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The amplified cancer gene LAPTM4B promotes tumor growth and tolerance to stress through the induction of autophagy

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Autophagy is a fundamental salvage pathway that encapsulates damaged cellular components and delivers them to the lysosome for degradation and recycling. This pathway usually conducts a protective cellular response to nutrient deprivation and various stresses. Tumor cells live with metabolic stress and use autophagy for their survival during tumor progression and metastasis. Genomic instability in tumor cells may result in amplification of crucial gene(s) for autophagy and upregulate the autophagic pathway. We demonstrate that a cancer-associated gene, LAPTM4B, plays an important role in lysosomal functions and is critical for autophagic maturation. Its amplification and overexpression promote autophagy, which renders tumor cells resistant to metabolic and genotoxic stress and results in more rapid tumor growth.

Keywords: LAPTM4b, breast cancer, autophagy, lysosome-mediated death, metabolic stress

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Lysosomal-associated protein transmembrane-4 β (LAPTM4B) is a putative novel oncogene located at chromosome 8q22, a region frequently amplified in breast cancers. Amplification and overexpression of LAPTM4B promotes anthracycline resistance in primary breast cancer and is associated with poor clinical outcomes in patients. Although it is known that LAPTM4B overexpression promotes retention of anthracyclines in the cytoplasmic compartment and decreases drug delivery to the nucleus, amplification of this gene in primary tumors prior to drug exposure raises the possibility of other roles for this protein, perhaps to promote cell survival or provide a growth advantage to cancers.

We explored the role of LAPTM4B in lysosome stability and found that knockdown of LAPTM4B triggers lysosome membrane permeabilization (LMP) with cathepsin release, which leads to lysosomal-mediated cell death in breast cancer cells. Furthermore, depletion of LAPTM4B results in increased lysosome pH, similar to what is observed after treatment with the lysosomotropic drug chloroquine. However, exogenous expression of LAPTM4B does not alter lysosome pH, and alteration of lysosome pH by chloroquine does not induce lysosome membrane permeabilization. These findings suggested that the change in lysosome pH after depletion of LAPTM4B is a secondary effect of LMP, resulting in deacidification of the lysosome. Apparently, threshold levels of LAPTM4B are required for lysosome stability and prevention of lysosome-mediated initiation of apoptosis. This may be important after exposure to chemotherapy drugs.

Lysosomes also play roles in the completion of autophagy. We examined the effect of modulating LAPTM4B expression on the process of autophagy during starvation in breast cancer cells. The combination of LAPTM4B depletion and starvation results in marked cytoplasmic accumulation of enlarged LC3-positive autophagosomes that do not colocalize with LAMP-2-positive lysosomes. This suggests that depletion of LAPTM4B results in failure of autophagosome-lysosome fusion and autolysosome formation. Immunoblotting LC3-II and p62 reveals an increase of both proteins after depletion of LAPTM4B or treatment with chloroquine, consistent with increased autophagosome number but a decrease in

autophagy flux. These results suggest LAPTM4B is required for later stages of autophagy maturation, in which autophagosomes are fused with lysosomes to form autolysosomes. Accumulation of autophagosomes leads to programmed cell death. The combination of LMP and blocked autophagy flux triggered by LAPTM4B depletion results in more pronounced caspase 3 activation and cell death. To investigate cell survival in periods of stress, we depleted LAPTM4B and found a pronounced decrease in the number of viable cells in low serum or low glucose.

In parallel experiments, we used cells stably overexpressing exogenous LAPTM4B. In these cells, starvation-induced autophagy was greatly promoted and overexpression of LAPTM4B results in higher cell viability during growth in low serum or glucose. This effect is disrupted by the autolysosome inhibitor chloroquine. These results are consistent with the notion that overexpression of LAPTM4B promotes increased autophagy flux in cancer cells exposed to metabolic stress. Finally, we found that tumor xenografts with overexpressed LAPTM4B grow much faster than control xenografts, and have a higher LC3-II/LC3-I ratio and decreased p62 levels, consistent with increased autophagic flux. This result suggests that LAPTM4B overexpression promotes actual tumor growth in vivo, perhaps by promoting greater tolerance to stress by increased autophagy.

LAPTM4B represents the first example of a gene crucial for autophagy maturation and flux that is amplified in cancer and associated with treatment resistance and poor clinical outcome in various cancers. Notably, the gene MTDH is a neighbor on 8q22 and is co-amplified with LAPTM4B. MTDH induces production of autophagosomes in different cell lineages through a noncanonical pathway independent of Beclin 1 and class III phosphatidylinositol 3-kinase by increasing expression of ATG5 and activating AMP kinase. Thus, the coordinate amplification of the two genes may activate and promote these two alternate pathways of autophagy and enhance tumor survival in metabolic and genotoxic stress. Amplified 8q22 may represent a novel "autophagy-promoting amplicon" in cancer. However, this amplicon may conversely offer a therapeutic window. Over-reliance on overexpression of 8q22 genes may represent an Achilles' heel for cancers that have amplified these genes. Furthermore, nonmalignant cells have less metabolic stress and may not rely on autophagy for survival. Targeting tumors that have 8q22 amplification and reliance on autophagy-mediated survival may preferentially kill marker-positive tumor cells with a high therapeutic index. Abnormal autophagosome accumulation caused by disrupting lysosome fusion with autophagosomes, rather than simple inhibition of initiation of autophagosome production, may induce tumor cell death. Such a strategy may synergize with other chemotherapeutic drugs, and overcome drug resistance. Drugs like chloroquine may represent only a lead compound and direct the way to development of new drugs that target autophagy.