell competition in heart development and regeneration

Esteban-Martínez L, Sierra R, Torres M.
Spanish National Centre for Cardiovascular Research, Madrid.

Cell Competition (CC) is the process by which viable cells are eliminated from tissues by comparison with neighbouring cells. Myc overexpression in a mosaic fashion induces CC in heart, a mechanism by which Myc-high cardiomyocytes actively eliminate neighbouring wildtype cardiomyocytes.

Aim: exploring the role of metabolism and autophagy during Myc-dependent CC and to analyse if cardiomyocytes are able to compete by comparison of their genetic background for autophagy. Materials and methods: CC induction in embryonic hearts was made by using the previously described iMOS-Myc mice (Cell Reports, 2014) and modulation of metabolism by maintaining pregnant females under hypoxia or treating them with monocarboxylate transporters (MCTs) inhibitors. Autophagy flux was evaluated by intraperitoneally injection of cloroquine and autophagy/mitophagy blockade was made by generating the iMOS-Myc; Atg7+/f and iMOS-Myc; Oma1+/f and crossing them with Nkx2.5Cre mice. Generation of an inducible-traceable strategy for restricting autophagy in a mosaic manner in postnatal heart was made by using iSureCre; αMHC-MerCreMer and Atg7f/f mice. Results: autophagy is activated during CC in embryonic hearts in both wildtype and Myc overexpressing neighbouring cardiomyocytes and its inhibition by Atg7 or Oma1 deletion protects wildtype cardiomyocytes from apoptotic cell death. More interestingly, hypoxia and metabolic communication shut down by MCTs inhibition completely abolish autophagy and CC. On the other hand, the fitness comparison between cardiomyocytes with differences in autophagy induces the reduction of Atg7+/- cardiomyocytes proportion in a ROS dependent manner. Intriguing, this effect only happens during the regenerative stage of neonatal heart. Conclusions: Together, these results suggest that some metabolites that are released through MCTs and whose levels can vary upon hypoxic conditions are implicated in mitophagy regulation during Myc-dependent CC of the embryonic heart. Moreover, by comparison of autophagy levels, postnatal cardiomyocytes are able to compete in a ROS dependent manner, which could have potential implications for cardiac regeneration.

An alternative metabolic link connects glutamine and autophagy to control glutamoptosis

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Objective: A wide variety of cancer cells exhibit a high consumption rate of the amino acid glutamine, quickly becoming addicted to this amino acid. However, the unbalanced activation of glutamine metabolism during nutrient restriction inhibits autophagy and kills cancer cells through an apoptotic mechanism termed “glutamoptosis”. While the cellular bioenergetic sensor AMPK is known to play a critical role in autophagy regulation, AMPK pathway and the bioenergetics status of the cell during glutamoptosis are still unknown.

Material and Methods: Using a metabolomic approach, we identified a parallel path through the asparagine synthetase (ASNS) and the GABA shunt.

Results: Our results indicated that glutamine sufficiency increased the ATP levels of the cell and prevented AMPK activation in the absence of other amino acids. Surprisingly, we observed that the sufficiency of glutamine to increase ATP levels and to inhibit AMPK pathway did not require alpha-ketoglutarate production through glutaminolysis. Indeed, glutaminase is not necessary to sustain ATP levels in glutamine sufficiency condition. Targeting glutaminase and ASNS prevented the inhibition of autophagy. We also observed that the inhibition of AMPK was a necessary step for the activation of mTORC1 and the subsequent inhibition of autophagy by glutamine metabolism.

Conclusions: We propose a new model in which ASNS and GABA shunt play a critical role in the control of autophagy by glutamine.

Post-ER degradation of misfolded GPI-anchored proteins is linked with microautophagy

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Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are membrane-conjugated cell-surface proteins with diverse structural, developmental, and signaling functions and clinical relevance. Typically, after biosynthesis and attachment to the preassembled GPI anchor, GPI-APs rapidly leave the endoplasmic reticulum (ER) and rely on post-ER quality control. Terminally misfolded GPI-APs end up inside the vacuole/lysosome for degradation, but their trafficking itinerary to this organelle and the processes linked to their uptake by the vacuole/lysosome remain uncharacterized. In a yeast mutant that is lacking Pep4, a key vacuolar protease, several misfolded model GPI-APs accumulated in the vacuolar membrane. In the same mutant, macroautophagy and the multi-vesicular body (MVB) pathway were intact, hinting at a hitherto-unknown trafficking pathway for the degradation of misfolded GPI-APs. To unravel it, we used a genome-wide screen coupled to high-throughput fluorescence microscopy and followed the fate of the misfolded GPI-AP: Gas1∗. We found that components of the early secretory and endocytic pathways are involved in its targeting to the vacuole and that vacuolar transporter chaperones (VTCs), with roles in microautophagy, negatively affect the vacuolar uptake of Gas1∗. In support, we demonstrate that Gas1∗ internalizes from vacuolar membranes into membrane-bound intravacuolar vesicles prior to degradation. Our data link post-ER degradation with microautophagy.

Wednesday, November 9th

18:00-19:00h Session 2

"**Physiological Roles of Autophagy"**

Chair: **Nadeza Apostolova**

Functional differences in microglial and neuronal macroautophagy under proteotoxic stress

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Autophagy is a catabolic process involved in many different cellular functions, with the result essential for maintaining cellular homeostasis. However, the molecular pathways governing different cellular functions of autophagy, remain poorly understood. The objective of this work was to investigate the role of autophagy in the context of proteotoxic stress in microglial and neuronal cells. Proteotoxic stress was induced by proteasome inhibition and different cellular pathways were studied. Our results demonstrated that proteotoxic stress produced massive apoptotic cellular death in microglial cells, as well as unbalanced induction of UPR, without activation of the IRE1α-sXbp1 arm, but predominant activation of the PERK-CHOP pathway. In contrast, neuronal cells were more resistant and showed canonical UPR activation, with a predominance of the IRE1α-sXbp1 arm. Importantly, both cell lines activated autophagy, but with different kinetics and cellular functions. Autophagy flux under basal conditions was higher in microglia, but under proteotoxic stress, neurons showed faster autophagy activation. Despite autophagy activation in both cell lines, polyubiquitinated proteins accumulated in a time-dependent manner in microglia, but not in neurons, indicating a different functional role of autophagy in both cell lines. At the molecular level, we observed predominant activation of the mTORC2-AKT-□-catenin pathway in microglia, but PDK1-AKT-FOXO3 in neurons. These molecular differences could explain the functional differences in autophagy observed in microglia and neurons. In conclusion, proteotoxic stress activates autophagy in both microglia and neurons, but through different signaling pathways and with different cellular functions.

cAMP-Protein Kinase A and Stress-Activated MAP signaling mediate transcriptional control of autophagy in fission yeast during glucose limitation or starvation

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The molecular mechanisms that induce autophagy during glucose starvation have been extensively explored in the budding yeast Saccharomyces cerevisiae, nonetheless Little is known about how this coping response is regulated in the evolutionary distant fission yeast Schizosaccharomyces pombe. Here, we show that S. pombe autophagy in response to glucose limitation relies on an entirely functional mitochondrial electron transport chain (ETC), but, in contrast to S. cerevisiae, the AMP-activated protein kinase (AMPK) and DNA damage response pathway components do not modulate fission yeast autophagic flux under these conditions. In the presence of glucose, the cAMP-protein kinase A (PKA) signaling pathway constitutively represses S. pombe autophagy by downregulating the transcription factor Rst2. Furthermore, the stress-activated protein kinase (SAPK) signaling pathway, and its central mitogen-activated protein kinase (MAPK) Sty1, positively modulates autophagy upon glucose limitation at the transcriptional level through its downstream effector Atf1 and by direct in vivo phosphorylation of Rst2 at S292. Thus, our data indicate that the signaling pathways that governs autophagy during glucose shortage or starvation have evolved differently in S. pombe and uncover the existence of sophisticated and multifaceted mechanisms that control this self-preservation and survival response.

Finding Nemo: discovering novel autophagy modulators with ebrafish

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Objective: The ATG5-ATG16 complex is key during the formation of the phagophore, allowing its elongation and the association of LC3. Also, this complexation links autophagy and the ubiquitin proteasome system, which have been implicating in apoptosis and some pathological scenarios such as cancer and Parkinson. As it happens with other autophagy components, we are currently lacking effective small molecule modulators to study their functions or as potential therapeutic agents.

Methods: Using virtual high throughput screening we identified two compounds: A14 (2,4-dichloro-N-(4-phenylbutyl)benzamide) and A62 (1-(3-methylphenyl)-N-[4-(1-pyrrolidinyl)benzyl]methanesulfonamide) that block the ATG5-ATG16 complexation. To further test their biological activity and toxicity we exposed zebrafish embryos to A14 and A62 at different developmental stages and concentrations.

Results: Both compounds are safe at quite high doses (10 □M), although produce a slight delay of development. Interestingly, these drugs have distinct impacts on autophagy. Using western blot and immunohistochemistry, we showed that A62, but not A14, disrupt autophagy flux measured after exposing embryos to NH_4Cl. However, A14 decreases the formation of autophagosomes. We also observed that A14 disrupts yolk physiology. As animals with external development, zebrafish maintain their nutrients, mostly lipids, in the yolk. Using BODIPY staining, we showed that fat is retained in the yolk instead of being mobilized to cells, suggesting that A14 might disrupt lipophagy.

Conclusions: These novel compounds efficiently disrupt autophagy, but through different mechanisms and have distinct effect on cellular biology. This work demonstrates that zebrafish are useful to study novel autophagy modulators and to decipher their exact mechanisms.

Thursday, November 10th

10:00-11:00h Session 3

"Physiological Roles of Autophagy"

"Autophagy and Disease"

Chair: **Raúl Durán**

"Physiological Roles of Autophagy"

Photosynthetic assimilation of CO_2 regulates TOR activity

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The target of rapamycin (TOR) kinase is a master regulator of cell growth in all eukaryotes, activating anabolic and repressing catabolic processes in response to nutrients and energy. It is well stablished that nutrients such as amino acids and glucose are major regulators of TOR in yeast and metazoans, but how TOR responds to nutrients in photosynthetic organisms is less understood. Our studies in the model single-celled alga *Chlamydomonas reinhardtii* have shown that TOR promotes protein synthesis and negatively regulates autophagy. We have also reported that phosphorous regulates TOR signaling via LST8, a conserved protein associated with TOR. Specifically, phosphorus limitation leads to a sharp decrease of LST8 protein abundance, which in turn results in downregulation of TOR kinase activity and autophagy activation. Moreover, we have recently shown that the carbon source is a main regulator of TOR activity in photosynthetic organisms. Our results demonstrated that the photosynthetic assimilation of CO_2 efficiently activates TOR signaling through the synthesis of key amino acids such as Gln, Glu, Ala, Val and Leu. These findings might have biotechnological and ecological implications since TOR controls biomass and cell growth coupled to CO_2 removal. Taking together, our results reveal that carbon and phosphorous are major inputs in the regulation of TOR signaling and autophagy in photosynthetic organisms.

