

REVIEW

LAPTM4B: an oncogene in various solid tumors and its functions

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The oncogene Lysosome-associated protein transmembrane-4 β (*LAPTM4B*) gene was identified, and the polymorphism region in the 5'-UTR of this gene was certified to be associated with tumor susceptibility. LAPTM4B-35 protein was found to be highly expressed in various solid tumors and could be a poor prognosis marker. The functions of LAPTM4B in solid tumors were also explored. It is suggested that LAPTM4B could promote the proliferation of tumor cells, boost invasion and metastasis, resist apoptosis, initiate autophagy and assist drug resistance.

Oncogene (2016) 35, 6359–6365; doi:10.1038/onc.2016.189; published online 23 May 2016

INTRODUCTION

Carcinogenesis is a complicated process that involves multiple stages with gene mutations accumulated, which results in deregulation of proliferation, invasion and metastasis, recurrence and drug resistance, leading to a poor prognosis. Despite the fact that more and more oncogenes have been found and various therapies targeting these oncogenes also have been developed in recent years, the cure rate of cancers is not satisfactory. Therefore, a lot of work about oncogenes and their relationship with carcinogenesis still needs to be done and is challenging.

In this review, we introduced the oncogene Lysosome-associated protein transmembrane-4 β (*LAPTM4B*) gene, which was first cloned in hepatocellular carcinoma (HCC) cells. A series of research findings, such as structures of *LAPTM4B* gene and protein, transcription regulations of *LAPTM4B* gene, relationships between polymorphisms of LAPTM4B and tumor susceptibility and the functions of LAPTM4B in solid tumors, were summarized, and they are shown in this article.

CLONING AND IDENTIFICATION OF THE *LAPTM4B* GENE

A gene involved in the proliferation and/or differentiation of hepatocytes was originally cloned using rapid amplification of complementary DNA ends and reverse transcription-PCR in HCC tissues by Zhou Rouli in 2000.¹ It was highly expressed in HCC, as well as in paired noncancerous liver and fetal liver cells, but showed very low expression in normal adult liver cells.¹ The gene was designed by HUGO Gene Nomenclature Committee, and it was named as *LAPTM4B* (GenBank ACCESSION NM: AY057051, NM_018407, Gene ID 55353).

BLAST program analysis shows that the *LAPTM4B* gene is mapped to chromosome 8q22.1, spanning at least 50 kb. It is composed of seven exons separated by six introns and contains an open reading frame including 951 nucleotides. Extron 1 contains the 5' untranslated sequence (5'-UTR) and the initiating methionine (Met). The mRNA of LAPTM4B is ~2.2 kb in length and

is in agreement with the size of the mRNA observed in Northern blots. There are two polyadenylation signal sites in the 3'-UTR, AATAAA and AATTAAA. The alternative polyadenylation (AATAAA) may result in another 1.42-kb mRNA variant.²

STRUCTURE OF THE LAPTM4B PROTEIN

The full-length complementary DNA of LAPTM4B contains two translational initiation codons (ATG) with an interval of 273 bp, and encode two protein isoforms, LAPTM4B-35 and LAPTM4B-24, with molecular weights of 35 kDa and 24 kDa, respectively. LAPTM4B-35 contains 317 amino acid residues and has a pI at 9.07 because of its high content of arginine residues. LAPTM4B-24 comprised 226 amino acid residues and has a pI at 4.65 because of its high content of acidic amino acid residues.

Computer analysis shows that LAPTM4B is an integral membrane protein, with four transmembrane regions at 117–133, 163–179, 200–216 and 243–259 amino acids, respectively (Figure 1). It also has two extracellular domains (EC1 and EC2): one N-terminal and one C-terminal tail in the cytoplasm. The full amino acid sequence contains one N-glycosylation site, eight phosphorylation sites, six putative sites in the cytoplasm and four N-myristoylation sites. Structurally, LAPTM4B-35 differs from LAPTM4B-24 in that it contains extra 91 amino acid residues at the N terminus that harbors a proline-rich domain, PPRP. It serves as the binding site of the SH3 domain of some signaling molecules and has critical roles in the proliferation and metastatic potentials of tumor cells.³ Moreover, LAPTM4B contains several putative lysosomal targeting motifs in the C termini, including tyrosine-based (YXX ϕ), PY (L/PPXY) and dileucine ([DE]XXXL[L/I]) motifs. Except for PY motifs, these motifs are recognized by major adapter proteins, which are involved in transporting from the Golgi to lysosome.⁴ Its C-terminal PY motifs can interact with the E3 ubiquitin ligase neuronal precursor-cell expressed developmentally down-regulated 4(Nedd4), participating in the lysosomal and plasma membrane sorting.⁵

