Molecular mechanisms of autophagy-based drug resistance in cancer cells

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Background and Aims. Endocrine therapy with tamoxifen (Tam) is the validated treatment for metastatic and non-metastatic estrogen receptor-positive (ER^+) breast cancer (BC). However, onset of resistance to endocrine therapy is a frequent complication that severs the prognosis of BC patients. In recent years, autophagy has emerged as a key factor for drug resistance although, so far, the underlying mechanisms are far from being exhaustively understood.
Aim of this research is to investigate the role of autophagy in resistance of BC cells against Tam toxicity, and the underlying molecular mechanisms.
Methods. Tam-resistant MCF7 (TamR) were selected by culturing the parental cells with Tam at concentrations up to 5 µM. Cells were treated with Tam up to 20 µM, and LMP evaluated by lysosome staining with LysoTracker Red. Metallothionein 2A (MT2A) and Hsp70 were overexpressed by transfection. Autophagic flux was assessed by using a confocal microscopy following transfection of a plasmid encoding mRFP-GFP-LC3. Gene expression was assessed by Real-Time PCR.
Results. TamR cells grow in the presence of 5 µM Tam, are less susceptible to Tam-induced LMP, have an increased autophagic flux and a greater amount of MT2A and ferritin heavy chain (FtH) mRNAs. Tam resistance does not rely on downregulation of the ERα and β, whose mRNA level was unchanged compared to MCF7. Autophagy ablation restores susceptibility of TamR cells to Tam, indicating that higher levels of autophagy and of iron-binding proteins are both required for resistance to Tam. To verify this finding, MT2A and Hsp70 were overexpressed in WT cells: the results showed that Tam-induced LMP is markedly attenuated in cells overexpressing these proteins. In keeping with the above results, we also observed that MT2A and Hsp70 accumulates in LysoTracker Red-positive dots, indicating that autophagy-mediated lysosomal relocation of these proteins is essential in protecting the lysosomes against Tam-induced LMP.
Conclusions. Our data indicate that activation of autophagic flux, overexpression and autophagy-mediated lysosomal relocation of iron-binding proteins concur to drug resistance of BC cells. These factors combined restrain Tam toxicity and outline a potential mechanism by which autophagy might promote drug resistance in cancer cells.