Genetic ablation or pharmacological inhibition of autophagy suppresses intrinsic resistance of breast cancer to HER2-targeted therapies

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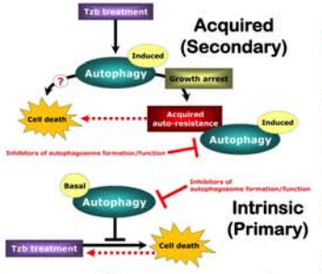
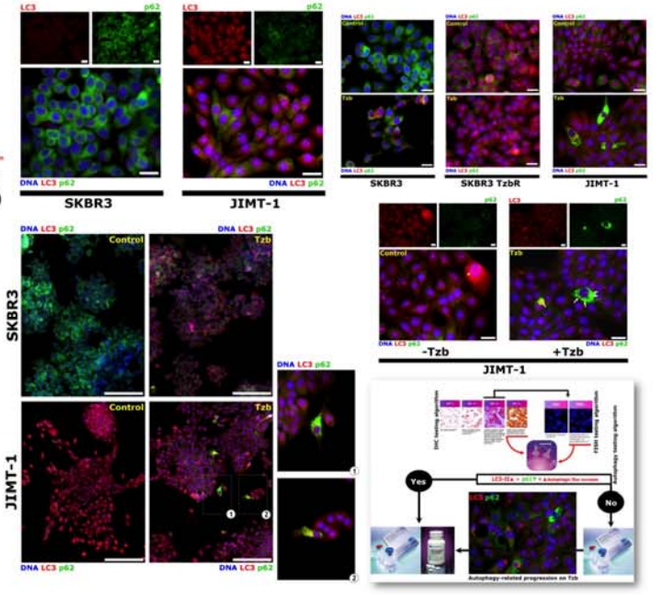
Autophagy, an evolutionary conserved catabolic process whereby cells generate energy and building blocks by promoting large-scale recycling of cytoplasmic macromolecules and organelles, represents a novel drug-targetable molecular mechanism underlying *de novo* (primary) refractoriness of *HER2* gene-amplified breast cancer (BC) to HER2 inhibition. JIMT-1 cells, which were obtained from a *HER2*-positive BC patient that rapidly progressed on trastuzumab (Herceptin™) *ab initio* and that show cross-resistance to multiple HER1/2 inhibiting drugs including lapatinib (Tykerb™), were found to constitutively exhibit an enhanced autophagic vesicle content as assessed by immunoblotting of endogenous ATG8/LC3 lipidation and confocal imaging of the recruitment of ATG8/LC3 to autophagic vesicles. A significant decrease in the expression status of the specific autophagy receptor p62/SQSTM1 -a protein selectively degraded by autophagy- confirmed further a constitutive activation of the autophagic flux in trastuzumab-refractory JIMT-1 cells. When the Human Autophagy RT2 Profiler™ PCR Array was employed to profile the expression of 84 genes involved in autophagy, *ATG12* was the most differentially up-regulated gene in JIMT-1 cells (>10-fold) as compared with trastuzumab-responsive SKBR3 cells. Upon collection of the transcriptional profile of the *ATG12* gene across two sets of > 50 widely used BC cell lines, HER2+ BC cells with well-established *de novo* resistance to trastuzumab were characterized by expressing significantly higher levels of *ATG12*. When lentiviral-delivered small hairpin RNA was employed to stably & specifically knock-down *ATG12* gene, JIMT-1 ATG12-shRNA cells were more significantly growth-inhibited by trastuzumab (up to 5-fold) than parental JIMT-1 cells. Moreover, the half-maximal inhibitory concentration (IC_{50}) values for the small-molecule HER1/2 Tyrosine Kinase Inhibitors gefitinib, erlotinib and lapatinib were drastically reduced by up to 10 times in response to *ATG12* gene silencing. Knockdown of autophagy-regulatory genes other than *ATG12* (e.g. *ATG5, ATG8/LC3B*) similarly suppressed refractoriness to trastuzumab as well as co-existing resistance to other HER1/2-targeted agents. Knockdown of autophagy-specific genes, however, did not alter basal sensitivity of JIMT-1 cells to multiple cytotoxics including doxorubicin, cisplatin, paclitaxel and vinorelbine. When lysosomal degradation was pharmacologically inhibited by using the antimalarial lysosomotropic drug chloroquine we found a massive accumulation of abnormal autophagolysosomes that promoted synergistic re-sensitization of JIMT-1 cells to the growth inhibitory and apoptotic activity of trastuzumab. In summary, autophagy-specific genes appear to play a potent protective role against HER2 inhibiting drugs currently in use. Given that genetic and pharmacological targeting of autophagy was found to be detrimental to intrinsic BC refractoriness to HER2-targeted therapies, our current findings may provide rationale for novel, chloroquine-based therapeutic approaches aimed to circumvent primary-resistance and potentiate the efficacy of both trastuzumab and lapatinib in patients treated for HER2-positive BC disease.

