ABSTRACT

Cell survival in response to environmental changes requires the maintenance of a dinamic equilibrium between protein synthesis and degradation. The main function of protein degradation, apart of regulating different cellular processes, is the removal of useless products or products which accumulation may be toxic to the cell. The amino acids resulting from this degradation are reused for the synthesis of new molecules or are otherwise metabolized for energy obtaining. Alteration of intracellular proteolysis can produce the accumulation of defective organelles or proteins (or other molecules) that can accumulate in insoluble aggregates what can lead to the development of different pathologies. During the last years there has been a great advance in the knowledge about intracellular degradation of proteins and their main mechanisms. However, a lot of molecular details are still unknown. It is therefore necessary to shed new light on these processes that could be of relevance to identify new therapeutic targets and to develop more effective treatments for diseases resulting from alterations in them.

Protein degradation can occur by different mechanisms that can be classified based on the involvement of lysosomes. Among them, macroautophagy (usually referred to as autophagy) and the ubiquitin-proteasome system are the most important. In essence, the ubiquitin-proteasome system consists in the polyubiquitination of proteins that are subsequently degraded in the proteasomes. Autophagy, on the contrary, consists in the sequestration of cytoplasmic portions by double membrane structures that will close forming an autophagosome that will ultimately fuse with endosomes and lysosomes forming autolysosomes. Is inside these structures where the engulfed material is degraded by the lysosomal proteases and cathepsins.

Autophagy is regulated by a wide variety of signaling transduction pathways that are activated in response to different environmental factors. Among them, nutrient starvation is the most potent inducer of autophagy. During nutrient deprivation, such as of amino acids, the cell is subjected to an energetic stress that will try to overcome by producing ATP from new sources. To this purpose, autophagy is activated in order to degrade cellular components, such as proteins, into its monomers that will be then metabolized. On the other hand, it has been repeatedly demonstrated that when amino acids are available, autophagy is inhibited. Although the effect of amino acids on autophagy has been extensively studied in many laboratories, the effect of glucose was not clear when we aborded this study and data were controversial.

In this work, we have clearly established that glucose is an inducer of autophagy by using different techniques such as quantification of LC3-II levels, as a marker of autophagy, by

Western-blot in the presence or absence of lysosomal inhibitors, quantification of the total amount of protein degraded and its percentage due to autophagy by pulse-chase experiments, morphometric quantification of autophagic structures (similar to autophagosomes and autolysosomes) by electron microscopy and quantification of the lysosomal mass by fluorescence. We have also observed that, in addition to autophagy, glucose induces the ubiquitination of proteins and their degradation in the proteasomes. Our data clearly demonstrate that glucose induces autophagy in all cell types analyzed and in all conditions tested. This effect is diminished when other factors are present, such as amino acids or fetal bovine serum, what could explain part of the contradictory data found in the literature.

Glucose provides the energy needed for the proper functioning of autophagy that will take place when a minimum threshold of ATP is reached. A drop in the energy availability caused by glycolysis inhibition blocks the autophagy induced by glucose. However, autophagy induction by glucose is not only due to the ATP provided. Although AMPK is phosphorylated in response to the decrease in ATP levels and the increase in calcium during glucose deprivation, it does not participate in the induction of autophagy by glucose, as well as mTOR. We have identified MAPK p38α as the kinase responsible for the induction of autophagy by glucose, as assessed by the use of chemical inhibitors and by the use of RNA interference and knockout MEFs. We consider that these results contribute to clarify the regulation of autophagy by nutrients and, specifically, by glucose.