UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.7

Proponent: Prof. Paola Coccetti

Project Title: Investigating new funcions of AMPK in the regulation of autophagy and lipophagy

Scientific background and 'open issues'

Autophagy is a conserved eukaryotic degradation pathway in which cytoplasmic components are degraded in the vacuole/lysosomes and recycled. In addition, autophagy removes damaged organelles and misfolded proteins and thus plays a crucial role in maintaining cellular homeostasis. Lipophagy, which targets lipid droplets, has recently emerged as another specific selective autophagy, whose regulation still needs to be clarified (doi.org/10.14348/molcells.2020.0046).

Strikingly, autophagic activity decreases with age in eukaryotic systems, moreover adequate autophagy is recognized as a central biological pathway that promotes health and longevity. (https://doi.org/10.1038/s43587-021- 00098-4). During starvation, autophagy is intensively induced by activation of AMP-activated kinase (AMPK) and SIRT1 (NAD-dependent protein deacetylase sirtuin-1), while it is suppressed by TORC1 in growth conditions. SNF1/AMPK is the yeast homologue of AMPK which is conserved from *Saccharomyces cerevisiae* to *Homo sapiens*. SNF1/AMPK plays a crucial role in cellular energy metabolism, regulating a variety of metabolic processes among which the growth in respiratory conditions, stress response and catabolic pathways.

AMPK plays a pivotal role in activating lipophagy, thus reducing and removing surplus of lipid droplets, alleviating lipotoxic stress and providing additional energy resources (doi:org/10.1038/srep41606). Additionally, AMPK contributes to the prevention of further lipid accumulation by deactivating key enzymes involved in the synthesis of fatty acids and cholesterol (doi: 10.3389/fphys.2022.970292). Interestingly, the activation of AMPK by specific bioactive natural compounds represents a promising therapeutic avenue for managing metabolic disorders characterized by excessive lipid storage and impaired autophagy (doi:10.3390/ijms20194801). These natural compounds trigger a cascade of cellular events that lead to autophagy, enabling cells to effectively minimize unwanted lipid accumulation. This strategy not only supports cellular homeostasis but also opens up new pathways for treating metabolic disorders, highlighting the potential of AMPK activation and autophagy induction also in delaying cellular senescence.

Objectives

By *in vivo* phosphoproteomic analyses, we recently published a complete dataset of Snf1/AMPK targets (https://doi.org/10.7554/eLife.84319), which pinpointed several new Snf1-dependent residues within proteins which regulate autophagy. Importantly, their functions cover both steps of autophagic machinery and lipophagy as well as negative regulators of TORC1 signaling. The main objectives of the present project are:
i) to dissect new molecular mechanisms by which Snf1/AMPK impinges on autophagy regulation by investigating the proteins identified by the above phosphoproteomic approach;
ii) to investigate AMPK-dependent (or independent) lipophagy and its impact on cellular senescence.
iii) to identify new potential natural bioactive molecules able to activate AMPK, to prevent excessive lipid storage and impaired autophagy and able to promote longevity. Indeed, several plant-derived extracts as well as specific isolated compounds are now available in our laboratory thanks to the collaboration with the Italian National Biodiversity Future Centre (NBFC) (to which Coccetti's research group is also affiliated).

Methodologies

The role of AMPK in the autophagic process will be studied in two different models, well established and used in our laboratory. The first one in the *Saccharomyces cerevisiae*, through which our group has studied AMPK regulation for several years, having developed several tools and mutants that could be useful for the present project. The second is represented by human hepatoma cells (HepG2), in which AMPK activation in response to specific nutrient changes has been studied recently from our laboratory. This latter is a very useful model in particular for the study of lipophagy, due to the role of the liver in lipid and cholesterol metabolism (doi:10.3390/ijms20194801).
In both models, cellular biology techniques will be employed to study autophagy (cell growth assays, lifespan measurements, flow cytometry analysis to measure lipid content), as well as biochemical analysis (enzymatic assays, in vitro kinase assays, immunoprecipitation and western blot analysis) and molecular biology techniques (DNA and RNA purification, Real-time PCR, site-direct mutagenesis and CrispR/Cas9 gene editing).
Immunofluorescence analysis and single cell live imaging will be employed to visualize autophagy activation and progression over time. Live cell imaging techniques have revolutionized the study of cellular processes, including the dynamics of lipid droplets across various models. These techniques have been already developed in our laboratory, and will enable us to visualize and analyze the behavior of lipid droplets and autophagy activation in real-time in various models. In addition, multi-omics analysis will be performed, thanks to international and national collaborations with other research groups, with expertise in metabolomics and proteomics, that have already been established and will continue for the entire period of the present PhD project.

Project's Sustainability & Mobility

Our research activity concerns the study of the molecular mechanisms that regulate cell cycle, signal transduction and cell growth, with a focus on energetic metabolism and its dysfunctions linked to human diseases. Metabolism has been studied using different cellular systems also through proteomics and metabolomics as well as systems biology approaches.

Possible destinations for mobility will involve:
- the laboratory of Professor Paula Ludovico, PhD, at University of Minho, Portugal will be possible for the PhD student. Paula Ludovico has a long-standing experience in cell signaling and autophagy.
- the laboratory of Professor Claudio De Virgilio, at University of Freiburg, Switzerland, wil, also be possible, given the ongoing collaboration with his group.

References

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