

Bisphosphonates induce autophagy by depleting geranylgeranyl diphosphate

Multiple studies have implicated the depletion of isoprenoid biosynthetic pathway intermediates in the induction of autophagy. However, the exact mechanism by which isoprenoid biosynthesis inhibitors induce autophagy has not been well established. We hypothesized that inhibition of farnesyl diphosphate synthase (FDPS) and geranylgeranyl diphosphate synthase (GGDPS) by bisphosphonates would induce autophagy by depleting cellular geranylgeranyl diphosphate (GGPP) and impairing protein geranylgeranylation. Herein, we show that an inhibitor of FDPS (zoledronate) and an inhibitor of GGDPS (digeranyl bisphosphonate, DGBP) induce autophagy in PC3 prostate cancer and MDA-MB-231 breast cancer cells as measured by accumulation of the autophagic marker LC3-II. Treatment of cells with lysosomal protease inhibitors [(2S,3S)-trans-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester (E-64d) and pepstatin A] in combination with zoledronate or digeranyl bisphosphonate further enhances the formation of LC3-II, indicating that these compounds induce autophagic flux. It is noteworthy that the addition of exogenous GGPP prevented the accumulation of LC3-II and impairment of Rab6 (a GGTase II substrate) geranylgeranylation by isoprenoid pathway inhibitors (lovastatin), zoledronate and DGBP). However, exogenous GGPP did not restore isoprenoid pathway inhibitor-induced impairment of Rap1a (a GGTase I substrate) geranylgeranylation. In addition, specific inhibitors of farnesyl transferase and geranylgeranyl transferase I are unable to induce autophagy in our system. Furthermore, the addition of bafilomycin A1 (an inhibitor of autophagy processing) enhances the antiproliferative effects of digeranyl bisphosphonate. These results are the first to demonstrate that bisphosphonates induce autophagy. Our study suggests that induction of autophagy in PC3 cells with these agents is probably dependent upon impairment of geranylgeranylation of GGTase II substrates.