Autophagy discussion in Lyon
CFATG11, November 8th- November 10th 2023

INTRODUCTION

Two elected members of the board of the Club Francophone de l'AuTophaGie (CFATG), Prof. Carole Kretz-Remy (Université Claude Bernard Lyon 1) and Dr Flavie Strappazzon (CNRS researcher, Lyon) from the Pathophysiology and Genetics of Neuron and Muscle laboratory (PGNM-INMG) were in charge of organizing the "11th Scientific Days on Autophagy, CFATG11", that were held in Lyon from the 8th to the 10th of November 2023. The conference was organized in conjunction with a local committee of researchers and lecturers from various research institutes of Lyon.

The CFATG (www.cfatg.org) was set up in 2011 to promote scientific research in autophagy, to stimulate exchanges between researchers and to ensure the dissemination of knowledge in this innovative and highly multifaceted field. To this end, each year a meeting is organized in France, for national and international researchers interested in the autophagy process.

Autophagy is a vital mechanism for the recycling of cellular components. Its importance was highlighted in 2016 when Prof. Yoshinori Ohsumi (University of Tokyo) was awarded the Nobel Prize in Physiology and Medicine for his discoveries on how autophagy works. This field of research is currently expanding rapidly, as shown by the 20,000 or so publications over the last 2 years, and covers a wide range of topics: Cell Biology, Mechanistic and Signaling, Immunity and Infection, Development, Metabolic Disorders, Cancer, Neuroscience, Ageing, etc.

The 11th CFATG congress gathered five internationally renowned speakers: Prof. Claudine Kraft, Freiburg, Germany; Dr Lisa Frankel, Copenhagen, Denmark; Dr Carmine Settembre, Naples, Italy; Dr Harald Wodrich, Bordeaux, France; Prof. Masaaki Komatsu, Tokyo, Japan. Prof. Komatsu was one of the first researchers to study the physiological function of an autophagic gene *in vivo*. In particular, his group constructed conditional knockout mice for the Atg7 gene. In subsequent studies, Prof Komatsu's group discovered that the Nrf2-Keap1 pathway plays an essential role in the process of liver damage caused by autophagic defects.

Around 130 participants were present at the meeting; many of them were young researchers, as the meeting was open to Master 2 students in biology from the University of Lyon as well as doctoral and post-doctoral students. This gave them the opportunity to attend an international-level meeting and, for some of them, to be selected for an oral or poster presentation in front of established researchers. This desire to give young researchers the opportunity to present their work is reflected in the fact that the conference was free of charge for Master2 students from Lyon and that many fellowships were awarded to cover the registration fees of doctoral and post-doctoral students.

The meeting was also the opportunity to promote the Auvergne-Rhône-Alpes region, the 2nd most dynamic region in France in terms of scientific research, to participants (industrialists, researchers, students) from all over France as well as from several European or even outside Europe countries. The decision to hold the conference at the Palais Hirsch, the historic and

prestigious birthplace of the University of Lyon (inaugurated on the 5th of December 1896) also underlined Lyon's position as a student city.

The CFATG 11 received a great deal of financial supports coming from the University of Lyon, the CNRS, various research institutes and institutions of Lyon as well as from a number of charitable organizations, learned societies and industrial sponsors.

Plenary lecture & Session 1

A key topic that has emerged during the keynote lecture and the molecular mechanisms & signaling session was the role of phase separation in proteostasis and more specifically in autophagy.

A lot of cellular membrane-less structures containing highly concentrated and weakly-interacting proteins and/or RNAs (P-bodies, stress granules …) are formed by liquid-liquid phase separation (LLPS). The condensates formed allow compartmentalization in the cytosol but also temporal control. LLPS is modulated by diverse factors such as ionic strength, pH, temperature, pathogenic conditions, post-translational modifications of their constituents etc. … and eventually can evolve towards gel-like or solid states.

P62/SQSTM1 is known to play a key role in the autophagic process notably as a poly-ubiquitinated protein receptor. Of interest, p62 bodies are formed by LLPS but little is known about their content. During the plenary lecture, Pro. Komatsu informed us that his lab now masters the purification of p62 bodies, which can contribute to the elucidation of disease/stress response mechanisms. For instance, the analysis of p62 droplets content by mass spectrometry allowed the identification of ULK1 and ULK2 kinases (Unc-51 like kinases) and the discovery of their involvement in the direct phosphorylation of p62, which activates NRF2 (Nuclear factor erythroid-2-related factor 2) in a redox-independent manner, a process important for nutritional intake (Ikeda et al., 2023). The analysis of p62 bodies composition also allowed the identification of the major constituent of Vault supramolecular complex (MVP). MVP recruits Vault to p62 bodies, which allows Vault degradation by selective autophagy, a defective “vault-phagy” being related to nonalcoholic steatohepatitis (NASH)-derived hepatocellular carcinoma (Kurusu et al., 2023).