Funding: This work was supported in part by Ministerio de Ciencia y Tecnología (Grant PGC2018-099048-B-I00).

"Autophagy and Disease"

Role of autophagy in the hepatoprotective role of the antiretroviral drug Rilpivirine: when less is more

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[d]*Departamento de Fisiología, Facultad de Medicina, Universidad de Valencia, Valencia*

Objective: Understanding the mechanisms through which hepatic stellate cells (HSC), become activated/inactivated is paramount to the search for novel therapeutic targets for liver fibrosis (LF). The antiretroviral drug Rilpivirine (RPV) has demonstrated a hepatoprotective effect in several animal models of chronic liver injury that is related to its antifibrogenic and apoptotic action in HSC. Here, we evaluate whether autophagy is implicated in this effect.

Material and methods: Two standard mouse models of chronic liver injury - fatty liver disease and carbon tetrachloride (CCl_4)-induced hepatotoxicity -and cultured HSC activated with the profibrotic cytokine TGF-β.

Results: RPV enhanced autophagy in the whole liver of both high fat diet- and CCl_4-exposed mice and in activated HSC (increased protein expression of autophagy markers, autophagosome content and lysosomal mass). Increased autophagic flux was observed in RPV-exposed HSC as revealed by tandem fluorescence-tagged LC3 and p62, and analysis of LC3-II accumulation in chloroquine-exposed cells. Importantly, autophagy was involved in the cytotoxic effect of RPV on HSC, as treatment with wortmannin or depletion of specific autophagy proteins (ATG5, Beclin-1 and SQSTM1/p62) by means of RNA interference rescued the cytotoxic effect of high concentrations of RPV on activated HSC. Finally, RPV compromises the viability of TGF-β-induced HSC independently of its antifibrogenic effect, observed as reduced collagen 1A1 synthesis which does not include RPV´s modulation of autophagy.

Conclusions: As a contributor to the mechanisms involved in the hepatoprotective action of RPV, autophagy may be a good candidate to explore when developing novel therapeutic avenues for LF.

"Autophagy and Disease"

Selective autophagy plays a protective role against acute and age-related retinal degeneration

Jiménez-Loygorri JI[1], *Ramírez-Pardo I*[1], *Villarejo-Zori B*[1], *Viedma-Poyatos A*[1], *Sierra-Filardi E*[1], *Gómez-Sintes R*[1] *and Boya P*[1]

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Objective. Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people in the developed world, and the number of people affected is expected to almost double by 2040. The retina presents one of the highest metabolic demands that is partially or fully fulfilled by mitochondria in the neuroretina and retinal pigment epithelium (RPE), respectively. Together with its post-mitotic status, this context requires a tightly-regulated housekeeping system that includes selective mitochondrial autophagy. We want to assess the effects of selective autophagy deficit or induction in the retina given an AMD-like paradigm.

Materials and methods. Eyes from *Ambra1*$^{+/+}$, *Ambra1*$^{+/gt}$ and mitoQC mice were analysed using flatmount or cryosection immunostaining. Sodium iodate (SI) was used as a model of AMD-like damage and Urolithin A (UA) as a mitophagy inducer. ARPE-19 human cells were used as an *in vitro* model and analysed by immunostaining, flow cytometry and RT-qPCR. Bioinformatic analysis of public human datasets was also performed.

Results. *Ambra1*$^{+/gt}$ autophagy-deficient mice present alterations in the RPE, similar to those observed in human AMD patients, such as abnormal morphology or lipofuscin accumulation, which appear in an age-dependent manner. Furthermore, *Ambra1*$^{+/gt}$ mice are more sensitive to acute SI-induced retinal degeneration than their *Ambra*$^{+/+}$ littermates. UA induced mitophagy *in vivo* and prevented degeneration both in the neuroretina and RPE. This amelioration was also associated with decreased lipid peroxidation, gliosis and increased photoreceptor survival. *In vitro*, inhibition of mitophagy, or general macroautophagy, abolished this rescue.

Conclusions. Selective autophagy plays a protective role in the retina and can be exploited to preserve vision in physiological or pathological conditions.

Thursday, November 10th

12:40-13:40h Session 4

“Autophagy and Disease”

Chair: **José Manuel Fuentes**

Characterization of the molecular mechanism of autophagy inhibition by HER2

Jetsy Karina Montero Vergara[1], *Verena Jendrossek*[1], *Silvia Vega-Rubín-de-Celis*[1,*]

[1]*Institute for Cell Biology (Cancer Research), University of Duisburg-Essen, Essen, Germany*

Objective: Approximately 20% of breast cancer (BC) cases have amplifications in HER2 which correlates with worse prognosis. We previously showed that Beclin 1 and HER2 interact and that such binding leads to autophagy inhibition and tumour development. Therefore, we aim to understand the molecular mechanism by which HER2 regulates autophagy and tumorigenesis.

Material and methods:

In vitro approaches including the GFP-LC3 puncta formation assay, western-blot (total levels of p62, LC3BI/II ratio) and HiBiT-LC3 reporter system were used to measure the autophagic flux in both HER2+ and HER2- cell lines. Besides, *in silico* studies lead to identify important regions in Beclin 1 and other key proteins implicated in regulating autophagy in this context. The role of these proteins in tumorigenesis was determined *in ovo*, through the Chick Chorioallantoic Membrane (CAM) assay.

Results: Our data demonstrate that the Beclin 1/HER2 complex includes a novel Beclin 1 interacting protein. Knockdown of this novel protein induces autophagy and upregulates the catalytic activity of Vps34 independently of mTORC1 and the Bcl2-Beclin 1 binding. To ascertain the molecular mechanism of Beclin 1 binding and autophagy regulation we used multiple mutants based on *in silico* analysis and we found that key regions for such binding include the ECD domain of Beclin 1. Furthermore, our *in ovo* CAM studies indicate that knock-down of this new interactor greatly inhibits tumour growth.

Conclusions: Taken together, our data suggest that a new Beclin 1 interacting protein mediates the effects of HER2 and Beclin 1 in autophagy and tumour growth.

Dysregulation of the autophagic-lysosomal pathway in Parkinson's disease associated to GBA

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2: Hospital Universitari Vall d'Hebron, Biochemistry Service, Barcelona, Spain
3: Autonomous University of Barcelona, Bellaterra, Spain

Objective: The first genetic risk factor for developing PD is the presence of mutations in the GBA gene that encodes the lysosomal enzyme glucocerebrosidase (GCase). An inverse relationship between the loss of GCase activity and the accumulation of alpha-synuclein has been demonstrated in different PD models and samples from PD patients carrying GBA mutations. The objective of this study was to generate and characterize a new in vitro neuronal model of Parkinson's diseases associated to GBA that allowed us to investigate the link between the loss of GCase and alpha-synuclein pathology. Methods: We have generated a set of differentiated and stable human dopaminergic cell lines that express the two most prevalent GBA mutations, i.e. N370S and L444P, as well as GBA knock out as an in vitro disease modeling system. We performed a deep analysis of the consequences triggered by the presence of mutant GBA and the loss of the GCase activity in the ER, mitochondria but especially focusing in the lysosomal compartment. Results: A variety of events triggered by the initial loss of GCase activity lead to intralysosomal accumulation of sphingolipids and cholesterol, lysosomal dysfunction, and impairment of chaperone-mediated autophagy (CMA), along with other events previously described in PD-GBA models. These pathogenic mechanisms contribute, directly and indirectly, to an increase in the accumulation and aggregation of alpha-synuclein. Conclusions: We describe a new molecular mechanism to understand how the initial loss of GCase activity can lead to general lysosomal dysfunction and to alterations in lysosomal lipid composition that impair CMA activity and promote abnormal accumulation of alpha-synuclein.

Does AMBRA-1 play onco-suppressive functions in skin squamous cell carcinomas?

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Activating molecule in Beclin-1 regulated autophagy (AMBRA1) participates in the regulation of different cellular processes including autophagy and cell proliferation. Previous research has shown that *AMBRA1* plays a tumor suppressor role. In this work we aimed at analyzing the role of AMBRA1 in the origin and progression of skin squamous cell carcinomas (SCC).

Results presented here show that *AMBRA1* genetic inhibition increases the proliferative and invasive capacity of SCC cells. Likewise, we found that SCC cells in which AMBRA1 has been genetically inactivated acquire a mesenchymal phenotype associated with a reduction in the expression of epithelial markers and an increase in the expression of mesenchymal markers. We also found that this effect relies on an increased activation of the TGF-β signaling pathway. Our findings support the notion that AMBRA1 plays an important role in the regulation of skin squamous cell carcinoma by controlling the epithelial to mesenchymal transition through TGF-β signaling pathway.

Thursday, November 10th

15:30-17:00h Session 5

"**Junior PI Symposium and WIA Presentation"**

Chair: **Patricia Boya**

Microvascular endothelial cell autophagy regulates neutrophil trafficking in inflammation

Reglero-Real N[1,7*], Pérez-Gutiérrez L[1,7], Yoshimura A[2], Rolas L[1], Barkaway A[1], Boulanger C[3], Muller WA[4], Nightingale TD[1], Perretti M[5], Tooze SA[6], Collinson L[2], Aksoy E[6] and Nourshargh S[1*]

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Objective: A key feature of an inflammatory reaction is tissue infiltration of neutrophils, a response that requires breaching of endothelial cells (ECs) that line the vascular lumen and must be tightly regulated to avoid excessive tissue damage. The role of autophagy as an essential regulator of immunity is well accepted, and defects in autophagy are linked to the development of numerous inflammatory conditions. However, while there is ample evidence of immune cell autophagy-related genes regulating inflammation, less is known about the role of EC autophagy in this context. Here, we explored the role of microvascular EC-autophagy in neutrophil trafficking within multiple acute models of inflammation.

Methods and Results: To investigate autophagy in ECs *in vivo*, we established a high-resolution confocal microscopy method to detect LC3-punctae in postcapillary venules using GFP-LC3 transgenic mice. With this approach, we found that inflamed venular ECs exhibited enhanced levels of LC3-puncta that localised exclusively at EC contacts, an event aligned temporally with the peak of neutrophil trafficking. Furthermore, mice with selective EC deletion of the key autophagy gene *Atg5* exhibited increased neutrophil extravasation in multiple inflammatory models. Real-time and high-resolution analysis of neutrophil-EC interactions by 4D confocal intravital microscopy revealed significantly exaggerated and faster neutrophil transendothelial migration across autophagy deficient ECs, while pharmacological induction of autophagy inhibited neutrophil migration. Mechanistically, autophagy machinery regulates the remodeling of EC junctions and expression of key EC adhesion molecules, facilitating their intracellular trafficking and degradation.

