Lysosomal Transmembrane Protein LAPTM4B Promotes Autophagy and Tolerance to Metabolic Stress in Cancer Cells

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Abstract

Amplification of chromosome 8q22, which includes the gene for lysosomal associated transmembrane protein LAPTM4B, has been linked to de novo anthracycline resistance in primary breast cancers with poor prognosis. LAPTM4B overexpression can induce cytosolic retention of anthracyclines and decrease drug-induced DNA damage. In this study, we tested the hypothesis that LAPTM4B may contribute to tumor cell growth or survival in the absence of a chemotherapeutic exposure. In mammary cells, LAPTM4B protein was localized in lysosomes where its depletion increased membrane permeability, pH, cathepsin release, and cellular apoptosis. Loss of LAPTM4B also inhibited later stages of autophagy by blocking maturation of the autophagosome, thereby rendering cells more sensitive to nutrient deprivation or hypoxia. Conversely, enforced overexpression of LAPTM4B promoted autophagic flux and cell survival during in vitro starvation and stimulated more rapid tumor growth in vivo. Together, our results indicate that LAPTM4B is required for lysosome homeostasis, acidification, and function, and that LAPTM4B renders tumor cells resistant to lysosome-mediated cell death triggered by environmental and genotoxic stresses. Cancer Res; 71(24); 7481–9. ©2011 AACR.

Introduction

Chemoresistance is often an acquired characteristic of recurrent tumors either induced or selected by exposure to a drug. Alternatively, de novo drug resistance can occur due to intrinsic features of the primary tumor which are selected by contributing a proliferative or survival advantage to tumor cells. The possible mechanisms of chemoresistance may include altered drug uptake, intracellular distribution, efflux, and turnover. Lysosomal retention of drugs is associated with drug resistance and lysosomal concentration of anthracyclines is thought to increase drug efflux and decrease drug nuclear localization, thereby preventing effective chemotherapy-induced DNA damage (1).

Lysosomes are organelles that contain hydrolytic enzymes such as proteases, nucleases, and lipases. Lysosomal membrane permeabilization (LMP) can pose a threat to cellular homeostasis through release of lysosomal contents and is a recognized trigger of cell death (2). For example, a selective and limited release of lysosomal cathepsins B or D may activate the mitochondrial cell death pathway by either generating reactive oxygen species and lipid mediators or triggering mitochondrial outer membrane permeabilization. Cathepsin B is a major mediator of apoptotic pathways activating the proapoptotic protein Bid by truncation. Truncated Bid (tBid) translocates to mitochondria to active the caspase cascade and promotes apoptosis. Cathepsin D is an aspartate protease that directly activates caspase (3, 4). The current knowledge of the proteins on lysosomal membranes critical for affecting or regulating these various lysosomal functions is limited.

Autophagy is a conserved lysosome-mediated intracellular trafficking pathway that degrades and recycles intracellular components (5). Autophagy is also a homeostatic mechanism that regulates metabolism and energy production and may be upregulated in response to a variety of cell stresses (5–7). As cancers develop and disseminate, the process of autophagy (autophagy flux) may be upregulated to support tumor cell survival and allow tumors to adapt to these stresses (8–10). In addition, autophagy has been shown to promote cell survival in response to chemotherapeutic agents (11–13). Conversely, too much autophagy may catabolize essential components resulting in autophagic cell death (14, 15). Autophagy is regulated by a signaling cascade involving mTOR pathway inhibition, the autophagy proteins (Atgs), and two ubiquitin-like conjugation systems (16–20). During autophagy initiation, a portion of the cytosol is surrounded by a cisternal membrane, the phagophore (21), that closes to form a double-membraned vesicle, the autophagosome (22). During autophagosome formation, cytosolic LC3 is cleaved by a protease and then conjugated to phosphatidylethanolamine to form autophagosomal membrane–associated LC3II (23); the level of LC3II correlates with the number of autophagosomes (24, 25). After their formation, autophagosomes fuse with endosomes to form amphisomes.
(26, 27) and then with lysosomes to form autolysosomes where sequestered material is degraded (5, 28–30). Blockade of autophagosome maturation and fusion with the lysosomal compartment results in accumulation of autophagosomes and interruption in the flux of material through the autophagic pathway (31). Autophagic flux can be monitored by measuring the level of substrates normally degraded by autophagy such as p62/SQSTM1 (25). The p62 protein is a ubiquitin-binding scaffold protein that binds directly to LC3 and is itself degraded in autolysosomes (32). Inhibition of autophagic degradation results in an increase in p62 levels and increased autophagy flux is indicated by decreased p62 levels (33). Many molecules have been implicated in these later stages of autophagosome maturation (30).

