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View Abstract

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ABSTRACT

TITLE: UBIQUITINATION SYSTEM AND THE REGULATION OF AUTOPHAGY IN HUMAN TUMOR CELLS

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ABSTRACT BODY:

Abstract Body: Autophagy is a tightly regulated catabolic process involved in the degradation and recycling of proteins and organelles. Ubiquitination plays an important role in the regulation of autophagy. VMP1 is an essential autophagy protein whose expression in pancreatic cancer stem cells, carrying activated KRAS, enables therapeutic resistance. Using biochemical and cellular approaches we investigated the role of VMP1 ubiquitination in the regulation of autophagy in human tumor cells. In silico analysis of VMP1 structure revealed three possible ubiquitination sites. Immunofluorescence of ubiquitin in VMP1-GFP transfected cells showed significant colocalization between VMP1 and ubiquitin. Immunoprecipitation with anti-FLAG in FLAG-Ub and VMP1-GFP co-transfected cells showed ubiquitinated VMP1-GFP in the eluates. Also, Ub-FLAG was found in the eluates of immunoprecipitation with anti-V5 in VMP1-V5 and Ub-FLAG cotransfected cells. However, VMP1 is not primarily degraded by the ubiquitin-proteasomal nor by lysosome systems since the inhibition of

the proteasome using MG132 and the lysosome with Chloroquine did not affect VMP1 levels. On the contrary, ubiquitination regulates the role of VMP1 in autophagy. VMP1 co-distributes with ubiquitin in ATG5 -/- MEF cells, indicating that ubiquitination of VMP1 is independent of ATG5 and it occurs upstream to LC3 conjugation. We found a highly significant association between VMP1 and ubiquitin in LC3-labeled autophagosomes, as well as in LAMP1-marked autolysosomes indicating that VMP1 is ubiquitinated during the whole autophagic process. Moreover, only ubiquitinated VMP1 is involved in autophagosome biogenesis. Finally, by proteomic analysis and co-immunoprecipitation assay we found that the subunit Cdt2 of the E3 ligase CRL4/Cdt2 is an interactor of VMP1, which relocates from the nucleus to the perinuclear region under VMP1 expression. In conclusion, we identified the ubiquitination as a post-translational modification for VMP1, which is present during the whole autophagy pathway. Moreover, Cdt2 is a novel interactor and the E3 ligase candidate for ubiquitination of VMP1. VMP1 ubiquitination is independent of ATG5 and is related to autophagosome biogenesis. Our results indicate that ubiquitination is a novel post-translational modification of VMP1, regulating autophagy in human tumor cells. And suggest that modulating VMP1 ubiquitination would be of clinical relevance in the manage of tumor cell therapeutic resistance.

(No Image Selected)

DISCLOSURE

The following authors have completed their 2022 DDW disclosure: Felipe Renna: No Answer. | Mariana Tadic: No Answer. | Tamara Orquera: No Answer. | Carolina Vecino: No Answer. | Alejandro Ropolo: No Answer. | Maria Vaccaro: Disclosure completed