Conclusion: Collectively, our results identify EC autophagy as an essential cellular process to limit physiological neutrophil trafficking during acute inflammation.

Control of mitosis by lysosomes to develop CIN-targeting cancer therapy

Mauvezin C

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Introduction. Mitosis dictates the faithful transmission of the genetic material among generations, which precludes chromosomal instability, a hallmark of cancer. Correct mitotic progression relies on the orchestrated degradation of mitotic factors, and whether autophagy and lysosome-dependent degradation are involved in mitotic coordination remains a controversial question. We recently established that lysosome-dependent degradation is an essential process which prevents chromosomal instability (CIN) (Almacellas et al., Autophagy 2021), providing new perspectives in cancer therapeutics. In addition, we characterized the toroidal nucleus (TN), a particular phenotype with perforated nucleus, as a novel biomarker for the identification of CIN, inherent in cancer cells.

Objective. Our current research focuses on promoting the toroidal nucleus as a robust marker for CIN in genotoxicity screenings.

Results. First, we aimed at better understanding the mechanisms driving the formation of the toroidal nucleus. We tested whether perturbations of centrosome orientation, DNA damage and/or cohesion defects could trigger the formation of TN upon inhibition of mitotic lysosomes. Next, we aimed at creating tools to automatically detect the TN using machine-learning trained algorithm. Finally, we are currently reassessing CIN levels in the 4 subtypes of breast cancer cells to evaluate the efficiency of inhibition of autophagy in combination or not with current treatment used in the clinic.

Conclusions: Our results establish a connection between two influential fields in cancer research: autophagy and chromosomal instability. Our findings serve as precedent for the characterization of the regulatory mechanisms, involving autophagy and lysosomes, required for chromosomal stability to establish novel CIN-targeted cancer therapy.

Anacardium occidentale extract promotes autophagy and protects against glycative stress derived cytotoxicity

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Sugars or their metabolites react with biomolecules through a non-enzymatic process called glycation resulting in advanced-glycation end products (AGEs). AGEs are toxic and play a pathogenic role in the progression of cellular aging. New literature supports a major role for autophagy in fighting glycation-derived cytotoxicity by clearance of cytosolic AGEs. There is scanty information about natural products that enhance autophagy in the context of glycative stress.

Objective: *Anacardium occidentale* (AO) has been traditionally used to manage diabetes mellitus and treat its sequelae in Caribbean folk medicine. New literature suggests that phytocompounds of AO could enhance autophagy. In this study, we evaluate the impact of AO in the autophagic process and its potential protective role against glycative stress.

Method: We have exposed human lens epithelial cells (HLECs) and MEFs to glycating reagent methylglyoxal (MGO) in the presence/absence of chloroquine and to AO microwave aqueous extract. We evaluated autophagy function using immunofluorescence against endogenous LC3 and p62 and quantified cytotoxicity using Cell Titer Blue. Finally, the phytochemical profile of the AO extract was explored.

Results: AO promoted autophagy in different cell types in a short-term period and concentration- and time-dependent experiments revealed that AO extract is cytoprotective against MGO-derived cell death.

Conclusions: Our findings indicate that *in vitro* glycative stress-induced cellular damage can be mitigated by AO extract that promotes autophagic activity. Identification and use of AO phytocompounds might be a therapeutical strategy to combat the age-related decline of autophagy and diminishes the detrimental impact of AGEs on tissue fitness, especially in those with low regeneration capacity.

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A mammalian target of rapamycin-perilipin3 (mTORC1-Plin3) pathway is essential to activate lipophagy and protects against hepatosteatosis

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Background and Aims: NAFLD is the most common hepatic pathology in western countries and no treatment is currently available. NAFLD is characterized by the aberrant hepatocellular accumulation of fatty acids in the form of lipid droplets (LDs). Recently, it was shown that liver LD degradation occurs through a process termed lipophagy, a form of autophagy. However, the molecular mechanisms governing liver lipophagy are elusive. Here, we aimed to ascertain the key molecular players that regulate hepatic lipophagy and their importance in NAFLD.

Methods: We analyzed the formation and degradation of LD in vitro (fibroblasts and primary mouse hepatocytes), in vivo and ex vivo (mouse and human liver slices) and focused on the role of the autophagy master regulator mTORC1 and the LD coating protein perilipin (Plin) 3 in these processes.

Results: We show that the autophagy machinery is recruited to the LD on hepatic overload of oleic acid in all experimental settings. This led to activation of lipophagy, a process that was abolished by Plin3 knockdown using RNA interference. Furthermore, Plin3 directly interacted with the autophagy proteins FIP200 and ATG16L, suggesting that Plin3 functions as a docking protein or is involved in autophagosome formation to activate lipophagy. Finally, we show that mTORC1 phosphorylated Plin3 to promote LD degradation.

Conclusions: These results reveal that mTORC1 regulates liver lipophagy through a mechanism dependent on Plin3 phosphorylation. We propose that stimulating this pathway can enhance lipophagy in hepatocytes to help protect the liver from lipid-mediated toxicity, thus offering a therapeutic strategy in NAFLD.

WIA presentation

Marina García

Thursday, November 10th

18:15-19:15h Session 6

"Autophagy and Disease"

Chair: **Ricardo Escalante**

Differential process of mitophagy in neurons and astrocytes associated to Parkinson's disease related to a-synuclein

Lastres-Becker, I.[1,2], *Castro- Sánchez*[1], *S. Silva-Llanes*[1], *I., Teresak*[3], *P., Lanciego, J.L.* [4], *Sierra, E.* [3], *Boya, P*[3].

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Objective: to determine the role of the different mitophagy pathways involved in PD, a key question that is still unresolved. We will investigate whether a-synuclein (α-SYN) WT and with the A53T mutation can modulate (induce/repress) mitophagy in neurons and astrocytes *in vivo* and *in vitro*.

Background: Mitochondria are organelles that perform essential functions in cells regulating metabolism, reactive oxygen species generation, and most importantly the production of most of its energy. Mitophagy is a quality control pathway whereby mitochondria are specifically targeted by autophagosomes for degradation within lysosomes. Mitochondrial dysfunction is a well-established pathological hallmark of Parkinson's disease (PD), probably due the high dependence on mitochondrial metabolism of neurons. However, whether mitophagy is altered in this disease is still a matter of intense debate.

Methods: we will assess mitophagy *in vivo* in MitoQC reporter mice after stereological injections of WT and mutated A53T α-SYN adeno-associated expression vectors. Along with mitophagy assessment, other mitochondrial parameters will also be evaluated to characterize mitostasis. Similar *in vitro* analysis will be performed in N2a cells that constitutively express α-SYN or immortalized astrocyte cell line (IMA2.1) treated with recombinant α-SYN.

Results: Results showed that *in vivo* α-SYN-WT or α-SYN-A53T neuronal overexpression leads to a decreased mitophagy in dopaminergic neurons of SNpc, whereas increased mitophagy occurs in reactive astrocytes surrounding area. *In vitro*, N2a expressing α-SYN showed increased mRNA expression of PINK1/PARKIN while reducing the expression of BNIP3/NIX, indicating the involvement of different mitophagy pathways.
Conclusions: Our results indicate that α-SYN (WT or A53T) overexpression induces differentiated mitophagy mechanisms between neurons and astrocytes, key for the development of PD.

Deciphering the formation and clearance of glycogen aggregates in Lafora disease

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Objective

Lafora disease (LD) is a severe neurodegenerative condition caused by mutations in two genes: EPM2A, which encodes laforin, a dual specificity phosphatase, and EPM2B, which encodes malin, an E3-ubiquitin ligase. The hallmark of the disease is the accumulation of cytoplasmic aggregates of poorly branched glycogen called Lafora bodies (LBs) in astrocytes and neurons. The accumulation of these aggregates underlies the pathophysiology of LD. In addition to glycogen, LBs contain a number of proteins, including ubiquitinated proteins and the autophagy adaptor p62. The presence of these proteins suggests that, like other insoluble molecular aggregates characteristic of neurodegenerative diseases, LBs could be targets for autophagic clearance. The mechanisms that drive the formation and clearance of LBs have not been identified yet.

Materials and methods

To study the formation and clearance of LBs, we have generated several mouse models, including a malin knockout mouse devoid of p62.

Results and conclusions

Our results demonstrate that p62 is involved in the formation of LBs. In the absence of p62, neuroinflammation and susceptibility to epilepsy are exacerbated. These observations identify p62 as a key player in the cellular protective response against glycogen aggregates. We also demonstrate that malin is dispensable for glycophagy, the selective autophagy of normal glycogen.

Targeting autophagy vulnerabilities for cancer treatment

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Autophagy is essential for maintaining cellular homeostasis and is implicated in many physiological and pathological conditions, including protection against disease, such as cancer. A dual role of autophagy in cancer has been described, and whereas it plays a major role in maintaining cellular homeostasis and therefore inhibiting tumor growth at early tumorigenesis stages, it also has pro-tumorigenic functions in established tumors. Thus, it is essential to understand the specific function of autophagy in each tumor type and stage.

We previously demonstrated that modulating autophagy in receptor-tyrosine kinase altered cancers led to decreased tumor development and progression. Through multiple bioinformatic tools, including expression arrays analysis as well as RPPA (Reverse phase protein arrays) analysis, we identified a new function of a tumor suppressor (mutated in multiple cancers arising from different tissues), in autophagy regulation. Our studies in vitro in cells derived from multiple cancer types suggest a transcriptional regulation of oncogenic kinases that regulate autophagy through Beclin 1. Both in vitro and in ovo experiments indicate that a combination of kinase inhibitors and autophagy-modulating drugs compromise cell growth and tumorigenesis in cancers containing mutations in some tumor suppressors.

In summary, we uncover a new autophagy-regulating axis that can stratify patients from a variety of tumor entities that could potentially benefit from autophagy modulating drugs.