Lysosomal associated protein transmembrane-4 β (LAPTM4B) is a putative novel oncogene (34, 35) frequently amplified and overexpressed in primary treatment-naive breast cancers (36). Like other LAPTM family members, LAPTM4B protein has a lysosome localization motif (34) and colocalizes with markers of late endosomes and lysosomes (37). Increased expression of LAPTM4B has been shown in hepatocellular carcinoma and lung, ovarian, uterine, and gastric cancers (35, 38–40). Overexpression of the LAPTM4B-35 isoform in hepatocellular carcinoma cell lines promotes apoptosis resistance in vitro and growth and metastasis of hepatocellular carcinoma xenografts in mice (41). We reported overexpression of LAPTM4B in primary breast tumors is associated with resistance to chemotherapy, specifically anthracyclines, and may serve as a predictive biomarker for distant recurrence in patients treated with adjuvant chemotherapy (36). By sequestering drug in the cytoplasmic compartment and enhancing eflux of drugs from cancer cells, overexpression of LAPTM4B decreases nuclear localization of drug and drug-induced DNA damage and thereby reduces drug effectiveness (36, 42). However, the potential mechanisms by which LAPTM4B promotes tumor growth and survival in treatment-naive cancers is not well studied.

This study tests the effect of modulating LAPTM4B expression on cell survival under various stress conditions including nutrient deprivation, pH alteration, hypoxia, chemotherapy exposure, and in vivo tumor growth. Our results show that high expression of LAPTM4B inhibits lysosome-mediated death pathways, promotes autophagy, and leads to stress tolerance, thereby enhancing tumor cell growth and survival. LAPTM4B may be an important new therapeutic target for inhibiting cancer growth or sensitizing tumors to chemotherapy.

Materials and Methods

Cell lines, siRNA transfection, and gene transfer

Breast cell lines BT549 and MDA-MB-468 were obtained from American Type Culture Collection and were authenticated by Promega PowerPlex 1.2 short tandem repeat profiling. Tert-immortalized human mammary epithelial cells (HMEC) were from Clontech and provided by the Zhao laboratory. BT549 cancer cells have amplification and overexpression of LAPTM4B (43) and were used for siRNA knockdown experiments. MDA-MB-46B (MDA468) cancer cells have normal DNA copy number and expression of LAPTM4B (43) and were chosen for LAPTM4B transfection for overexpression and tumor xenograft experiments. Cell culture condition, siRNA transfection, expression construct carrying LAPTM4B cDNA, and stable transfer of this construct into MDA468 were conducted as described (36).

Analysis of LMP

Cells were transfected with control and LAPTM4B siRNA. After 48 hours, control and LAPTM4B expression–modified cells were incubated with fluorescein isothiocyanate (FITC)-labeled dextrans of different molecular weights from 4, 40, and 70 kDa (Sigma) for 2 or 12 hours, followed by wash out of excess dextran with culture medium, examination on a Zeiss HAL100 fluorescence microscope and photographed with a Zeiss camera.

Tumorigenicity assay

Two groups of mice were injected with LAPTM4B expression–manipulated MDA468 cells or the control counterpart cells into the inguinal mammary fat pads. Mice were routinely monitored for health and tumor size; mice were sacrificed when tumors reached 2 cm. Tumor growth rates were measured and compared between LAPTM4B-manipulated and control tumors. LAPTM4B expression level and the autophagosome marker LC3 were measured by immunoblot in tumor lysates prepared from xenograft tumors.

Statistical analysis

Difference in cell viability, tumor size, and ratios of LC3II/LC3I, or of p62/actin between control and testing groups was evaluated by the Student t test and P values derived from this test were used to determine the significance of the difference. Additional methods are described in Supplementary Material.

Results

LAPTM4B localizes to lysosomes and downregulation of LAPTM4B triggers LMP

LAPTM4B is a member of a family of proteins which contain a lysosome targeting motif at the C terminus (34). To determine lysosome localization in mammary epithelial cells, we examined HMECs stably expressing exogenous 6-His epitope–tagged LAPTM4B by immunofluorescence microscopy and showed expression of His-LAPTM4B in the lysosomal compartment defined by the lysosome-marker Lysotracker DND-99 (Supplementary Fig. S1A).

