LABORATORY OF MOLECULAR EMBRYOLOGY

AUTOPHAGY IN HEALTH AND DISEASE

Lab Head: Prof. Francesco Cecconi

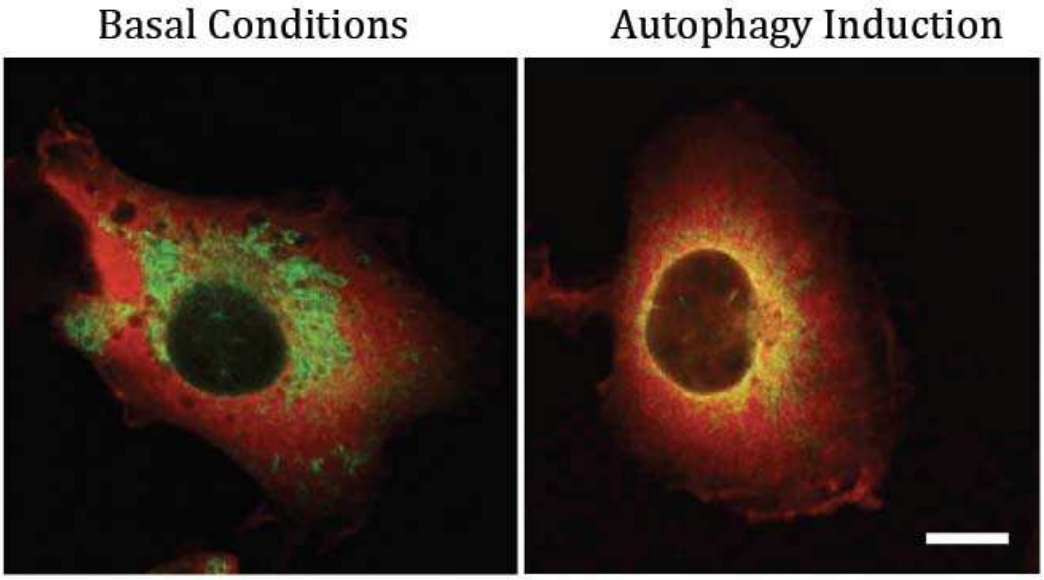
Autophagy is a highly conserved catabolic process, by which cytoplasmic material (e.g., proteins, lipids and organelles) is transported to lysosomes for degradation by means of double-membraned vesicles, the autophagosomes. Its importance in a vaste range of diseases has been postulated, including disorders of the immune system, cancer and neurodegeneration. However, although the main pathways of autophagy have been elucidated at a deep molecular level, the relative importance of this process in controlling the switch between death and survival, the rate of cell growth and the regulation of cell differentiation, all need more investigation. Our Lab is committed in unravelling the upstream regulation of autophagy and elucidating the role of this process in three different pathological conditions: neurodegeneration, autoimmunity and cancer.

UNIT 1. AUTOPHAGY SIGNALLING NETWORK

***Postdoctoral Fellows*: Dr. Francesca Nazio, Dr. Flavie Strappazzon, Dr. Cristina Valacca**

***PhD students*: Maria Cristina Capizzi, Somayeh Poorpirali**

Macroautophagy is an intracellular degradation process that has a well documented role in the maintenance of tissue homeostasis and in the response to stressful environments. In recent years, our lab has identified a novel protein called AMBRA1 (Activating Molecule in Beclin 1-Regulated Autophagy), that acts as an activator of autophagy and is involved in the development of the nervous system. In our laboratory we are studying AMBRA1 from different point of views: 1) analysis of AMBRA1 promoter that aims at the identification of transcriptional factors regulating both negatively and positively AMBRA1 gene expression; 2) analysis of the AMBRA1 gene at a post-transcriptional level by studying the role of AMBRA1-splicing variants and by identifying specific miRNAs that are able to down-regulate AMBRA1 expression; 3) analysis of AMBRA1 stability and interactions by studying its post-translational modifications and their role in AMBRA1 functionality during autophagy induction and by identifying the role of different AMBRA1 cellular pools, such as mitochondrial AMBRA1, in autophagy signalling.