Autophagy and resistance to trastuzumab

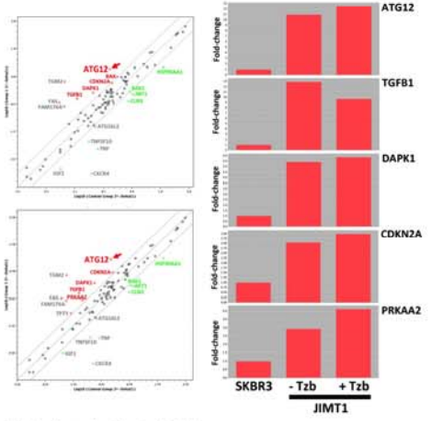

Autophagy Facilitates the Development of Breast Cancer Resistance to the Anti-HER2 Monoclonal Antibody Trastuzumab



Autophagy activation in BC models with *De novo* resistance to trastuzumab

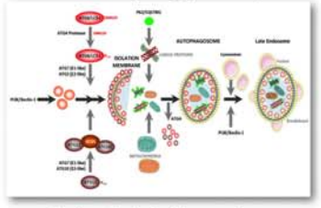


Up-regulation of autophagy-specific genes and *De novo* resistance to trastuzumab

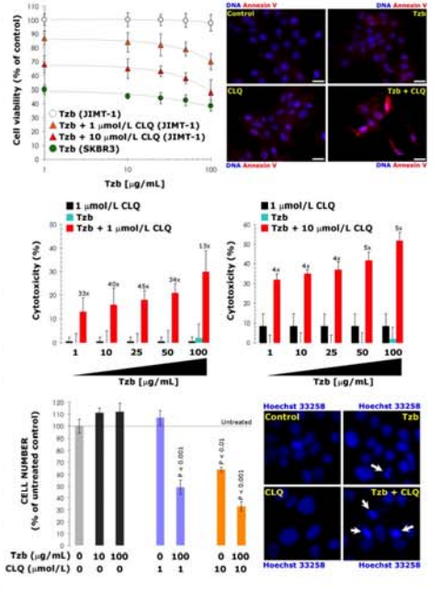


Pharmacological inhibition of autophagy flux and reversal of primary resistance to trastuzumab

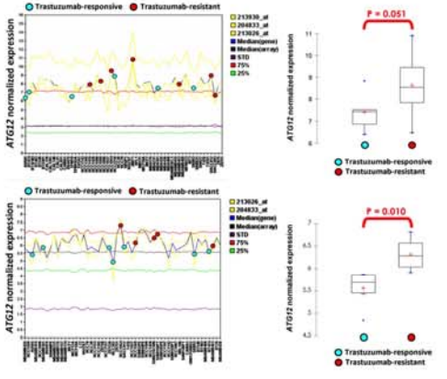
The autophagy pathway

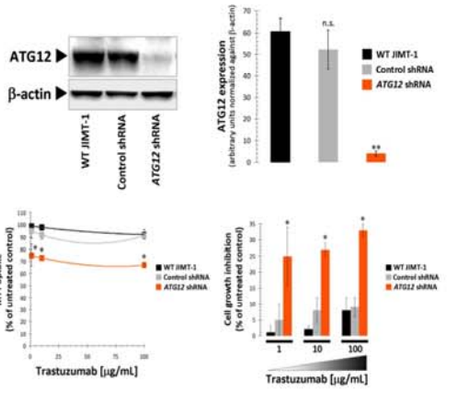


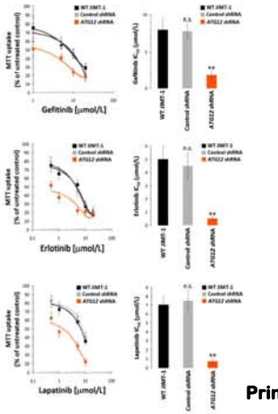
Antimalarial chloroquine: A strategy for targeting autophagy



Genetic ablation of the autophagy-specific gene *ATG12* and reversal of primary resistance to anti-HER2 therapies







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