To understand how LAPTM4B might modulate lysosome function, we investigated the relationship between LAPTM4B expression and LMP. LMP was evaluated by measuring endocytic uptake and release of a nondigestible fluid-phase substrate, FITC-dextran. In BT549 breast cancer cells transfected with a scramble control siRNA, after 2 hours of exposure to FITC-dextran, the fluorescent dextran particles were detected in a punctate cytoplasmic pattern consistent with lysosomes and were retained in the lysosomal compartment 12 hours later (Fig. 1A; Supplementary Fig. S2A and S2B). In BT549 cells transduced by Promega PowerPlex 1.2 short tandem repeat profiling. Tert-immortalized human mammary epithelial cells (HMEC) were from Clontech and provided by the Zhao laboratory.
transfected with LAPTM4B-specific siRNA, the fluorescence pattern was similar to control cells at 2 hours; however, after 12 hours, the 4 and 40 kDa FITC-dextran molecules were in a diffuse distribution throughout the cell consistent with release from the lysosomal compartment (Fig. 1B; Supplementary Fig. S2C and S2D). The distribution of the larger molecular weight 70 kDa FITC-dextrans were mostly unchanged after 12 hours (Fig. 1B, bottom). These results suggest that LAPTM4B exerts lysosome-stabilizing properties to retain low and intermediate molecular weight macromolecules. Doxorubicin is small molecule with a molecular weight of approximately 580 Da. As shown in Fig. 1C, after 2 hours of drug exposure, doxorubicin autofluorescence is colocalized with Lysotracker in both the control-treated and LAPTM4B-siRNA–treated BT549 cells consistent with initial uptake of drug into lysosomes. Doxorubicin is retained in the lysosomal compartment 24 hours later in the control siRNA–treated or untreated BT549 cells (Fig. 1D, top; Supplementary Fig. S1B). However, in the BT549 cells treated with LAPTM4B-siRNA, after 12 to 24 hours, doxorubicin has been redistributed and predominantly colocalizes with nuclear 4',6-diamidino-2-phenylindole (DAPI) staining (Fig. 1D, bottom). This result suggests that knockdown of LAPTM4B does not completely abolish the uptake of doxorubicin by cytoplasmic organelles but significantly weakens the capability of lysosomes to retain the drug, resulting in its more rapid release and redistribution to the nucleus.

Doxorubicin is also a weak base that can be trapped in acidified lysosomes. To examine whether LAPTM4B expression has an effect on lysosomal pH, we used a pH sensing dye, LysoSensor Green DND-189, which is taken up into lysosomes and fluoresces with greater intensity in lower pH. As shown in Fig. 2A, BT549 cells treated with LAPTM4B-siRNA have a higher lysosomal pH (lower fluorescent intensity) than control cells. The degree of increase in lysosomal pH is similar to BT549 cells treated with 50 μmol/L chloroquine, a lysosomotropic agent that increases the lysosomal pH (Fig. 2B). However,
SKBR3 breast cancer cells with forced stable overexpression of LAPTM4B had similar lysosomal pH compared with control cells (Fig. 2C). To determine whether change in lysosomal pH alone could account for increased permeability of the lysosome, we treated BT549 cells with 50 μmol/L chloroquine to increase lysosomal pH and found no alteration in the uptake of dextran at 2 hours and dextran was not released into the cytosol at 12 hours (Supplementary Fig. S2E and S2F). These results suggest that LAPTM4B may not directly regulate lysosomal pH, but the increase of lysosomal pH in LAPTM4B knockdown cells may be a secondary effect of increased LMP resulting in deacidification of the lysosome.

**Knockdown of LAPTM4B by siRNA provokes lysosomal mediated programmed cell death through induction of cathepsin release**