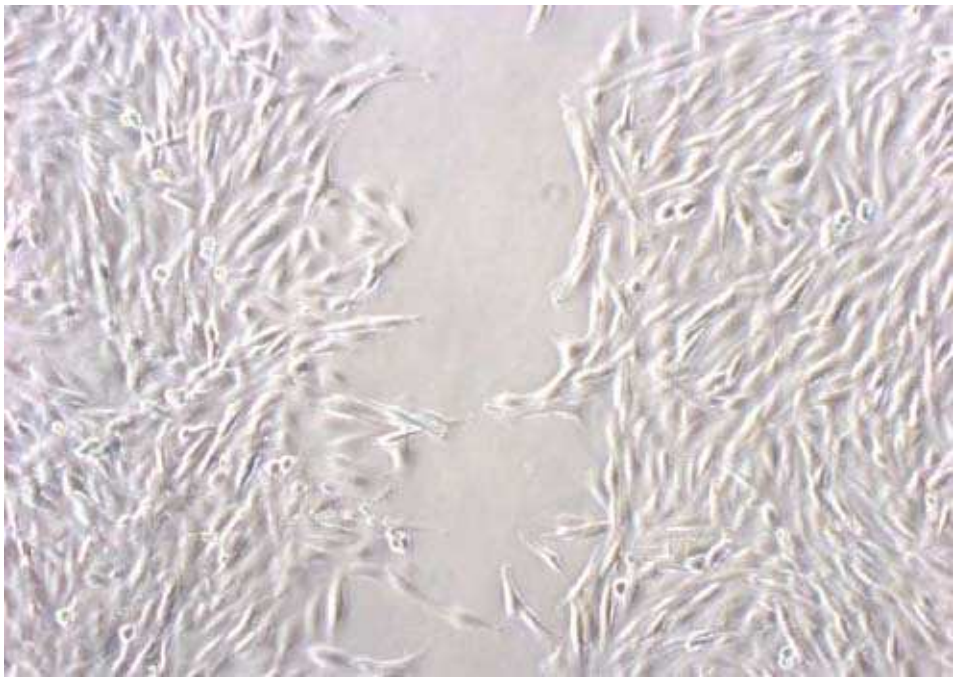


Red: AMBRA1
Green: ER marker

UNIT 2. AUTOPHAGY IN CELL MIGRATION
Staff Scientist (Ricercatore): **Dr. Sabrina Di Bartolomeo**
Postdoctoral Fellow**: Dr. Cristina Valacca**

Cell migration is a basic process, associated with physiological conditions, such as during embryo development or wound healing, as well as with pathological situations, like cancer spreading. This process can be regulated by a myriad of extracellular stimuli, including growth factors or environmental changes. After stimulation, specific intracellular signaling are activated and a cellular response is induced. In our lab, we are studying the relationship between autophagy and cell migration, especially regarding the role that they play in cancer (e.g., glioma and breast cancer). Cell migration is, indeed, a crucial process during metastasis formation, and understanding the processes involved in this event is of the utmost importance in order to develop anti-cancer drugs and design new therapies. The existing relationship between autophagy and migration is a quite novel concept and the characterization of the molecular program linking these two processes in a tumor context will contribute to our knowledge of tumor progression.

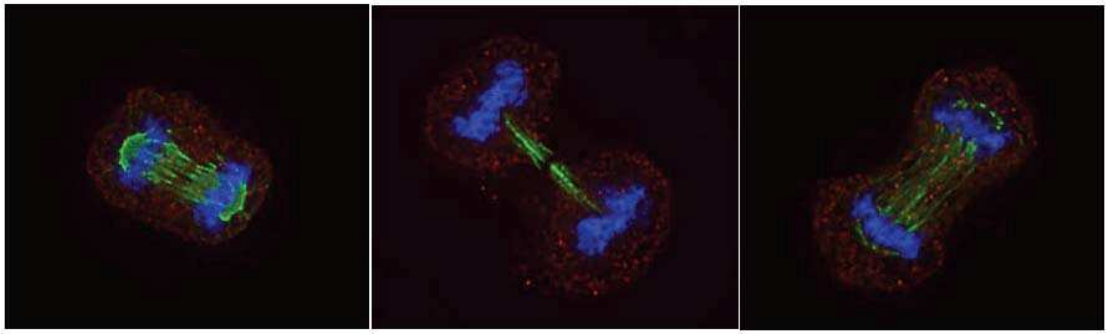
Wound healing assay showing glioma cells re-filling the cell-free gap after18 hours from scratch



UNIT 3. AUTOPHAGY AND CANCER (DON'T MESS WITH THE CELL CYCLE!)
***Staff Scientist (Ricercatore*): Dr. Sabrina Di Bartolomeo**
***Postdoctoral Fellow*: Dr. Valentina Cianfanelli**
***PhD student*: Ashraf uz Zaman**