The clustering and compartmentalization processes are also important, in yeast, for the formation of the phagophore assembly site (PAS). Indeed, Prof. Claudine Kraft, full professor at the University of Freiburg (Germany), who opened the molecular mechanisms and signaling session as a guest speaker, showed us that the vacuole anchored protein Vac8 acts as an assembly hub at the vacuole, recruiting ATG1, ATG11-bound cargo-receptor complexes but also phosphatidylinositol 3-kinase, which promotes PAS assembly (Hollenstein et al., 2021). This pathway is driven by multiple avidity interactions in which local concentration or confinement of otherwise low-binding interactions partners is translated into higher functional affinities. The study of the omegasome biogenesis in mammals is also a current mater of study debated during the session by Dr Daniele Campisi (Paris, France).

Then, Dr Anaïs Franco-Romero (Padova, Italy) informed us about the characterization of a new regulator of muscle mass integrity and aging, called MYTHO (Macroautophagy regulator and

YouTH Optimizer) (Leduc-Gaudet et al., 2023). The gene encoding this protein is transcriptionally upregulated by FoxO (member of the forkhead family of transcription factors) in catabolic conditions such as skeletal muscle starvation. MYTHO is required for skeletal muscle autophagy and is down regulated in myotonic dystrophy type I. How MYTHO regulates autophagy in muscles is under current investigation. The analysis of the involvement and regulation of ER-phagy in the maintenance of muscle integrity was also presented by Marine Daura, a doctoral student from Lyon (France), as was the function of the LC3/GABARAP protein family in autophagy (Leboutet et al., 2023a; Leboutet et al., 2023b). Indeed, Dr Renaud Legouis (Gif sur Yvette, France) and his lab, using site-directed mutagenesis in *C. elegans*, demonstrated that, despite the widespread belief that the main function of this protein family depends on its lipidation with the phagophore membrane lipid phosphatidylethanolamine, the lipidation of LGG1 protein (homolog of the mammalian GABARAP family) is not required for autophagy. This discovery should prompt us to revisit our autophagosomes detection methods.

Session 2

Professor Carmine Settembre, Full Professor of Histology in the Department of Clinical Medicine and Surgery at the University of Naples "Federico II" (Italy), opened the Physiopathology section as guest speaker. In his presentation, he highlighted recent discoveries on lysosome-dependent signaling pathways and emphasized that a deeper understanding of lysosome composition and function could provide fundamental insights into human physiology and disease. He explained how lysosome dysfunction contributes to various pathologies and how inherited mutations that compromise lysosomal hydrolysis, transport or signaling components lead to multi-organ disorders with severe metabolic and neurological impact. From a functional point of view, he explained how the lysosome senses nutrient availability through its association with the rapamycin complex 1 (mTORC1).

He also highlighted the role of the transcription factors microphthalmia/transcription factor E (MiT/TFE) in the feedback regulation of lysosome biogenesis (Settembre and Perrea, 2023) and presented a novel molecular mechanism explaining of how cells control ER-Phagy.

Afterwards, Emilio-José Vélez (St Pée sur Nivelle, France) spoke about Chaperone-mediated autophagy (CMA), a type of autophagy that selectively recognizes cytosolic proteins containing the KFERQ sequence. Mechanistically, these KFERQ containing proteins are linked by HSPA8/HSC70 (heat shock cognate 71 kDa protein, member of the heat shock protein 70 family) and co-chaperones and addressed to the lysosome thanks to a specific binding to the cytosolic tail of LAMP2A (lysosomal associated membrane protein 2A). The group directed by Iban Seiliez demonstrated that a CMA-like process exists in fish species and that *lamp2a* knockout induces severe defects of carbohydrate and lipid metabolism. In the presented study, they explored the CMA function in a fish species considered as an evolutionary model system to study natural cases of impaired glucose homeostasis, namely the rainbow trout (RT, *Oncorhynchus mykiss*); they demonstrated that high levels of glucose induce CMA flux that is mediated by mitochondrial reactive oxygen species generation and involves the antioxidant transcription factor Nfe2l2/Nrf2. These presented results offer novel insights in both the role and regulation of CMA during glucose-related metabolic disorders.

This section was followed by a round table chaired by two Associate professors of University Lyon1 (Dr Ludivine Walter and Dr Aurore Rozières). This round table was devoted to gather

two patient associations entitled "autour du BPAN" and "BPAN France" and researchers working on autophagy, to increase mutual understanding. This was an opportunity to talk about the Beta-propeller protein-associated neurodegeneration (BPAN), a rare disorder due to mutations in the WDR45 (WD repeat domain 45) gene. The pathology is typically characterized by early-onset seizures, infantile-onset developmental delay, intellectual disability, absent-to-limited expressive language, motor dysfunction (ataxia), and abnormal behaviors often similar to autism spectrum disorder. A work focusing on the BPAN pathology was presented by Prof. Bertrand Mollereau (Lyon, France). Indeed, the WDR45 gene mutated in BPAN patients, is a regulator of macroautophagy. The BPAN disease is thus emerging as one of the rare monogenic human neurological diseases targeting a regulator of autophagy defining it as a relevant model for directly assessing the role of autophagy in neurological diseases (Mollereau et al., 2023).