The maintenance of lysosomal membrane integrity is important for cell survival. LMP leads to cathepsin release followed by caspase activation. This process initiates apoptosis which triggers further lysosomal destabilization to induce lysosomal mediated cell death in a positive feedback loop (2). To determine whether LAPTM4B knockdown led to cathepsin release from lysosomes, we conducted immunoblot analysis for cathepsins on cell cytosol fractions of BT549 tumor cells after transfection with control or LAPTM4B-specific siRNA. Depletion of LAPTM4B resulted in appearance of cathepsin B and cathepsin D in the cytosol of cells (Fig. 3A) and resulted in Bid truncation, caspase-3 activation, and PARP cleavage (Fig. 3B) consistent with initiation of apoptosis. Pan inhibitors of cathepsins (EST and pepstatin A) protected BT549 cells from LAPTM4B knockdown–induced Bid truncation and apoptosis. The pan-caspase inhibitor z-VAD-FMK was sufficient to abrogate caspase-3 activation but did not prevent Bid truncation. Treatment with 50 μmol/L chloroquine alone was not sufficient to induce cathepsin release and resulted in no added effect to LAPTM4B knockdown on cathepsin release or apoptosis induction (Supplementary Fig. S3, lanes 4 and 8). However, the release of cathepsin B and D and the activation of caspase-3 were more pronounced in cells also treated with the DNA-damaging drug, doxorubicin (Fig. 3A and B; Supplementary Fig. S3, lanes 2 and 6). There was no potentiation of cathepsin release or caspase-3 activation in cells also treated with the microtubule-stabilizing agent taxol (Supplementary Fig. S3, lanes 3 and 7), consistent with previous studies showing that taxol-induced apoptosis may be independent of caspase-3 and -9 activation (44). These results suggest that a certain level of LAPTM4B is required to prevent lysosome-mediated initiation of apoptosis. This requirement is more notable in the setting of additional cellular insult, such as exposure to certain chemotherapy agents.

**LAPTM4B overexpression promotes autophagy**

Autophagy plays a critical survival role by supporting energy requirements and sustaining viability under adverse conditions and may also promote resistance to chemotherapy-induced genotoxic stress. To determine whether modulation of LAPTM4B expression has an effect on autophagy, we examined markers of autophagosome maturation and flux during starvation-induced autophagy in BT549 breast cancer cells with or without depletion of LAPTM4B. In parental BT549 cells (with inherent overexpression of LAPTM4B), autophagy was induced by starvation as indicated by the appearance of punctate LC3 staining in the cytoplasm. Many of the LC3-puncta colocalized with the

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**Figure 3.** Suppression of LAPTM4B induces cathepsin-dependent cleavage of Bid, caspase-3, and PARP in breast cancer cells that overexpress LAPTM4B. A, BT549 cells transfected with a scrambled control siRNA (Ctrl) or LAPTM4B-specific siRNA (L4B siRNA) incubated in medium alone (−) or with 1 μmol/L doxorubicin (Doxo). Proteins (100 μg per lane) from cytosol preparations were fractionated on SDS-PAGE; blots were probed with anti-cathepsins B, D, and anti-actin. B, BT549 cells pretreated with cathepsin inhibitors, pepstatin A and EST (Cath inh), or pan-caspase inhibitor z-VAD-FMK (Casp inh) then transfected with control or LAPTM4B-specific siRNA and treated with medium alone (−) or 1 μmol/L doxorubicin (Doxo). Proteins (100 μg per lane) from cell lysates were fractionated on SDS-PAGE; blots were probed with anti-Bid, anti-caspase-3, anti-PARP, and anti-actin antibodies.
lysosome marker LAMP2 indicating normal autophagosome maturation and fusion to lysosomes (Fig. 4A). In contrast, in cells in which LAPTM4B has been depleted by siRNA, starvation resulted in marked cytoplasmic accumulation of enlarged LC3-positive autophagosomes, some of which were not colocalized with LAMP2-positive lysosomes (Fig. 4B). This suggests that depletion of LAPTM4B resulted in a block in autophagosome–lysosome fusion and blocked autolysosome formation.

We next analyzed autophagy in these cells by immunoblotting for LC3II and p62. The siRNA depletion of LAPTM4B had no effect on the level of LC3II in BT549 cells grown in nutrient-rich medium but resulted in significant increase in the level of LC3II when cells were stressed by serum starvation (Fig. 4C; Supplementary Fig. S4A). LC3II is an indicator of autophagosome number which can increase due to increased autophagosome formation or from a block in autophagosome maturation or both (31). To determine whether autophagy flux was increased or blocked, we measured levels of the autophagy substrate, p62. In control cells, serum starvation increased autophagy flux as indicated by lower p62 levels in the serum-starved cells than in cells grown in nutrient-rich medium. However, in cells depleted of LAPTM4B, there was no change in the level of p62 after serum starvation, indicating a block in starvation-induced autophagy flux (Fig. 4C; Supplementary Fig. S4B). The effect of knockdown of LAPTM4B on LC3II and p62 levels was similar to what was observed in serum-starved cells treated with chloroquine. In conjunction with the immunofluorescence findings, these results suggest that LAPTM4B is required for the later stages of autophagy maturation in
which autophagosomes are fused with lysosomes to form autolysosomes. Abortion accumulation of autophagosomes may act as a cell death messenger to trigger caspase-dependent or -independent cell death. We found an increase in cell death as indicated by caspase-3 activation and cleaved PARP in LAPTM4B-depleted cells with starvation-induced aggregation of autophagosomes (Fig. 4C). Although downregulation of LAPTM4B alone may trigger LMP and to a lesser extent caspase activation, the combination of LMP and blocked autophagy results in more pronounced caspase activation and cell death.