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Received 23 March 2016; revised 18 April 2016; accepted 18 April 2016; published online 23 May 2016

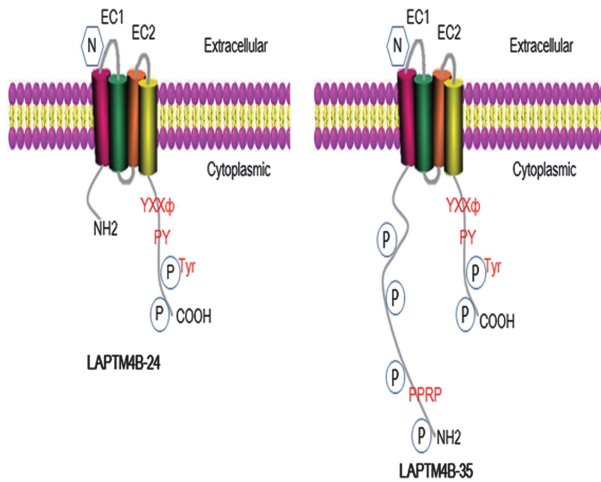


Figure 1. Topology of LAPTMB-24 (left) and LAPTMB-35 (right) proteins. N encircled by a hexagon represents N-glycosylation site; P encircled by a circle represents phosphorylation site.

EXPRESSION ANALYSIS OF LAPTMB

Expression of LAPTMB mRNA has been evaluated via Northern blotting, reverse transcription-PCR and hybridization *in situ*. LAPTMB mRNA is expressed in a variety of human normal tissues: its expression is high in the testis, heart, skeletal muscle and uterus; moderate in ovary, kidney and pancreas; low in liver, spleen and thymus; and lowest in lung and peripheral leukocytes.⁶ It is also expressed highly in fetal kidney, heart and spleen (Figure 2).

Immunohistochemistry with an antibody specifically proved that LAPTMB-35, but not LAPTMB-24, is upregulated in a wide range of cancers including hepatocellular cancer, breast cancer, gastric cancer, lung cancer, colon cancer, gallbladder cancer, extrahepatic cholangiocarcinoma and ovarian carcinoma. In addition, the ratio of LAPTMB-35 to LAPTMB-24 may be related to the development of various cancers.³ Note that there was a significantly positive correlation between LAPTMB-35 expression levels and the probability of metastasis to lymph nodes or distant organs, whereas LAPTMB-35 expression levels were inversely associated with the differentiation status of cancer tissues and the overall and disease-free postresectional survival of patients with gallbladder cancer,⁷ extrahepatic cholangiocarcinoma,⁸ ovarian cancer,⁹ HCC,¹⁰ gastric cancer¹¹ and cervical cancer.¹²

TRANSCRIPTIONAL REGULATION OF LAPTMB

Moreover, overexpression of LAPTMB in solid cancers may result from transcriptional regulation by transcription factors or micro-RNAs. In recent years, an increasing number of researches have focused on the upstream regulation of LAPTMB. SP1 binds 558bp upstream of LAPTMB transcription initiation site, and it is correlated with high expression of LAPTMB in hepatocellular carcinoma.¹³ In addition, cyclic AMP responsive element-binding protein-1 (CREB1) is a transcription factor that has a vital role in cell proliferation, differentiation and survival. It was also regarded as an oncogene that promotes tumor cell growth and proliferation. A previous study showed that CREB1 binds to the +157–+165 fragment of LAPTMB promoter region and then upregulates the transcription of LAPTMB in breast cancer.¹⁴ MiR-188-5p also suppresses the expression of LAPTMB through binding to its 3'-UTR area, which results in the inhibition of cell proliferation, invasion and migration in prostate cancer.¹⁵ Moreover, a study revealed that enforced expression of the homeobox transcription factor, which has an important role in hematopoietic stem cell