Friday, November 11th

10:00-11:20h Session 7

Chair: **José Luis Crespo**

“Autophagy and Disease”

“Mechanisms of Autophagy”

"Autophagy and Disease"

Astrocyte-derived small extracellular vesicles exert a protective effect on euron and astrocytes primary cultures damaged with epoxomicin

Alarcon-Gil J.[1], Posada-Gracia M.[1], Nogales M.D.C.[1], Escobar-Peso A.[1], Ponce-Mora A.[3], Casarejos M.J.[1], Gómez A.[1], Asensio M.J.[1], Yuste A.[3], O´Loghlen A.[2], Bejarano E.[3] and Rodríguez-Navarro J.A.[1]

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Small extracellular vesicles such as exosomes are highly relevant in aging and neurodegenerative diseases. These exosomes are lipid bilayer particles smaller than 150 nm, which are released by cellular multivesicular bodies. Their contents can differ substantially according to the cell type and health status. Specifically, astrocyte-derived exosomes (AsEVs) exert neuroprotective effects on aging. However, their activities and mechanisms of action are not fully understood. We aimed to study the effects of AsEVs from early passages of primary astroglial cultures on primary neuronal and astroglial cultures. Both cultures were also treated with epoxomicin (10 nM) to generate a model of disrupted proteostasis. Thus, the results obtained from the lactate dehydrogenase and Griess assays indicated a protective effect of AsEVs against epoxomicin in both neuronal and astroglial cultures. Next, we studied the possible mechanisms through which AsEVs exert their protective actions. Therefore, we observed by immunocytochemistry and immunoblotting that AsEVs improved p62 and LC3 autophagic flux in control and epoxomicin-damaged astroglial cultures but not in neuronal cultures. We also confirmed that AsEVs ameliorated the decrease in GSH and GSTM-2 levels and mitochondrial function in epoxomicin-treated neuronal and astroglial cultures. In brief, AsEVs exerted a protective effect on epoxomicin-damaged neurons and astrocytes by improving antioxidant response and mitochondrial function. Moreover, AsEVs improved the autophagic flux in control and epoxomicin-damaged astrocytes. Therefore, it is necessary to further study the effects and mechanisms of action of AsEVs for their possible use in aging and neurological disorders.

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"Autophagy and Disease"

Diabetes type 3: role of human amylin and therapeutic approaches

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Objective: To analyze the cellular mechanisms involved in human amylin (hIAPP) elimination and to study the possible beneficial effects of resveratrol administration.

Material and methods: we have used rat insulinoma cell lines, INS1-E WT and INS1-E overexpressing hIAPP. To assess hIAPP detoxifying mechanisms, we have focused on INS1-E hIAPP cell line using different compounds, such us the autophagy flux blocker chloroquine (CQ) and the exosomes formation inhibitor GW4869. Exosomes were obtained from both, INS1-E WT and INS1-E hIAPP cell lines, which were isolated using a commercial kit from cell culture media and analyzed by Dynamic Light Scattering. Human amylin-enriched media were administered to HT-22 hippocampal cells and amylin uptake was observed. Protein expression has been evaluated by Western blot and immunofluorescence assays.

Results and conclusions: By forcing the production of amylin, in a hyperglycemic context, we observed a greater export through exosomes and said amylin is subsequently captured by HT-22 cells, forming β-amyloid aggregates inside them. In summary Resveratrol administration not only improved different cell signaling pathways involved in cell homeostasis such as mitochondria dynamics, reticulum stress or the mTORC1 pathway but also facilitated its removal via exosomes.

"Mechanisms of Autophagy"

Molecular mechanisms of the interaction between the PROPPIN Atg21 and Atg16

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Atg8/LC3 lipidation is essential for phagophore expansion during autophagy. WIPI2 in mammals and its yeast homolog Atg21, mediate the recruitment of the lipidation machinery to the phagophore membrane through their interaction with PI3P and ATG16L1/Atg16. Our work aims to further study the molecular mechanism of the Atg21-Atg16 interaction in yeast and the differences and similarities with the mammalian homologs.

For this purpose, we used two-hybrid techniques to identify the residues in both Atg16 and Atg21 that mediate this interaction. Additionally, we used the reporters Pho8Δ60, GFP-Atg8, and Ape1 to analyze the effect of mutations of these residues on bulk and selective autophagy.

We show that disruption of Atg21-Atg16 binding blocks selective autophagy, but also strongly impairs bulk autophagy. We find that the Atg16 binding site on Atg21 is conserved in human WIPI2 and involves the same binding surface identified in yeast WIPI4 or Hsv2 as responsible for Atg2 binding, thus indicating that WIPI proteins use the same mechanism to interact with different proteins of the autophagic machinery.

Furthermore, our data indicate that Atg16 dimerization is required for Atg21 binding, and that residues in both Atg16 monomers mediate Atg21 binding. Finally, our findings support the idea that the WIPI2 binding site in ATG16L1 has been lost in yeast Atg16, and has been replaced by a site in the coiled-coil domain involving residues in both Atg16 monomers.

"Mechanisms of Autophagy"

TP53INP2 regulates selective autophagy

Saška Ivanova[1,2,3], Sandra Macedo Ribeiro[4], Marta Martinez-Vicente[5], and Antonio Zorzano[1,2,3]

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TP53INP2 is a versatile protein involved in different processes such as transactivation of a number of nuclear hormone receptors, myogenic and adipogenic differentiation, autophagy and apoptosis among others (1-4). In addition, TP53INP2 mRNA and protein levels are down-regulated in muscle and adipose tissue in diabetic and obese mice and humans (1, 2). TP53INP2 positively regulates autophagy by binding to all Atg8 proteins through LIR motif, and expression of TP53INP2 increases the number of autophagosomes in basal and starvation conditions (3). On the contrary with the increased autophagy, TP53INP2 up-regulates protein levels of some autophagy receptors as for example p62 and NBR1 (1), and decreases their degradation through autophagy. Moreover, in TP52INP2 expressing cells p62 phase separated (in 30% of the cells), which as well as increased protein levels of p62 was abolished by mutations in LIR sequence of TP53INP2. Surface Plasmon Resonance studies showed that the LIR sequence of TP53INP2 has the highest affinity for the Atg8 proteins that any other LIR described so far, which explains why TP53INP2 displaces p62 and some other autophagy receptors from the autophagosomes. Mass spectrometry of the purified autophagosomes revealed that TP53INP2 expressing cells have increased levels of mitochondria proteins in the autophagosomes, suggesting an increase in mitophagy. We are currently investigating if TP53INP2 is an autophagy receptor.

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Friday, November 11th

12:20-13:40h Session 8

"**Mechanisms of Autophagy"**

"Chaperone-Mediated Autophagy"

Chair: Marta Martínez

"Mechanisms of Autophagy"

H_2S regulates selective ER-phagy and mitophagy through persulfidation

Angeles Aroca, Cecilia Gotor

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Hydrogen sulfide (H2S) is an endogenously generated gaseous signaling molecule, which has been implicated in autophagy regulation in both plants and mammals through persulfidation of specific targets. In previous works, it has been showed that persulfidation is the molecular mechanism through which sulfide regulates autophagy in plant cells. ATG18a is a core autophagy component that is required for bulk autophagy and is required for reticulophagy during ER stress. Thus, persulfidation of ATG18a may regulate ER stress–induced autophagy in Arabidopsis. In this research, we have revealed the role of sulfide in ER stress as a negative regulator of autophagy in plants. We demonstrate that sulfide regulates ATG18a phospholipid-binding activity by reversible persulfidation at Cys103, and this modification activates ATG18a binding capacity to specific phospholipids in a reversible manner. Our findings strongly suggest that persulfidation of ATG18a at Cys103 regulates autophagy under ER stress and the impairment of persulfidation affects both the number and size of autophagosomes.

In mammals, sulfide regulates parkin, an E3 ubiquitin ligase that plays a critical role in mitophagy through persulfidation and H2S also modulates mitochondrial morphology to promote mitophagy in endothelial cells under high glucose. Mitophagy in plants has been scarcely studied, therefore, our aim is to shed light to the role of sulfide in the regulation mitophagy in plants and to broaden the range of plant targets for mitophagy in Arabidopsis. Our results demonstrated that plants pretreated with H2S donors showed healthier mitochondria that plants treated only with the mitochondrial uncoupler dinitrophenol (DNP). Although further research must be performed, these results demonstrate that sulfide is a negative regulator of mitophagy in Arabidopsis.

"Chaperone-Mediated Autophagy"

Chaperone-mediated autophagy controls proteomic and transcriptomic pathways to maintain glioma stem cell activity

Ander Matheu[1], *Jaione Auzmendi-Iriarte*[1], *Maddalen Otaegi-Ugartemendia*[1], *Felix Elortza*[2], *Ana Maria Cuervo*[3],

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Chaperone-mediated autophagy (CMA) is a homeostatic process essential for the lysosomal degradation of a selected subset of the proteome. CMA activity directly depends on the levels of LAMP2A, a critical receptor for CMA substrate proteins at the lysosomal membrane. In glioblastoma (GBM), the most common and aggressive brain cancer in adulthood, high levels of LAMP2A in the tumor and tumor-associated pericytes have been linked to temozolomide resistance and tumor progression. However, the role of LAMP2A, and hence CMA, in any cancer stem cell type or in glioblastoma stem cells (GSC) remains unknown. In this work, we show that LAMP2A expression is enriched in patient-derived GSCs, and its depletion diminishes GSC-mediated tumorigenic activities. Conversely, overexpression of LAMP2A facilitates the acquisition of GSC properties. Proteomic and transcriptomic analysis of LAMP2A-depleted GSCs revealed reduced extracellular matrix (ECM) interaction effectors in both analyses. Moreover, pathways related to mitochondrial metabolism and the immune system were differentially deregulated at the proteome level. Furthermore, clinical samples of GBM tissue presented with overexpression of LAMP2, which correlated with advanced glioma grade and poor overall survival. In conclusion, these results identify a novel role of CMA in directly regulating GSCs activity via multiple pathways at the proteome and transcriptome levels.

SIGNIFICANCE

A receptor of chaperone-mediated autophagy regulates glioblastoma stem cells and may serve as a potential biomarker for advanced tumor grade and poor survival in this disease.

"Chaperone-Mediated Autophagy"

Role of pericytes´ chaperone-mediated autophagy in tissue repair after injury/inflammatory damage

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Introduction: Pericytes (PC) are perivascular cells known to present mesenchymal stem cells (MSCs) like-properties that contribute to tissue regeneration during injuries and diseases. Our team has recently demonstrated that glioblastoma (GB) induces an aberrant up-regulation of chaperone-mediated autophagy (CMA) in PC that modulates their immune function and some MSCs-like properties to support tumor growth. CMA ablation in PC make them useful as anti-tumor treatment. To better understand the PC biology dependent on CMA that let us to characterize the role of CMA in the MSCs-like function of PC, and therefore to determine if CMA in PC might also be a useful target for brain tissue remodeling therapy, we analyzed the MSCs-like properties in CMA-deficient PC compared to controls. **Methods:** Bioinformatic analyses from RNA-seq studies were related to stemness genes and validated. To analyze PC pluripotent properties, we characterized the cell lineage and cell differentiation assays were done using MSCs and adding secretome from WT PC or KO PC. To analyze if CMA in PC was modulated in response to damaged/repaired tissue, LAMP-2A expression was measured in PC of the damaged areas of a demyelinating mouse model and related to the tissue reparation after intravenous PC therapy. **Results:** we have found that CMA-deficient PC lose their pluripotent properties and their secretome does not seem to affect the stemness of MSCs. CMA in PC is modulated in response to inflammation and tissue remodeling. **Conclusion:** CMA seems to be required to maintain the MSCs like-function of PC that contributes to tissue reparation.