In a complementary experiment, stable overexpression of LAPTM4B in MDA468 cells with inherently low levels of LAPTM4B, resulted in greater starvation-induced autophagy as indicated by higher levels of LC3II and lower levels of p62 (Fig. 4D; Supplementary Fig. S4C and S4D). This effect was blocked 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.

**Overexpression of LAPTM4B promotes tumor cell survival and resistance to apoptosis induced by low serum concentration or glucose deprivation**

The previous experiments suggest that overexpression of LAPTM4B may result in decreased sensitivity of tumor cells to insults that trigger LMP, lysosome-mediated programmed cell death, or induce autophagy. We examined the effect of modulating LAPTM4B expression levels on cell survival in cells exposed to various environmental stressors. In BT549 cells with amplification and overexpression of LAPTM4B, siRNA depletion of LAPTM4B resulted in approximately 50% decrease ($P = 0.0267$) in viable cells when cultured in nutrient-rich medium. Depletion of LAPTM4B resulted in a similar or more pronounced decrease in viable cells when grown in low serum or low glucose medium ($P = 0.001$ and 0.0260, respectively; Fig. 5A). MDA468 breast cancer cells have inherently low normal levels of LAPTM4B expression. MDA468 cells with forced overexpression of LAPTM4B had similar cell viability to parental cells when cultured in nutrient-rich media ($P = 0.105$). These results suggest that a certain level of LAPTM4B may be required for tumor cell survival, but its overexpression does not directly promote *in vitro* cell growth under normal culture conditions. However, the cells with overexpression of LAPTM4B showed higher cell viability than control cells when cultured in low serum or low glucose medium ($P = 0.0256$ and 0.0960, respectively; Fig. 5B). As shown in Fig. 5C, the decreased cell viability of BT549 cells after knockdown of LAPTM4B was associated with increased activation of caspase-3 and PARP cleavage. Similarly, the enhanced survival of MDA468 cells overexpressing LAPTM4B in nutrient-deprived conditions was associated with much lower levels of active caspase-3 and PARP cleavage. These results support our hypothesis that high expression of LAPTM4B potentiates the growth and survival of breast cancer cells under metabolic stress.

Next, we tested whether modulation of LAPTM4B affects survival of cells exposed to other types of stress. As shown in Supplementary Fig. S5A and S5B, expression levels of LAPTM4B had no effect on cell viability when cells were cultured in acidified medium. Exposure to hypoxic stress showed variable results. Forced overexpression of LAPTM4B in MDA468 cells had no significant effect on cell viability of cells grown in low oxygen (Supplementary Fig. S5C). However, BT549 cells with LAPTM4B depletion were more sensitive to hypoxia and had reduced viability compared with parental BT549 cells that overexpress LAPTM4B (Supplementary Fig. S3D).

We further examined the effects of LAPTM4B modulation on tumor growth and survival in an *in vivo* xenograft model.
MDA468 cells with stable expression of either a control vector or a vector driving LAPTM4B overexpression were inoculated into the cleared mammary fat pad of nude mice. After 2 months, we found that the tumor xenografts that overexpressed LAPTM4B grew much faster than control xenografts (t test, P = 0.033; Fig. 6A). Examination of the xenograft tumor explants showed that each continued to overexpress LAPTM4B compared with control xenograft tumors (Fig. 6B); the presence of the smaller proteolytic fragments indicates proper delivery of LAPTM4B to the lysosomes (37). We examined tumor lysates for markers of autophagy by immunoblot (Fig. 6C). LAPTM4B-overexpressing xenografts showed higher LC3II/LC3I ratio (P = 0.0040; Fig. 6D), decreased p62 levels (P = 0.0036; Fig. 6E), and higher LC3II/LC3I/p62 ratio (P = 0.0074; Fig. 6F) consistent with increased autophagic flux. This result suggests that LAPTM4B overexpression promotes tumor growth in vivo, perhaps by promoting greater tolerance to stress through increased induction of autophagy.