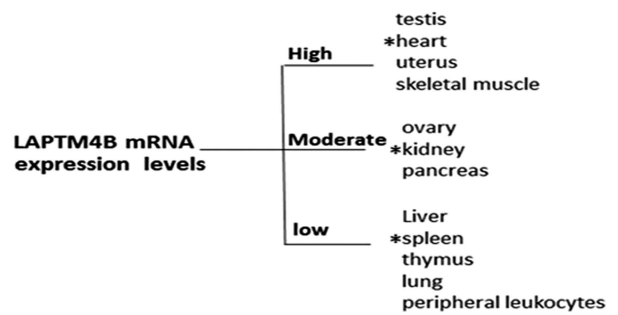


Figure 2. The expression levels of LAPTMB mRNA in human normal tissues. The asterisk represents the organ in which LAPTMB is highly expressed in fetal tissues.

self-renewal and expansion, could upregulate LAPTMB expression in hematopoietic stem cells rather than in mature hematopoietic cells.¹⁶

LAPTMB POLYMORPHISM AND ITS IMPORTANCE IN SUSCEPTIBILITY TO CARCINOMAS

There are two alleles of LAPTMB named LAPTMB*1 and LAPTMB*2 (GenBank numbers AY219176 and AY219177, respectively), encoding 35- and 40- kDa proteins, respectively. Allele *1 contains only one copy of a 19-bp sequence at the 5'-UTR of the first exon, whereas this segment of allele *2 is duplicated and tandemly repeated¹⁷ (Figure 3). Compared with allele*1, the extra 19-bp sequence changes the open reading frame of LAPTMB gene and makes allele *2 encode one more protein isoform, a 40-kD protein. The mRNA of allele *1 starts translation only at nucleotide 157, because there are in-frame termination codons at nucleotides 40 and 103. However, the mRNA of allele *2 starts translation at nucleotide 17, which produces a protein with an extra 53 amino acids at its N terminus than allele *1 (Figure 4). The function of the 40-kDa protein encoded by allele *2 and its correlation with disease has not been elaborated so far. A more in-depth research will be required to clarify these points.

Previous studies have shown that LAPTMB polymorphisms were related to susceptibility to HCC,^{18,19} breast cancer,^{20–22} non-small lung cancer,²³ gastric cancer,²⁴ cervical cancer,²⁵ endometrial carcinoma,²⁶ colorectal cancer,²⁷ lymphoma,²⁸ gallbladder carcinoma,²⁹ ovarian carcinoma³⁰ and malignant melanoma,³¹ but not to squamous cell carcinomas such as esophageal carcinoma, rectum carcinoma²⁷ and nasopharyngeal carcinoma.³² Lately, a meta-analysis in Chinese Han population revealed that LAPTMB allele *2 carriers exhibited a higher cancer risk compared with allele *1 homozygotes (for *1/2, odds ratio = 1.55, 95% confidence interval 1.367–1.758; for *2/2, odds ratio = 2.093, 95% confidence interval 1.666–2.629; for *1/2 + *2/2, odds ratio = 1.806, 95% confidence interval 1.527–2.137). Moreover, LAPTMB allele *2 has been proven to be a risk factor for cancer (odds ratio = 1.487, 95% confidence interval 1.339–1.651).³³

FUNCTIONS OF LAPTMB AND THE MECHANISMS THEREOF

LAPTMB gene promotes the growth and proliferation of cells in various kinds of tumors

Uncontrolled cell growth is the main biological property of tumors. The proteins of LAPTMB gene, LAPTMB-35 and LAPTMB-24, have an important role in promoting growth and proliferation of cells in many kinds of tumors. In HCC, the effect of LAPTMB-35 on xenograft tumor growth of HepG2 cells was examined in BALB/c nude mice. The tumor growth of mice with stable overexpression of LAPTMB-35 HepG2 cells was significantly more rapid than that in the Mock group, whereas the tumor