Autophagy contributes to a plethora of physiological and pathological processes, and has recently emerged as a key regulator of multiple aspects of cancer biology. The role of autophagy in cancer is complex, serving as a barrier to limit tumor initiation and as an adaptive response functional to malignant progression in the established neoplastic lesions. In addition to ties to the tumorigenesis, there are also evidence for a direct role for autophagy in controlling cell proliferation and vice versa, but less is known about this relationship. In this context, in our lab we are studying the function of the pro-autophagic protein AMBRA1 in cell cycle regulation and its implications in tumor insurgence. Moreover, we are investigating the cross-talk between autophagy and cell cycle regulation by characterizing the post-translational modifications of autophagy core-complex proteins during cell cycle progression. Finally, we are evaluating the role played by different diet regimens (e.g., Mediterranean, rich, iperproteic) in autophagy regulation an tumorigenesis.
Images showing a HeLa cell during the anaphase (a), the telophase (b and

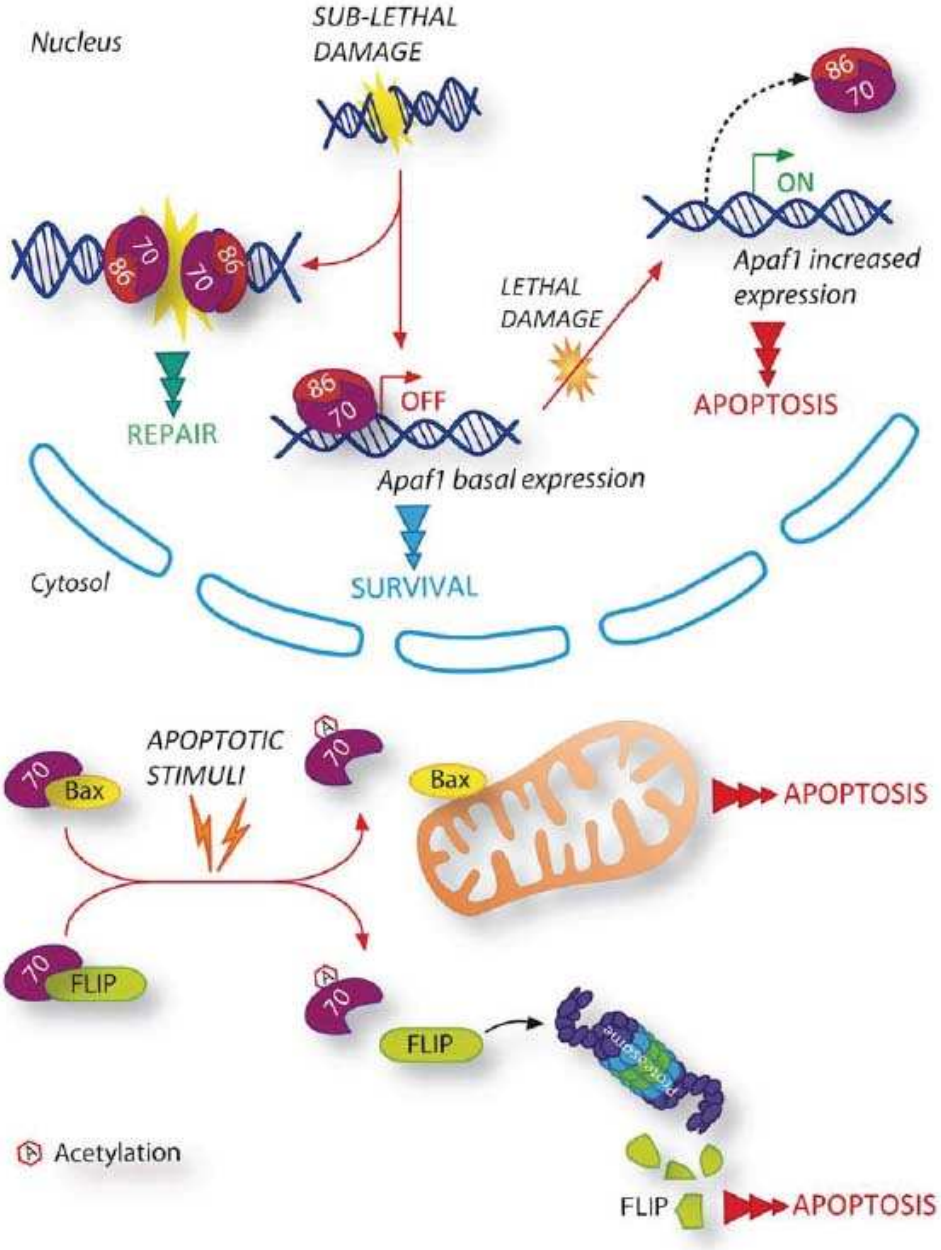
Images showing a HeLa cell during the anaphase (a), the telophase (b nd c) of mitosis in which AMBRA1 has been stained in red, beta-tubulin in green and DNA in blue.