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"Chaperone-Mediated Autophagy"

Dissecting the regulation of CMA in a natural model of impaired glucose homeostasis, the rainbow trout

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Chaperone-Mediated Autophagy (CMA) is a major pathway of lysosomal proteolysis recognized as a key player in the maintenance of cellular homeostasis, and whose defects have been associated with several human pathologies, including neurodegenerative diseases, cancers and diabetes. Although previously thought to be restricted to mammals and birds, we have recently provided evidence for a CMA-like process in medaka fish, thus reshuffling the deck on how the full evolution of CMA should be appreciated across metazoans. Now we propose to explore CMA function in a fish species considered a natural model organism of impaired glucose homeostasis, namely the rainbow trout (RT, *Oncorhynchus mykiss*). First, we adapted and validated a fluorescent reporter (KFERQ-PA-mCherry1) previously used to track CMA in mammalian cells, in an RT hepatoma-derived cell line (RTH-149). We found that incubation of cells with glucose (Glu, 25 mM) induced the translocation of the CMA reporter to lysosomes and/or late endosomes in a KFERQ- and Lamp2a-dependent manner, as well as reduced the half-life of the reporter compared to the control (5 mM), thus demonstrating increased CMA flux. Furthermore, we observed that activation of CMA upon Glu exposure was mediated by generation of ROS at the mitochondrial level and involves, at in part, the anti-oxidative stress transcription factor NRF2, hence affording the first glimpse into the mechanisms at play in this effect. Together, our results provide unequivocal evidence for CMA activity existence in RT and offer novel insights into both the role and regulation of CMA during glucose metabolism disorders.

Thursday, November 10th

11:15-12:30h **Coffee break and posters** (even ID numbers)

Anacardium occidentale extract promotes autophagy and protects against glycative stress derived cytotoxicity (ID_42)

Ponce-Mora A.[1] *Alarcón-Gil J.*[2]*, Méril-Mamert V*[3]*, Rodríguez-Navarro J.A*[2]*, Muriel Sylvestre*[3]*, Gerardo Cebrián-Torrejón*[3] *and Bejarano E*[1*]

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Sugars or their metabolites react with biomolecules through a non-enzymatic process called glycation resulting in advanced-glycation end products (AGEs). AGEs are toxic and play a pathogenic role in the progression of cellular aging. New literature supports a major role for autophagy in fighting glycation-derived cytotoxicity by clearance of cytosolic AGEs. There is scanty information about natural products that enhance autophagy in the context of glycative stress.

Objective: *Anacardium occidentale* (AO) has been traditionally used to manage diabetes mellitus and treat its sequelae in Caribbean folk medicine. New literature suggests that phytocompounds of AO could enhance autophagy. In this study, we evaluate the impact of AO in the autophagic process and its potential protective role against glycative stress.

Method: We have exposed human lens epithelial cells (HLECs) and MEFs to glycating reagent methylglyoxal (MGO) in the presence/absence of chloroquine and to AO microwave aqueous extract. We evaluated autophagy function using immunofluorescence against endogenous LC3 and p62 and quantified cytotoxicity using Cell Titer Blue. Finally, the phytochemical profile of the AO extract was explored.

Results: AO promoted autophagy in different cell types in a short-term period and concentration- and time-dependent experiments revealed that AO extract is cytoprotective against MGO-derived cell death.

Conclusions: Our findings indicate that *in vitro* glycative stress-induced cellular damage can be mitigated by AO extract that promotes autophagic activity. Identification and use of AO phytocompounds might be a therapeutical strategy to combat the age-related decline of autophagy and diminishes the detrimental impact of AGEs on tissue fitness, especially in those with low regeneration capacity.

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Anti-tumoral compound NNC-55-0396 affects autophagy at different levels through calcium signaling (ID_38)

Visa A, Alza L, Cantí C & Herreros J

Cell Calcium Signaling Lab, IRBLleida, University of Lleida, Lleida, Spain.

Agents deregulating autophagy, a key metabolic stress response of cancer cells, are being tested for cancer therapy. We previously demonstrated that the anti-tumoral drug NNC-55-0396 (NNC) kills glioblastoma (GBM) cells by activating the ER Unfolded Protein Response (UPR; specifically, the IRE1a branch) and causing inositol triphosphate receptors (IP_3R)-dependent Ca^{2+} overload.

Aim. We observed that cytotoxic concentrations of NNC promote a massive cytoplasmic vacuolation in GBM cells and sought to understand the relationship of this vacuolation with autophagy, UPR and Ca^{2+} signaling.

Material and Methods. We used the glioblastoma cell line A172 and patient derived-GBM cultures to study autophagy, using a variety of methods (including Western-blot, qPCR, LC3 tandem assay, immunocytochemistry, electron microscopy and gene silencing).

Results and conclusions. We found that NNC-induced vacuolation is linked to autophagy downstream of UPR and Ca^{2+} signaling. Thus, silencing *IRE1α*/*JNK1* or inhibiting Ca^{2+}/IP_3R signaling prevented the vacuolation induced by NNC. We also find that p62/SQSTM-1, a cargo receptor of the autophagosome, is regulated transcriptionally by Ca^{2+} mobilizing agents, including NNC. Therefore, NNC induces autophagy emanating from ER stress and Ca^{2+} signaling, involving the regulation of autophagic proteins. Nonetheless, tandem-fluorescent tagged LC3, ubiquitin and electron microscopy analyses indicate that NNC additionally blocks late-stage autophagy, leading to enlarged defective degradative autolysosomes. Mechanistically, NNC causes defective pro-cathepsin B processing that is restored by calcium chelation, pointing to lysosomal dysfunction as the cause of autophagy blockade. Our results underscore a multiple autophagic deregulation that underlies the anti-tumoral cytotoxic activity of NNC.

Autophagy as a central process regulating carbon storage in the new extremophilic microalga Chlamydomonas urium (ID_58)

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Extremophilic microalgae have the ability to grow and adapt their metabolism to extreme conditions including the presence of high concentrations of heavy metals, acidic pH, high or very low temperature. In addition to their ecological importance as primary producers in extreme environments, these organisms also have a biotechnological potential. Autophagy is a pro-survival process that allows eukaryotic cells to maintain cellular homeostasis through the degradation of damaged cellular components. In the model microalga *Chlamydomonas reinhardtii*, autophagy is upregulated upon nutrient limitation or other stress conditions such as metal toxicity or oxidative stress. However, whether autophagy plays a role in the adaptation of extremophiles to adverse environments has not been investigated. To address this question, we recently isolated a new microalga, *Chlamydomonas urium*, from the Tinto river (Nerva, Spain), a well-characterized extremely acidic river with a high content of heavy metals. Genome sequencing and annotation showed that core *ATG* genes are conserved in *C. urium*, suggesting that autophagy must be conserved in extremophilic organisms. Indeed, our studies by western blot analysis and ultrastructural microscopy indicated that autophagy is a highly dynamic process in this microalga. In addition, metabolomic analysis revealed a higher abundance of energy and TCA cycle metabolites in *C. urium* upon autophagy inhibition. This microalga also displays enhanced photosynthesis efficiency and large levels of triacylglycerols and starch, the two main carbon storage molecules in photosynthetic cells. Thus, taken together, our results pinpoint autophagy as a key process in the control of central metabolism, including carbon storage, in the new extremophilic microalga *C. urium*.

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Autophagy protein LC3C binding to phospholipid and interaction with lipid membranes (ID_26)

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Macroautophagy is a process in which the formation of the autophagosome is an essential step. Autophagosome generation is a complex event, in which many proteins are involved. Among the latter, yeast Atg8 or its mammalian orthologues are essential in autophagosome membrane elongation, shaping and closure. A subfamily of the human Atg8 orthologues is formed by the proteins LC3A, LC3B, and LC3C. Previous studies suggest that, at variance with the other two, LC3C does not participate in cardiolipin-mediated mitophagy. The present study was devoted to exploring the binding of LC3C to lipid vesicles, bilayers and monolayers, and the ensuing protein-dependent perturbing effects, in the absence of the mitochondrial lipid cardiolipin. All Atg8 orthologues are covalently bound to a phospholipid prior to their involvement in autophagosome elongation. In our case, a mutant in the C-terminal amino acid, LC3C G126C, together with the use of a maleimide-derivatized phosphatidyl ethanolamine, ensured LC3C lipidation. Ultracentrifugation, surface pressure measurements, spectroscopic and cryo-electron microscopic techniques revealed that lipidated LC3C induced vesicle aggregation and inter-vesicular lipid mixing, including inner-monolayer lipid mixing, consistent with *in vitro* vesicle-vesicle fusion. LC3C was also able to cause the release of vesicular aqueous contents. The data support the idea that LC3C would be able to help in autophagosome elongation/fusion in autophagy phenomena.

Chaperone-Mediated Autophagy regulates muscle stem cell functions during muscle regeneration (ID_36)

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Objective

Skeletal muscle has a remarkable capacity to regenerate by virtue of its resident stem cells (satellite cells), which are normally quiescent. Upon injury, quiescent satellite cells activate and proliferate, to subsequently differentiate and form new myofibers or self-renew. We previously showed that quiescent satellite cells have basal macroautophagy while loss of this activity causes satellite cell regenerative decline with aging. However, the role of other proteostatic pathways in satellite cells functionality remains unknown. Chaperone-mediated autophagy (CMA) is a form of autophagy specialized in selective protein degradation, whereby proteins containing a pentapeptide (KFERQ-like motif) are recognized by the cytosolic chaperone HSC70 and targeted to lysosomes via binding to LAMP-2A. We aim to investigate whether CMA also regulates satellite cell functions and whether this activity changes with aging.

Methods

We assessed CMA activity using a CMA-reporter mouse and transcriptomics profiling of satellite cells during the process of myogenesis. To determine the role of CMA in satellite cell functions, we generated a mouse for the specific and inducible deletion of *Lamp2A* in satellite cells (*Lamp2A*$^{\Delta Pax7ER}$).

Results

We found that CMA is maximal in quiescent satellite cells and is progressively downregulated as cells enter the cell cycle. We have detected no significant differences in satellite cells from *Lamp2A*$^{\Delta Pax7ER}$ muscle in homeostasis. However, after muscle injury, regeneration was significantly delayed in *Lamp2A*$^{\Delta Pax7ER}$ compared to wild type mice. Moreover, we found that cell-cycle entry from quiescence and proliferative capacity were reduced in *Lamp2A*$^{\Delta Pax7ER}$ satellite cells.