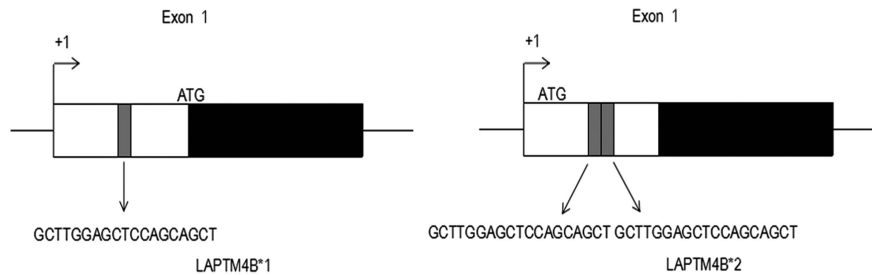


Figure 3. Schematic representation of the LPTM4B promoter and exon 1. Exon 1 is depicted as a box; the gray box indicates the 19-bp sequence. ATG loci are shown, respectively. The nucleotide sequence is numbered with transcriptional start site as +1. Allele *1 contains only one copy of the 19-bp sequence; meanwhile, allele *2 contains two copies of the 19-bp sequence in a tandem array.

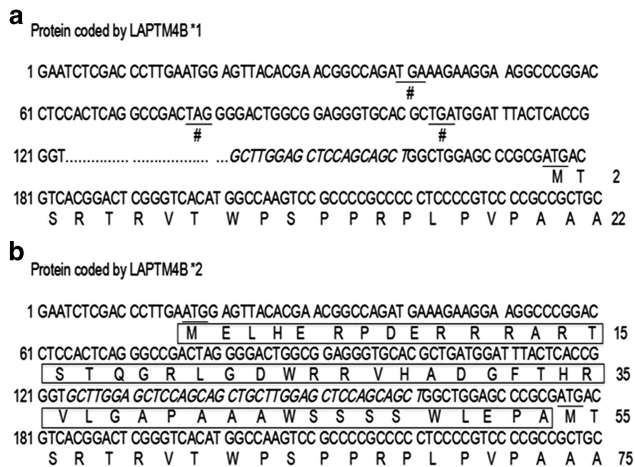


Figure 4. Comparison of the putative proteins encoded by LPTM4B*1 and LPTM4B*2. This schematic diagram shows the partial segments of the first exon in LPTM4B*1 (a) and LPTM4B*2 (b). The sequence numbers of the first nucleotide (left) and the final amino acid (right) in each row are shown, respectively. The nucleotide sequences are numbered with the putative transcription start site marked as +1. In-frame termination codons are underlined and marked by the symbol #, and the 19-bp sequences in both of the alleles are represented in italics. The mRNA of allele *1 can start translation only at nucleotide 157, because of the in-frame termination codons at nucleotides 40 and 103. However, allele *2 starts translation at nucleotide 17, producing an extra 53 amino acids, which are boxed at the N terminus of LPTM4B.

growth of mice with stable knockdown of LPTM4B-35 HepG2 cells was slower than that in the Mock group. In addition, the 3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay also showed that the growth rate of stable knockdown LPTM4B-35 HepG2 cells was reversed after transfecting the LPTM4B-35 protein expression plasmid.³⁴ In gallbladder carcinoma, MTT assay and flow cytometry experiment also demonstrated that overexpression of LPTM4B-35 can boost the proliferation of gallbladder carcinoma cells.⁷ Some other researchers used RNA interference to knock down LPTM4B-35 protein in Hela cells and showed that Hela cells with depletion of LPTM4B-35 had a low growth rate in the CCK8 assay.³⁵ The function of LPTM4B-35 was also certified in non-small cell lung cancer, where stable knockdown of LPTM4B in Calu-6 lung cancer cells statistically reduced anchorage-dependent and anchorage-independent colonies.³⁶ The mechanisms of LPTM4B-35 involved in promoting the growth and proliferation of carcinoma cells may attribute to affect proliferation-regulating proteins and activate the AKT signaling pathway. By activating