UNIT 4. DEATH AND SURVIVAL IN DNA STRESS RESPONSE
Staff Scientist (Ricercatore): Dr. Daniela De Zio

For many years, our studies have been focused on the apoptosis signaling with particular attention to the role of the pro-apoptotic protein Apaf1 in neurobiology and cancer diseases. Our last findings pointed out to Apaf1 transcriptional regulation mediated by the crucial DNA repair complex Ku. Ku is specifically involved in direct binding to the two broken DNA ends and in the promotion of the NHEJ (nonhomologous end-joining) pathway of DSBR (double strand breaks repair). Our findings have shown that Ku-mediated Apaf1 regulation is dynamically modulated upon DNA stress response. This has opened a new field of interest in our laboratory: DNA repair proteins involved in specific DNA repair pathways modulating function or activity of some apoptotic factors. In this context, the autophagic process has been also demonstrated to be activated upon genotoxic stress and it could be functional to the energy production that is necessary for DNA repair processes, contributing to the pro-survival phase of the DNA damage response. Thus, we are also investigating the possible crosstalk between DNA repair and autophagy, focusing our attention on the existence of functions associated to the DNA damage response of typical autophagic proteins.

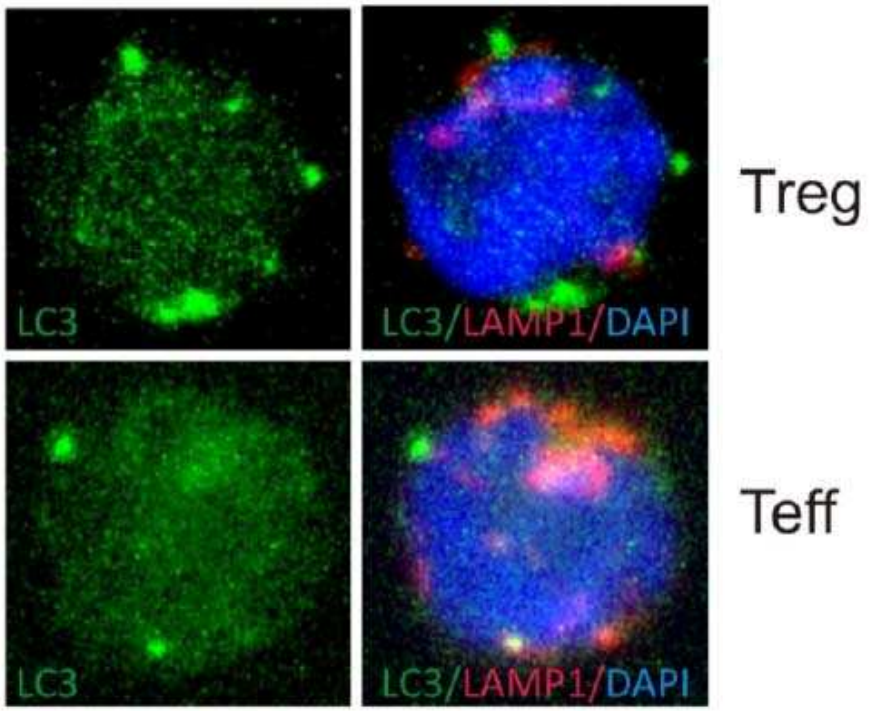
Ku involvement in the DSBR pathway and apoptosis


Nucleus
SUB-LETHAL DAMAGE
86
70
ON
Apaf1 increased expression
LETHAL DAMAGE
APOPTOSIS
REPAIR
OFF
Apaf1 basal expression
Cytosol
SURVIVAL
APOPTOTIC STIMULI
Bax
APOPTOSIS
FLIP
Proteasome
FLIP
APOPTOSIS
Acetylation

UNIT 5. AUTOPHAGY REGULATION IN THE IMMUNE SYSTEM
***Postdoctoral Fellow*: Dr. Juliane Becher**

Helper T cells can be divided into two major groups, thus the proinflammatory effector T cells and regulatory T cells which exert a suppressive function on the effector T cell population. T cell homeostasis is of particular importance to protect the organism from excessive inflammation and the development of autoreactive effector T cells correlates with the incidence of autoimmune disease such as multiple sclerosis. Thus studying the mechanisms underlying regulatory T cell differentiation is of marked interest. A central role of mTOR in directing T cell differentiation has been widely described. A predominantly inflammatory environment is associated with enhanced mTOR activation and triggers effector T cell but non regulatory T cell differentiation. We are thus investigating the role of autophagy, which in contrast is activated upon mTOR inhibition, on T cell differentiation. Preliminar results show, that regulatory T cells exhibit elevated levels of autophagy. Furthermore, induction of autophagy in an animal model of multiple sclerosis attenuates the course of the disease due to an increase in the regulatory T cell population. Freshly isolated regulatory T cells exhibit higher numbers of autophagosomes than effector T cells (LC3 positive dots). LC3 partially collocalizes with LAMP1, indicating the presence of utophagolysosomes