Conclusions

These results highlight the importance of CMA to preserve satellite cell functions along the myogenic process and during tissue regeneration.

Consequences of the decrease in EGF binding to EGFR in Mytotonic Dystrophy type 1 (ID_60)

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by a CTG repeat expansion in the 3' untranslated region of the dystrophia myotonica protein kinase gene. AKT dephosphorylation and autophagy are associated with DM1. Although autophagy has been widely studied in DM1, the endocytic pathway has not. AKT has a critical role in endocytosis, and its phosphorylation is mediated by the activation of tyrosine kinase receptors, such as the epidermal growth factor receptor (EGFR). EGFactivated EGFR triggers the internalization and degradation of ligandreceptor complexes that serve as a PI3K/AKT signaling platform. In this study, we used primary fibroblasts from healthy subjects and DM1 patients. Cells were stimulated with a high concentration of EGF to promote EGFR internalization and degradation. Thereafter, the morphology of organelles and the levels of protein were determined by immunofluorescence, electron microscopy and western blot. DM1-derived fibroblasts showed enlarged endosomes and lysosomes. Interestingly, the EGF binding to EGFR was reduced in DM1 cells and EGFR internalization was also slowed during the early steps of endocytosis. However, EGF-activated EGFR enhanced AKT and ERK1/2 phosphorylation levels in the DM1derived fibroblasts. Therefore, there was a delay in EGFstimulated EGFR endocytosis in DM1 cells. This alteration might be due to the decrease in the binding of EGF to EGFR, and not to a decrease in AKT phosphorylation.

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Increased autophagy markers and mitochondrial impairment in type 1 diabetes (ID_4)

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Objective: Type 1 Diabetes (T1D) is related to cardiovascular diseases (CVD). Hyperglycaemia is a risk factors for developing CVD in diabetic patients since it promotes oxidative stress, inflammation, and cellular death. These cellular stress conditions can activate different pathways, including autophagy. The objective of this study was to evaluate the rates of oxidative stress, mitochondrial function and autophagic flux in leukocytes from T1D patients and inflammatory parameters in their serum.

Material and methods: Sixty-seven healthy volunteers and fifty-six T1D patients were recruited. Anthropometric measurements were recorded, and blood samples were used for biochemical determination and molecular analysis. We measured TNF-α, IL-6 and myeloperoxidase (MPO) levels in patients' serum. In leukocytes, we assessed mitochondrial function and several oxidative stress parameters with fluorescent probes: DCFH-DA for total ROS production, MitoSOX for mitochondrial ROS production, and TMRM for mitochondrial membrane potential. The relative expression of the autophagic markers SQSTM1/p62, Beclin 1 and LC3 were evaluated by Western Blot.

Results: T1D patients presented higher glucose levels and HbA1c-DCCT than controls (both $p<0.001$). Serum levels of TNF-α ($p<0.01$) and MPO ($p<0.05$) were higher in T1D patients, but no difference in IL-6. In addition, leukocytes from T1D presented oxidative stress and mitochondrial alterations as increased levels of total and mitochondrial ROS production (both $p<0.05$) and enhanced mitochondrial membrane potential ($p<0.05$) were present. Finally, leukocytes from T1D subjects exhibited enhanced autophagic flux, since reduced protein levels of SQSTM1/p62, and increased levels of Beclin 1 and LC3-II/I ratio (all $p<0.05$) were detected.

Conclusions: Oxidative stress and mitochondrial alterations may induce inflammation and activate autophagy in the leukocytes of T1D patients.

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Induction of autophagy/mitophagy through bioactive compounds from acorn cupule. Neuroprotective mechanisms (ID_64)

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Autophagy is a highly conserved catabolic process that maintain cellular homeostasis by facilitating the renewal of cytoplasmic structures. Impaired autophagy is directly involved in multiple age-related diseases, including neurodegenerative disorders such as Parkinson's disease (PD), the second most common neurodegenerative disease worldwide. In PD, the cell degradation process is deregulated, inducing aggregates of several proteins, affecting the formation of autophagosomes or lysosomal biogenesis. The acorn, a staple food for thousands of years, is an important source of a natural compound known as ellagitannins, whose metabolism originates substances known as urolithins. Urolithins have significant beneficial antiproliferative, anticancer, and antiaging effects.

In this study we analyzed the possible beneficial effects that different bioactive compounds of acorn cupule may have in several models of PD, through the induction of autophagy and/or mitophagy.

The characterization and standarization of green (GAE) and ripened (RAE) acorn cupule extracts was determined by the ABTS method. Western blot and inmunofluorescence studies have been used for detection of specific proteins. Cell viability assays by flow cytometry was determined using propidium iodide (IP). The ultrastructural level analysis was performed by electron microscopy.

We observed an induction of autophagy and mitophagy with the acorn cupule extracts at different degrees of maturation in several cells lines. This induction was also observed in human fibroblasts from Parkinson's patients with the G2019S and R1441G LRRK2 mutations. GAE and RAE protect from sensitivity of these pathogenic mutations as well as the exposure to stress caused by paraquat (PQ).

Our results reveal that modulation of autophagy/mitophagy by bioactive compounds of acorn cupule, would help to treat or slow neuronal death occurs in neurodegenerative process.

This research was partially supported by the "Fondo Europeo de Desarrollo Regional" (FEDER) from the European Union. S.C-C and E.U-C were supported by a FPU fellowship [FPU19/04435 and FPU16/00684, respectively] from the Ministerio de Ciencia, Innovación y Universidades, Spain. G.D-G is supported by Program [RYC-2016–20883]. E, A-C is supported by a pre-doctoral fellowship of Valhondo Calaff Foundation. M. P-B and A.G-B received fellowships from the "Plan Propio de Iniciación a la Investigación, Desarrollo Tecnológico e Innovación (Universidad de Extremadura). M.N-S was supported by the "Ramon y Cajal" Program [RYC-2016–20883], and P. G-S, was funded by "Juan de la Cierva Incorporación" Program [IJC2019-039229-I], Spain. S.M.S.Y-D was supported by CIBERNED [CB06/05/0041]. RAGP received research support from "Junta de Extremadura", financed by the European Union [IB20031]. JMF received research support from "Instituto de Salud Carlos" III

Mitophagy boosting protects cells against MNU toxicity (ID_30)

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Objective: A pharmacological model to study the human disease *Retinitis Pigmentosa* is N-methyl-N-nitrosourea (MNU) injection which results in retinal degeneration. Our objective is to study the autophagy and mitophagy status of the cells in this disease model.

Materials and methods: The mitophagy reporter (MitoQC) and autophagy reporter mice (mCherry-GFP-LC3) have been used to determine the mitophagic and autophagic flux in the retina after MNU injection. Retinal pigment epithelium cell line (ARPE-19) MitoQC has been used to elucidate the cellular and molecular mechanisms after MNU treatment.

Results: Neurodegeneration events appear one day after intraperitoneal MNU injection: reduction of photoreceptors thickness layer and increase of TUNEL and GFAP staining. Mitophagosomes and autophagosomes are less frequent in the retina of mice treated with MNU, but they are bigger and they tend to accumulate in the external limiting membrane. ARPE-19 cells are also vulnerable to MNU in a dose-dependent manner producing DNA damage, cytoplasm vacuolization, organelle alterations and cell death. Mitophagy levels depend on the MNU dose: an increase of the mitophagic flux is observed at low doses, whereas it seems to be blocked at high doses. We demonstrate this mitophagy is PINK-Parkin dependent and boosting this pathway makes cells more resistant to MNU. Finally, we achieve to protect MNU-treated retinae explants with the mitophagy inductor, DFP.

Conclusion: MNU treatment induces activation of PINK-Parkin dependent mitophagy. We propose that mitophagy could act as a defense mechanism in this disease model.

Novel small molecule antioxidants improve stress-induced cell reprogramming while decrease autophagy and cell death in rapeseed: a physiology and transcriptomics study (ID_62)

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Plant *in vitro* regeneration systems are essential in modern crop breeding. Stress-induced microspore embryogenesis is an *in vitro* biotechnological process to rapidly obtain doubled-haploid plants to accelerate breeding. By application of stress conditions the microspore, precursor cell of the pollen grain, is reprogrammed to produce an embryo that regenerate a plant. In many crop species the process is highly inefficient, being increased cell death a major event that impairs the reprogramming process leading to decreased embryo yield.

In this work, we have analyzed autophagy and oxidative stress, and their involvement in cell death during microspore embryogenesis induction in the model crop species *Brassica napus*, where cell reprogramming is induced by a treatment of 32°C. After stress, *BnATG5* was upregulated, NBR1 degraded and accumulated upon E-64 inhibition, indicating autophagy activation. RNAseq-transcriptomics analyses showed sets of autophagy and oxidative stress-related genes differentially expressed, after embryogenesis induction. Autophagy inhibition by E-64 reduced cell death, while the autophagy inducer AZD8055, increased death, suggesting an autophagy-dependent cell death. Treatment with novel small molecule antioxidants reduced cell death and promoted embryogenesis initiation, while they lead to *BnATG5* and *BnATG8a* downregulation. Results indicate that oxidative stress and autophagy contribute to cell death during stress-induced microspore reprogramming, and that novel small molecule antioxidants can be a new tool to suppress autophagy-dependent cell death, improving *in vitro* embryogenesis efficiency for crop breeding.

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Reduced systemic autophagy by simultaneous loss of ATG4B, ATG4C and ATG4D leads to premature death in mice (ID_52)

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Objectives: In the last years, autophagy has emerged as an essential pathway for most of cellular functions, either as a cellular housekeeping or as quality control mechanism. In this way, the specific functions of the four mammalian orthologues of yeast Atg4 protease (ATG4A-D) remain largely unknown. Previously in our laboratory we characterized the ATG4B and ATG4C knockout mice and recently we described the roles of ATG4D in the context of autophagy and neuroprotection through the analysis of ATG4D-deficient mice. Here, through the generation and analysis of ATG4B-C-D triple deficient cells and mice (ATG4A-only mice), we are able to describe the functions of ATG4A when no other member of the ATG4 family is expressed.

Material and methods: This study was carried out using cell biology, electron microscope analyses and molecular biology techniques. Moreover, additional knockout lines were generated through the use of *CRISPR/Cas9* technology.

Results and conclusions: By studying ATG4A-only mice, we show that the presence of ATG4A alone is sufficient to overcome perinatal death, characteristic of murine models of total autophagy deficiency. However, ATG4A-only mice show premature death and multimorbidity characterized by features often observed in both aged individuals and in murine models of accelerated aging. Moreover, through the analysis of ATG4A-only cells, we show that ATG4A activity alone is not enough to maintain a correct dynamic between mATG8 priming and delipidation, which results in a sound reduction of autophagic flux due to an impairment in the formation of autophagosomes, which ultimately underlies the reduced lifespan and healthspan of ATG4A-only mice.