Discussion

The cancer gene LAPTM4B is one of two genes amplified and overexpressed from chromosome 8q22 which predicts for de novo anthracycline resistance and metastatic recurrence in patients with breast cancer (36). Our previous work suggested one mechanism by which LAPTM4B may promote chemotherapy resistance is by retention of anthracyclines in a cytoplasmic compartment thereby preventing nuclear drug localization and drug-induced DNA damage. The frequent occurrence of 8q amplification and overexpression of LAPTM4B in treatment-naive cancers raised the possibility that LAPTM4B may provide a growth or survival advantage to tumor cells in the absence of a therapy challenge, resulting in its selection and upregulation in primary (untreated) cancers. In this study, we provide evidence that LAPTM4B is localized in lysosomes of mammary cells and promotes lysosome membrane stability. Decreased expression of LAPTM4B leads to increases in LMP, lysosomal pH, lysosomal release of cathepsins, and lysosome-mediated cell death. This effect is more pronounced when cells are exposed to anthracycline chemotherapy but not when exposed to a taxane, consistent with the increased sensitivity to anthracyclines in tumors with low LAPTM4B expression (36). We find that LAPTM4B plays a critical role in autophagy and insufficient levels of LAPTM4B results in a failure of autophagosome–lysosome fusion causing a block in autolysosome formation and decreased autophagy flux. In contrast, overexpression of LAPTM4B in breast cancer cells that have normal DNA copy number of 8q22 and express normal levels of LAPTM4B results in increased autophagy flux. These observations suggest that the proper function of LAPTM4B is required for lysosome-mediated autophagy maturation. A recent study showed that transient overexpression of LAPTM4B without coordinate increase of the expression of its partner MCOLN1 in retinal epithelial cells led to increased LC3II levels because of an accumulation of autophagosomes (37). This finding could result from increased autophagy induction or a block in autophagy flux but is consistent with the notion that appropriate levels of LAPTM4B are crucial to proper lysosome-mediated autophagy.

Fusion of lysosome with autophagosome is a critical step for autophagy maturation. Rab7, SKD1, Lamp2, UVRAG, and Reb1con have been shown to be essential for this process.
(5, 29, 30, 37, 45, 46). Frequent frameshift mutations have been identified in the ULVRA6 gene in colon and gastric cancers (47); however, no significant aberrations in autophagy were found in cancer cells carrying this mutation (48). To the best of our knowledge, LAPTMB4 represents the first gene crucial for autophagy maturation and flux that is amplified in cancer (36, 49) and is associated with treatment resistance and poor clinical outcome in various cancers (34, 36, 39–41, 49). Metadherin was recently shown to induce autophagy in different cell lineages by increasing expression of atg5 and activating AMP kinase (50). Interestingly, the gene for metadherin, MTDH, is a neighbor to LAPTMB4 and is coamplified with LAPTMB4 in cancers. We propose that the coordinate amplification of the 2 genes may activate simultaneously the 2 major stages of the autophagy pathway and thereby enhance tumor survival.

Tumors frequently experience elevated metabolic stress from nutrient and oxygen deprivation due to insufficient angiogenesis (8). The high metabolic demands of cell proliferation and altered metabolism provide additional tumor cell intrinsic stress. Thus, tumors may be more dependent on survival mechanisms such as autophagy to maintain growth or to disseminate (10, 51). We show that the roles of LAPTMB4 in limiting lysosome-mediated cell death and promoting autophagy have significant survival effects in cancer cells, including greater resistance to nutrient deprivation, hypoxia, and chemotherapy-induced genotoxic stress. We further show that LAPTMB4 prosurvival effect is associated with greater in vivo tumor growth. The new knowledge from this study contributes to a better understanding of the molecular mechanisms of lysosome-mediated drug resistance and the particular dependency of tumors on lysosomal function for cell survival. As normal tissues are seldom under nutrient deficiency, there may be a significant therapeutic window for inhibiting LAPTMB4 in tumors that overexpress this gene and are dependent on it for survival. Over-reliance of cancer cells on LAPTMB4 for tolerance to stress may represent the "Achilles heel" for malignancies that have amplified this gene and provide a new therapeutic strategy for targeting these cancers.

Disclosure of Potential Conflicts of Interest

A.L. Richardson and Z.C. Wang have filed a patent on the detection of chr 8q22 genes including LAPTMB4 to predict anthracycline resistance in cancer (Dana-Farber Cancer Institute and Brigham and Women's Hospital). The other authors disclosed no potential conflicts of interest.

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