Freshly isolated regulatory T cells exhibit higher numbers of autophagosomes than effector T cells (LC3 positive dots). LC3 partially collocalizes with LAMP1, indicating the presence of autophagolysosomes



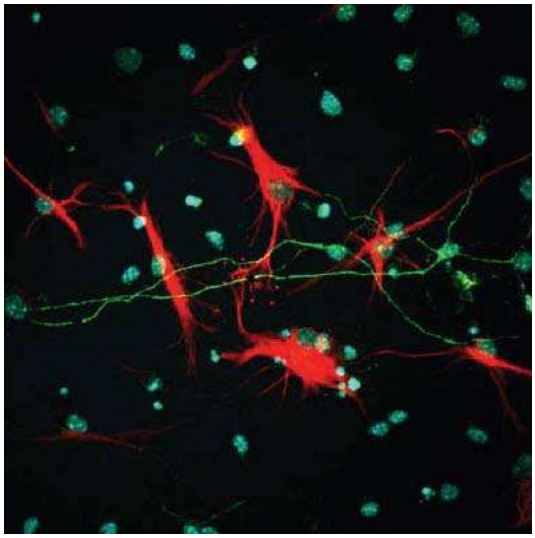
UNIT 6. AUTOPHAGY IN THE NERVOUS SYSTEM
***Postdoctoral Fellow*: Dr. Francesca Nazio, Dr. Paola Merlo**
***PhD student*: Meysam Yazdankhah**

Autophagy is the major pathway involved in degradation of long-lived proteins and organelles, cellular remodeling, and survival during nutrient starvation. In this context of studies,

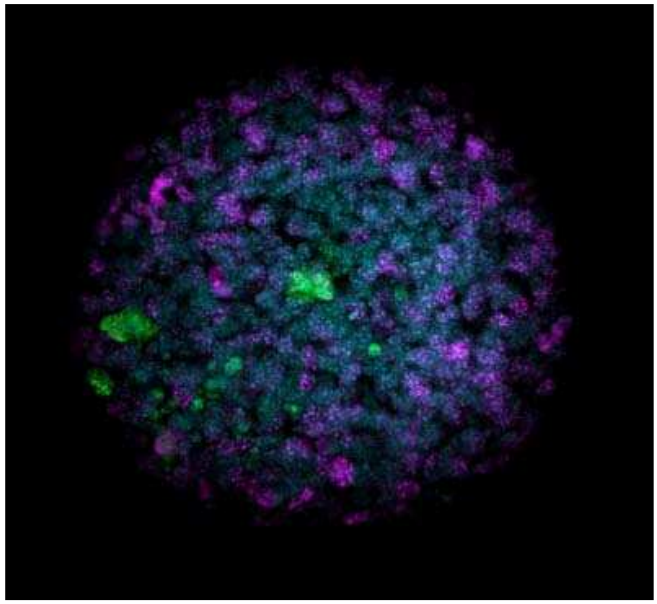
1. We aim at unravelling the role played by autophagy in the dendritic spine during the pathogenesis of Alzheimer's Disease. We have recently shown that spine degeneration, which characterizes the onset of this disease, is related to mitochondrial destabilization, local activation of caspase 3 and dephosphorylation of the AMPA subunit GluR1. We are now elucidating the roles played by mitochondria morpholgy factors and autophagy regulators in this degradative pathway.
2. Multipotent neural stem cells exist in adult brain, such as those found in the subventricular zone of the lateral ventricle and dentate gyrus of hippocampus.

Although, there is evidence about the role of autophagy in the development of central nervous system, the role of this process during generation of newborn neurons in adult brain is still uncharacterized. We are interested to know how autophagy machinery changes during neural stem cell differentiation and what is the role of these changes in differentiation and maintenance of neural stem cells.

Neural precursor cells can differentiate into astrocytes and neurons in differentiating condition. Red: GFAP and green: TuJ1, blue: DAPI. Scale bar, 10 µm.



Proliferation and apoptosis are observed in neurospheres. Green: cleaved caspase 3, pink: Ki67, and blue: DAPI.



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