Regulation of autophagy by the CPT1C-SAC1 complex in neurons (ID_48)

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Carnitine palmitoyl transferase 1C (CPT1C) belongs to the family of CPT1 enzymes but it lacks catalytic activity. It is mainly expressed in neurons and localizes to endoplasmic reticulum, where it acts as a sensor for malonyl-CoA. This metabolite is a lipid precursor whose levels decrease under glucose depletion or fat surplus. Our group has unveiled that CPT1C is able to bind SAC1 protein, a lipid phosphatase that dephosphorylates phosphatidylinositol-4-phostate (PI4P), and downregulates its activity in normal conditions but not when malonyl-CoA is depleted. Since PI4P has been involved in autophagy initiation and lysosome (Lys)-autophagosome (APG) fusion, we are interested in studying the possible role of CPT1C-SAC1 complex in autophagy. So, we observed that CPT1C expression in non-neuronal cells (RPE-1) raised PI4P levels and increased the number of autophagosomes. Moreover, CPT1C expression increased the number of FIP200 spots, a protein involved in autophagosome formation, and enhanced the ATG9A output from the cis-Golgi, suggesting that more PI4KIIIβ-loaded ATG9A vesicles could reach autophagy initiation sites. Accordingly, CPT1C deletion or SAC1 overexpression in primary cortical neurons impaired basal autophagy initiation, as malonyl-CoA depletion did in wild type ones. Our results suggest that the complex CPT1C-SAC1 regulates basal autophagy initiation in neurons depending on malonyl-CoA. Further experiments will unveil whether impaired autophagy contributes to the learning and motor deficits that are observed in CPT1C KO mice and in humans carrying CPT1C mutations.

SIRT1-dependent autophagy as a novel therapy for age-related memory decline (ID_44)

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Sirtuins (SIRT) are directly involved in the maintenance of neuronal homeostasis and functions throughout life. Among the SIRT1-7 members, the SIRT1 has been described as pivotal for the well-aging at the whole organism level. Importantly, its anti-aging effects require the cellular degradative process referred to as autophagy. Moreover, it is known that blood contains youthful factors that can restore age-related memory impairment via the activation of the autophagy machinery. Nonetheless, the role of SIRT1-dependent autophagy and the upstream systemic factors in memory fitness during aging is unknown. We have found that SIRT1 activity is reduced during aging and essential for novel memory integration, and restoring its activity ameliorates age-related cognitive decline. This phenotype is accompanied by restoration of the autophagy machinery in the aged HpC. Overall, this data paves the way for novel therapeutic avenues aimed at restoring memory fitness by modulating SIRT1-dependent autophagy.

Thursday, November 10th

17:00-18:15h **Coffee Break and posters** (odd ID numbers)

Ambra1 haploinsufficiency results in metabolic alterations and exacerbates age-associated retinal degeneration (ID_39)

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Objectives. The retina is a highly complex and metabolically active tissue in our eyes that senses the light. Persistent light-induced stimuli lead to constant turnover of the damaged cellular structures by proteostatic mechanisms such as autophagy. Our previous data demonstrates that during aging there is a gradual decrease of autophagy which compromises retinal homeostasis and visual function. AMBRA1 (autophagy and beclin-1 regulator 1) is a key protein involved in the initiation phase of autophagy pathway. To further determine the importance of autophagy along aging, we will use Ambra$^{1+/gt}$ heterozygous mice that display reduced autophagic response, mimicking the decrease of autophagy during the aging process.

Materials and methods. We have used young (3 months), middle-aged (12 months) and old (25 months) Ambra1$^{+/+}$ and Ambra$^{1+/gt}$ mice for this study. Visual function was determined by electroretinographic recordings (ERGs) along aging. Mice were euthanized and eyes were enucleated for histological analysis or for biochemical procedures. Retinal morphology and cellular components were assessed by immunofluorescence techniques on retinal cryosections or flatmounts. mRNA and protein lysates were obtained from isolated retinas. Metabolomic analysis were conducted using mass spectrometry and bioinformatic analysis was performed,

Results. Ambra$^{1+/gt}$ retinas display a reduced autophagic flux at young ages without changes in the retinal integrity and function. However, aged Ambra1+/gt animals show an exacerbated loss of visual function also evidenced by morphological changes as increased gliosis, reduced nuclear density and cone number. Interestingly, we observed increased bipolar cell protrusions that might indicate the loss of synapsis between retinal layers. Partial loss of autophagy results in increased damaged mitochondria evidenced by a reduction in the mitochondrial membrane potential (MMP) in young age, higher oxidative stress in middle-aged and accumulation of mitochondrial mass in old Ambra$^{1+/gt}$ retinas. Finally, by metabolomics, we demonstrate marked metabolic alterations suggestive of defective oxidative metabolism in Ambra$^{1+/gt}$ animals at 1 year of age.

Conclusions. Reduced autophagic response in Ambra1 haploinsufficient retinas leads to an exacerbated age-associated declines in retinal function, metabolic alterations and accumulation of damaged mitochondria. Thus, autophagy is a key process to maintain retinal homeostasis and its downregulation exacerbates the age-dependent decline in retinal morphology and function.

AMPK activation is not enough to boost autophagy in primary neural cells (ID_3)

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Autophagy is altered in many neurodegenerative proteinopathies, such as Alzheimer's disease (AD), characterized by the deposition of β-amyloid and hyperphosphorylated MAPT. During AD progression, autophagic vesicles accumulate within dystrophic neurites.

AMPK and AKT-MTORC1 signaling pathways are central nodes in the balance between anabolism and catabolism. It is generally accepted that MTORC1 activation leads to the inhibition of macroautophagy (hereafter called autophagy), whereas AMPK activation is supposed to enhance this process. In addition, some regulatory cross-talk between both pathways has been reported. Accordingly, autophagy inducers such as MTORC1 inhibitors may have beneficial effects in the clearance/prevention of protein aggregates in the brain, as we reported in the APP/PS1 AD mouse model.

The aim of this work is to determine the cellular contribution (astrocytes versus neurons) of this in vivo effect. At present we are analyzing astrocyte contribution, as we did with neurons.

Based on the increase of autophagy markers in neurons and astrocytes from APP/PS1 mice, we analyzed the effect of autophagic flux modulation. We found a slight increase of autophagy with rapamycin, a well-known MTORC1 inhibitor, in both cell types. Surprisingly, AMPK activation with metformin did not enhance autophagy, which could be due, at least in part, to an insufficient inhibition of MTORC1. Finally, we studied the direct effect of this modulation on autophagy-dependent amyloidosis.

These results suggest that AMPK activation, in contrast to mTORC1 inhibition, is not sufficient to enhance autophagy in primary neurons and astrocytes. Thus, the protective mechanisms of autophagy against neurodegeneration must be further examined.

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ATG4B mediates beta-catenin expression and 3D spheroid formation in pancreatic ductal adenocarcinoma (ID_65)

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid malignancies, with a high mortality rate of approximately 7% of diagnosed patients living more than 5 years (Siegel RL, 2019). Current treatment regimens have minimal impact on patient survival, highlighting a vital requirement for novel therapeutics. A growing body of evidence demonstrates that functional autophagy is required for growth and stress resilience of KRas-driven PDAC. In these conditions, genetic inhibition of autophagy protease, ATG4B, results in tumour regression in mouse cancer models (Yang, Cancer Discovery 2019) and can increase immune invasion (Yamamoto, Nature 2020).

Here we have studied the role of ATG4B in human PANC-1 cells using ATG4B knockout cell lines generated by CRISPR/Cas9 genome editing. We present that knockout of ATG4B triggers a reduction in beta-catenin expression in PANC-1 cells. This significantly reduced expression of beta-catenin can be rescued with the addition of proteosome inhibitor MG-132 or recombinant WNT3a. RNA sequencing indicates a highly significant reduction in several genes involved in WNT/beta-catenin signalling. Furthermore, ATG4B knockout in PANC-1 can prevent the ability for these cells to form 3D spheroids when cultured in ultra-low attachment u-bottom plates. Interestingly, this spheroid disintegration in PANC-1 ATG4B-/- can be rescued with the addition of extracellular matrix. To conclude, our data suggests that ATG4B is involved in WNT/beta-catenin signalling in PDAC, and we propose ATG4B modulation as a therapeutic strategy in PDAC.

Autophagy deregulation differs between muscle and motoneurons in Spinal Muscular Atrophy (ID_47)

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Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by reduced survival motor neuron (SMN) protein. In spinal cord motoneurons (MNs) low levels of SMN produce degeneration and progressive muscle weakness and atrophy. Molecular mechanisms that contribute to SMA neurodegeneration are not fully understood. However, autophagy deregulation and autophagosome accumulation observed in SMA MNs are associated with the neuropathological hallmarks observed in SMA.

Objective: Evaluate autophagy-related markers in SMA models comparing non-neuronal and neuronal tissues or cells.

Materials and methods: Spinal cord and muscle tissues were obtained from SMA severe mouse model. Human MNs were obtained by differentiation of iPSCs (SMA patient and unaffected control). SMA and control human fibroblasts were included in the study. Samples were analyzed by western blot or immunofluorescence to examine autophagy markers: LC3-II, Beclin 1, p62/SQSTM1 and mTOR.

Results: SMA mouse gastrocnemius presented decreased protein levels of LC3-II, Beclin 1, p62/SQSTM1, and reduced mTOR phosphorylation at the pre-symptomatic stage of the disease. Accordingly, LC3-II was reduced in SMA fibroblasts. However, in mouse and human cultured SMA MNs, mTOR phosphorylation and LC3-II levels were increased.

Conclusions: These results suggest differential regulation of the autophagy process in SMA muscle cells and MNs. These differences may reflect a specific response to SMN reduction, which could imply divergent tissue-dependent responses to current SMA therapies.

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Autophagy modulation in glial cells infected with herpes simplex virus type 1 (ID_53)

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Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that, occasionally, may spread to the central nervous system (CNS). A well-balanced autophagy is essential for the correct functionality of the CNS, but HSV-1 infection could alter this balance, which may lead to neurodegeneration. While the relationship between HSV-1 and autophagy has been extensively analyzed in neurons, studies in glial cells are scarce. We observed that autophagy is not stimulated in response to infection in human oligodendroglioma and astrocytoma cell lines, suggesting that autophagy may not play an important antiviral role in glial cells. Later in infection, autophagy markers were downregulated in oligodendroglioma cells, but not in astrocytoma cell line. These results manifest the strong cell-type dependence of the activity of HSV-1 anti-autophagic proteins. Finally, to analyse how autophagy influences HSV-1 infection, we suppressed the pathway with pharmacological modulators. Indeed, we generated autophagy-deficient glial cells with knockout (KO) of *ATG5* gene. Interestingly, even though autophagy is considered an antiviral mechanism against HSV-1, infection was severely impaired in *ATG5* KO cells. Thus, autophagy could play a proviral role in glial cells, contrary to what happens in neurons. On the other hand, *ATG5* KO cells treated with pharmacological modulators showed a decrease in infection compared to untreated cells. These results indicate that the reduction of infection by modulators is mostly due to their nonspecific effects. We argue that macroautophagy might not be a main antiviral mechanism in glial cells, whereas other types of autophagy might have a proviral function during HSV-1 infection.