This section also included two interesting presentations by doctoral student, Thomas El Jamal from Lyon, and by Dr Camilla Bean, (Udine,Italy), dealing respectively with mitochondrial respiration and mitophagy in familial sarcoidosis and the characterization of an ATP synthase mutation associated with lysosomal-autophagosomal alterations.

Session 3

The immunity and infection session was opened, as a guest speaker, by Dr Harald Wodrich (Bordeaux, France) who taught us more about the cellular response to membrane penetration in the case of adenoviral infection. Entering of adenovirus by endocytosis induces an autophagic response intended to clear the virus. This process is initiated by the lysis of the endosome by adenovirus protein VI and the exposure of its intracellular membrane glycanes. Glycanes are detected by Galectine8 that recruits and activates TBK1 by phosphorylation, which in turn, promotes autophagy stimulation. However, adenovirus is able to bypass this antiviral response by escaping from the lysed endosome (Pied et al.,2022). The study of the consequences of the adenovirus-induced membrane damage on the autophagic response has yet to reveal all its secrets.

In the same way, the possible involvement of autophagy proteins in noncanonical autophagy processes such as virus assembly was presented and discussed by the PhD student Marjorie Palaric (Paris, France); its involvement in secretion, or membrane repair is also a current topic. For instance, Dr Pierre Lapaquette (Dijon, France) described a strong recruitment of autophagy proteins at the penetration site (associated to host plasma membrane damages) of the opportunistic fungal pathogen C*. albicans* into epithelial cells. This recruitment is involved in plasma membrane repair via exocytosis of lysosomal membrane and thus in the protection of epithelial cells against *C-albicans* induced cell death (Lapaquette P et al., 2022). Time will tell if this protection mechanism can be bypassed by the pathogen.

On the immunity side, the involvement of autophagy proteins in the polarization of B cells but also in B cell receptor trafficking and antigen presentation was described by Quentin Frenger (PhD student, Strasbourg, France). Dr Aurore Rozières (Lyon, France) also taught us about the possible role of the autophagic flux alteration as a parameter involved in the inappropriate immune response towards gut microbes in patients suffering from the Crohn’s disease, an inflammatory bowel disease; all examples of the involvement of autophagy in the balance of innate and adaptative immunity, inflammation, infection and of our quite primitive understanding of the plethora of functions in which autophagy proteins are involved.

Session 4

Dr Lisa Frankel, Associate Professor at Copenhagen University, opened the cancer section as a guest speaker. She highlighted the important role of autophagy in controlling ribosome homeostasis in senescence. Indeed, her group has recently demonstrated that ribosomes are selectively degraded by autophagy during oncogene-induced senescence. From a molecular point of view, they demonstrated that this degradation is mediated by the release of USP10 from the ribosome, leading to ribosome ubiquitination. The autophagy receptor protein p62 in turn recognizes the ubiquitinated ribosomes, allowing them to be renewed. This process facilitates the metabolome and secretome associated with senescence (Lopez et al). She also discussed the general impact of autophagy on cellular RNA homeostasis, a topic that is covered in depth in Kolapalli et al.2023. This part of the meeting callously highlighted the fact that autophagy can regulate tumorigenesis through RNA homeostasis.

The section was rounded off by oral presentations from doctoral students explaining, respectively, the crucial role played by autophagy in the integrity of the intestinal stem cell genome (Caterina Luana Pitasi, Paris, France), the post-transcriptional control of autophagy in head and neck cancers (Axel Arthur - Toulouse, France) and the effect on tumor development of the inactivation of Atg5 in the bone microenvironment (Marie-Charlotte Trojani - Nice, France).

Finally, Prof. Mario Tschan from the University of Bern (Switzerland) focused on studies destinated to elucidate the function of autophagy and oncogenic splice variants in cancer cell motility. Altogether, the importance of post-transcriptional control of autophagy in cancer strongly emerged and further studies are needed in this area of research, which could lead to a better understanding of cell function and its deregulation in cancer

CONCLUSION

The CFATG11 provided an opportunity to bring together 130 scientists working in the field of autophagy in France and other French-speaking countries, as well as in Europe. This event brought together regional, national and international researchers of international renown that made the academic ecosystem visible and attractive and allowed to communicate on works related to Autophagy. These days enabled us to facilitate exchanges and collaborations, to disseminate the most recent knowledge in the field of autophagy and gave young scientists the opportunity to present their work. Moreover, the presence of researchers from industry made possible the emergence of partnerships between academia and industry. Finally, the presence of associations representing patients with mutations in the autophagy regulatory genes provided an opportunity to reflect on the consequences of autophagy studies on health and possible therapies.

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