Modulation of membrane interaction provides insights into the inhibition of autophagic flux in mammalian cells

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Abstract

INTRODUCTION: Autophagy plays an important role in cell homeostasis and there are clear evidences that modulators of autophagy may have a number of potential benefits, for example in cancer therapy. Many of them are lysosomotropic agents that block the last critical autophagy step and induce cell death. However, the mechanisms involved on the autophagic cell death still need clarification. **OBJECTIVES:** By studying the ability of isomeric molecules in perturbing membranes, we uncover a paradigm to control the autophagic flux and induce cell death. **MATERIAL AND METHODS:** For this purpose, we performed comparative analysis of the isomeric pentacyclic triterpenoids Betulinic acid (BA) and Oleanolic acid (OA).To identify the role of specific drug-membrane interactions, we did *in vitro* studies of cell membrane models and performed *in silico* molecular dynamics simulations using DPOC bilayer. The analysis of cell membrane permeability was based on the release of carboxyfluorescein from unilamellar liposomes. To study the effects of BA and OA on mitochondria and lysosome membrane, we used normal human keratinocytes (HaCaT) as cell model. **RESULTS AND DISCUSSION:** According to insights from *in vitro* studies and molecular dynamics simulations, BA and OA dramatically differed on their ability to interact with membranes. When the lysosomal membrane was impaired upon treatment with BA, autophagy ceased

and was no longer able to keep cell homeostasis. The observations indicated that the toxic effects of BA were associated with impairment of autophagic flux, instead of intrinsic apoptosis as proposed for decades. Contrarily, cellular homeostasis was maintained upon autophagic clearance of OA-damaged mitochondria. Nevertheless, the application of an autophagy inhibitor, such as Chloroquine or Bafylomicin-A1 enhanced the therapeutic efficacy of OA in inducing autophagic cell death. **CONCLUSION:** These results support the concept that an efficient strategy for inducing autophagic cell death is to promote parallel damage in mitochondrial and lysosomal compartments.