Ceramide enhances the binding of LC3/GABARAP autophagy proteins to lipid membranes (ID_25)

Varela Y. R. [1], *Iriondo M. N.* [1], *Gonzalez-Ramirez E. J.* [1], *Etxaniz A.* [1], *Ballesteros U.* [1], *Goñi F. M.* [1], *Alonso A.*[1]

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Sphingolipids have been related to autophagy, however the mechanism(s) that they follow to regulate the process are still unknown. Among sphingolipids, ceramides have been described to play an important role both in the autophagy that promotes survival and in the one that ends in cellular death. For this reason, we decided to study how the membrane properties induced by this lipid could modulate the autophagy response. Among all the proteins involved in autophagy, we chose to study the LC3/GABARAP family. These proteins have been found to take part in different steps of macroautophagy, such as in the autophagosome formation process or in cargo recognition. We combined Langmuir balance and liposome flotation assays to quantify the *in vitro* binding of those proteins to lipid mixtures, in the presence or absence of ceramide. Our results suggest that the soluble form of the proteins does not strongly bind ceramide. However, the presence of 10% ceramide in membranes that include cardiolipin (CL) is enough to increase the basal binding of our proteins towards CL-containing membranes. Further studies using giant unilamellar vesicles (GUV), calorimetry and atomic force microscopy (AFM) allowed us to compare the membrane properties of the bilayers and we were able to see that 10% ceramide induced lateral segregation in CL-containing membranes. These results could indicate that the remodeling of the bilayer caused by ceramide is the reason behind the higher binding of LC3/GABARAP.

Chaperone mediated autophagy as therapeutic target in retina in Parkinson's disease (ID_33)

Raquel Gómez-Sintes[1,2], Adrián Martín-Segura[2], Inmaculada Tasset-Cuevas[2], María Martín-Bartolomé[1], Sandra Alonso-Gil[1], Ana María Cuervo[2,3], Patricia Boya[1].

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Objective:

Parkinson's disease (PD) is characterized by α-synuclein deposits in neurons, leading to dopaminergic neuron loss in central nervous system (CNS) and we are particularly interested in the retina.

Disturbances in the protein quality control systems contribute to neurodegeneration by interfering with the removal of PD-related pathogenic proteins. We are focused on a selective degradation pathway known as chaperone-mediated autophagy (CMA).

We propose that restoring normal CMA activity in the retina of a mouse PD model will improve α -synuclein degradation, neuronal homeostasis and slow neuronal loss related to PD.

Material and methods:

The PD model used in this study is a mouse model of autosomal dominant PD conditionally expressing human A53T mutant α-synuclein in dopaminergic neurons.

Results:

Our first experiments suggested signs of degeneration as thinning of the inner nuclear layer, decreased TH staining, accumulation of α–synuclein and deficits in visual behavioural tests in the PD model.

Conclusion:

We have found several indicators of degeneration in PD model retinas and we believe that our suggested CMA activation approach would be able to reverse this phenotype, thus delaying the disease progression in the retina.

Characterization of proteins functionally related to Wdr45l in Dictyostelium (ID_49)

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WIPIs are a conserved family of proteins with a characteristic 7-bladed b-helix structure. Mutations in human WIPI4 cause BPAN, a rare disease characterized by developmental delay, motor disorders and seizures. The pathological mechanisms leading to BPAN are still unclear and it has been proposed that, in addition to autophagy, other pathways such as iron accumulation, mitochondria homeostasis and endoplasmic reticulum (ER) stress may also have a role. The social amoeba *Dictyostelium discoideum* is an excellent model to study the molecular mechanisms of autophagy and thus autophagy-related diseases. Its developmental cycle takes place in the absence of nutrients, making it dependent of autophagy. We have previously generated a *Dictyostelium* model of BPAN by disrupting Wdr45l, the WIPI4 homologue, in this model (1). We found that Wdr45l, the lipid transfer proteins Atg2A,B and the scramblase Vmp1 are essential for autophagy in *Dictyostelium*. Our results suggest that the three proteins function in the same stage of autophagosome formation. The possible role in autophagy of other Vmp1-related proteins such as Tmem41 and Tmem64 proteins has also been studied in this model.

1. Tornero-Écija, A., Tábara, L., Bueno-Arribas, M., Antón-Esteban, L., Navarro-Gómez, C., Sánchez, I., Vincent, O., and Escalante, R. 2021. A *Dictyostelium* model for BPAN disease reveals a functional relationship between the WDR45/WIPI4 homolog Wdr45l and Vmp1 in the regulation of autophagy-associated PtdIns3P and ER stress. *Autophagy* Jul 27: 1-17.

Impairment of autophagy in plasmanylethanolamine desaturase-deficient cells (ID_45)

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Plasmalogens are an abundant class of glycerophospholipids in human and animal bodies characterized by a vinyl-ether bond. Their biosynthesis begins in the peroxisome by the creation of the ether bond, which is reduced to a vinyl-ether within the endoplasmic reticulum by an enzyme plasmanylethanolamine desaturase (PEDS) recently identified. Impaired biosynthesis and regulation of plasmalogens may lead to certain neurological and metabolic diseases, and our previous data indicate their importance in the autophagosomal membrane. Therefore, we have studied the effect of PEDS depletion on autophagy.

For this purpose, Wild-type (HAP1) and Knock-Out cells for PEDS (PEDS KO) were incubated in media with normal or lipid-depleted serum (to avoid the presence of external plasmalogens). We studied the autophagic flux using western blot and immunocytochemistry techniques.

Our results show that, under normal conditions, both (WT and PEDS KO) cell types are able to respond adequately to serum deprivation and show normal autophagy. However, in the absence of exogenous lipids, after culture in media with lipid-free serum, the LC3 and p62 flux is impaired.

Plasmanylethanolamine desaturase is therefore essential for the proper functioning of autophagy. Still, it is necessary to further investigate the molecular mechanisms in order to understand more deeply the importance of plasmalogens in autophagy in health and disease.

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Implication of SIRT1 axis in Queen Bee Acid-induced autophagy (ID_61)

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Autophagy is a highly conserved intracellular catabolic pathway that removes toxic protein aggregates and cellular organelles to maintain neuronal homeostasis. The impaired regulation of autophagy leads to the accumulation of protein aggregates and, consequently, to neurodegeneration. Since then, the activation of autophagy has been considered as a therapeutic strategy against neurodegeneration by preventing or eliminating protein aggregates. Queen bee acid (QBA, 10-hydroxy-2-decenoic acid) is exclusively found in royal jelly and it constitutes its major fatty acid component. Additionally, QBA has anti-tumor, anti-inflammatory and antibacterial activities and promotes neurogenesis and neuronal health. In the present study, we investigated the mechanism by which QBA induces autophagy in both neuroblastoma cell line and ICR mice. In mice, the treatment was administered in drinking water or by intraperitoneal injection. Proteins levels were assessed by immunoblotting or immunoprecipitation and gene expression was analyzed by RT-qPCR. Our results showed that QBA upregulated the expression of several autophagy proteins through the activation of Sirtuin 1(SIRT1) and forkhead box transcription factor (FOXO). Furthermore, the inhibition of SIRT1 by EX527 decreased the effect of QBA in vitro. Indeed, QBA increased the expression of Sirtuin1 and its deacetylase activity. Therefore, SIRT1 might play a key role in QBAinduced autophagy.

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THE PARKINSONIAN LRRK2 R1441G MUTATION SHOWS MACROAUTOPHAGY-MITOPHAGY DYSREGULATION CONCOMITANT WITH ENDOPLASMIC RETICULUM STRESS

EFFECT OF LRRK2 R1441G MUTATION ON THE MECHANISM OF AUTOPHAGY IN HUMAN CELLS FROM PARKINSON'S PATIENTS (ID_31)

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Autophagy is a mechanism responsible for the degradation of cellular components to maintain their homeostasis. However, autophagy is commonly altered and compromised in several diseases, including neurodegenerative disorders. Parkinson´s disease (PD) can be considered a multifactorial disease because environmental factors, genetic factors and aging are involved. Several genes are involved in PD pathology, among which the LRRK2 gene and its mutations, inherited in an autosomal dominant manner, are responsible for most genetic PD cases. The R1441G LRRK2 mutation is, after G2019S, the most important in PD pathogenesis. Our results demonstrate a relationship between the R1441G LRRK2 mutation and a mechanistic dysregulation of autophagy that compromises cell viability. This altered autophagy mechanism is associated with organellar stress including mitochondrial (which induces mitophagy) and endoplasmic reticulum (ER) stress, consistent with the fact that patients with this mutation are more vulnerable to toxins related to PD, such as MPP^+.

Unveiling the role of autophagy in the chloroplast stress response in the model microalga Chlamydomonas reinhardtii (ID_57)

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Autophagy is a major catabolic process by which eukaryotic cells degrade and recycle intracellular material including protein aggregates or dysfunctional organelles to maintain cellular homeostasis and to cope with stress. A hallmark of autophagy is the formation of autophagosomes, double-membrane vesicles in which the cargo that will be ultimately degraded in the vacuole is engulfed. Our previous studies in the model microalga *Chlamydomonas reinhardtii* have demonstrated that autophagy is upregulated in response to a wide range of stress conditions including nutrient limitation, oxidative stress or chloroplast damage. To further analyze the role of autophagy in maintaining chloroplast homeostasis, we have generated in *C. reinhardtii* an ATG8-deficient mutant using CRISPR-Cas9 technology. On the one hand, a comparative metabolomic analysis of WT and *atg8* cells has revealed that the autophagy mutant displays a metabolic reprogramming according to carbon source. On the other hand, the absence of ATG8 causes hypersensitivity to cerulenin and norflurazon, two drugs that lead to ROS-linked chloroplast stress that trigger autophagy in Chlamydomonas. In addition, RNA-Seq and quantitative proteomic analyses suggest that the global adaptive response to chloroplast damage is severely affected in the *atg8* mutant. To investigate whether the decreased chloroplast function of the *atg8* mutant is linked to a redox imbalance, we have generated WT and *atg8* cells expressing ROS probes that allow us to monitor H_2O_2 production in all major cellular compartments of Chlamydomonas cells.

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