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INVESTIGATION OF THE SEQUENTIAL STAGES OF AUTOPHAGY BY TIME LAPSE MICROSCOPY

Abdul Alim Al-Bari[1,2] and Pingyong Xu[1]

[1]Institute of Biophysics, Chinese Academy of Sciences, China
[2]University of Rajshahi, Bangladesh

Autophagy, a genetically regulated catabolic process facilitates nutrient recycling via lysosomal degradation of unwanted cellular proteins and organelles. As cellular homeostasis, the damaged or defective organelles including mitochondria, endoplasmic reticulum or Golgi apparatus are degraded and recycled via autophagy. In recent years, accumulating evidences have focused to the significance roles of autophagy in various human diseases. Thus, the regulation of autophagy process is enormous important for not only basic research but also therapeutic values. Autophagy process is characterized by a series of events such as initiation, nucleation, and elongation of the isolation membrane (IM) and formation of autophagosomes. Autophagosomes finally sequester with cytoplasmic components, and following fusion with lysosomes, the cellular contents are degraded and released back into the cell by lysosome. Several sensitive and quantitative techniques have been developed for monitoring the particular steps of autophagy e.g. IM localization by lipidated LC3 fused with EGFP. However, LC3-based probes have several limitations such as only transiently detected. Thus, it is necessary to detect the sequential steps of autophagy by time-lapse imaging because single time point fluorescence images may miss the sequential events of autophagosome formation. In this study, we focused on the individual steps of autophagy process in sequential manner with time-lapse microscopy. For these purposes, we generated Beclin1 knockout COS7 cell with the help of CRISPR-Cas9 system. Then we added Tat-Beclin 1 to these COS7 cells to investigate the compensatory effects of Beclin 1 and various steps of autophagy under microscopes in time lapse manner. At the same time we also used Atg5 knockout MEF cells and purify Tat-Atg5 peptide for the same manner. In this way, we established the sequential events of autophagy process and provide an impact in regulation of autophagy for the therapeutic value in diverse diseases like cancers.

alimalbari347@ru.ac.bd