AKT, glycogen synthase kinase 3β can be phosphorylated, and it results in the attenuation of phosphorylation and degradation of c-Myc. Besides, another downstream target of activated AKT, forkhead box O4, the transcription factor of p27, can also be phosphorylated, and it results in the inactivation of forkhead box O4.³⁴ Recently, researchers found that LPTM4B-24 may also have a relationship with tumor growth. In Hela cells, LPTM4B-24 knockdown attenuated cell proliferation and reduced the cell size, which were restored by re-expression of LPTM4B-24.³⁷

LPTM4B gene promotes metastasis and invasion of tumor cells
LPTM4B gene can promote metastasis and invasion of tumor cells, which is line with a series of results. In HepG2 cells that overexpress LPTM4B-35, the Boyden chamber assay demonstrated that cell migration and invasion were promoted compared with mock cells.³⁴ In addition, LPTM4B knockdown may also suppress HeLa cell migration *in vitro*. The ability to invade Matrigel was assessed using a Transwell assay. In parallel, knockdown of LPTM4B decreased invasion and migration of related proteins, such as matrix metalloprotein 2 (MMP-2), matrix metalloprotein 9 (MMP-9), CDK12 and HIF-1α.³⁵ The promotion of metastasis and invasion by LPTM4B-35 may be contributed by the PPRP motif of LPTM4B-35, which can interact with SH3 domain-containing proteins that are involved in many signaling pathways.³ Besides, a series of clinical experiments showed that carcinomas derived from stomach, breast, colon, ovary, liver, pancreas, cervix, prostate, lung, endometrium and gallbladder with LPTM4B-35 overexpression may present more invasive characteristics.^{10,36,38–49}

LPTM4B gene inhibits apoptosis
Resistance to apoptosis is one of the classical characteristics of cancer cells. As it is an oncogene, overexpression of the LPTM4B gene has been shown to inhibit apoptosis function. After treating HCC cells with Adriamycin to induce apoptosis, the apoptosis rate was reduced in stable LPTM4B-overexpressing HepG2 cells but it was increased in stable LPTM4B knockdown HepG2 cells as compared with the mock group. In addition, restoration of LPTM4B-35 by transfection of LPTM4B-35 expression plasmid into stable LPTM4B knockdown HepG2 cells reversed the apoptosis rate. Besides, a series of apoptosis-related proteins such as cleaved caspase-3 and PARP were decreased in stable LPTM4B overexpression HepG2 cells but increased in stable LPTM4B knockdown HepG2 cells.³⁴ In gallbladder carcinoma cells, overexpression of LPTM4B can also attenuate epirubicin-induced apoptosis, which was certified by flow cytometry experiment and apoptosis-related protein analysis.⁵⁰ In breast cancer, the doxorubicin-induced apoptosis rate of MDA-MB-231 and BT549 breast tumor cells increased after small interfering RNA targeting LPTM4B was transfected into them.⁵¹ On the other hand, some researchers thought that low LPTM4B expression could also inhibit the classical apoptosis pathway. A431 cells stably

overexpressing LAPT4M4B were sensitized to caspase-3 activation after treatment with anthracyclines or paclitaxel. Conversely, A431 cells depleted of LAPT4M4B were protected from anthracycline- and paclitaxel-induced PARP cleavage. The same phenomenon was also seen in KPL-4 breast cancer cells. In this regard, researchers thought that LAPT4M4B might affect sensitivity to chemotherapeutic compounds and cell death mechanisms differently depending on its expression level and ceramide compartmentalization. Cells with high levels of LAPT4M4B expression displayed increased clearance of ceramide from late endosome, sensitizing cells to ceramide-induced apoptosis. In parallel, late endosome membranes were stabilized and cells were not sensitized to lysosome-mediated death. On the other hand, cells with low LAPT4M4B expression made ceramide sequestered in late endosome, protecting cells from ceramide toxicity in the late endosome but sensitizing cells to lysosome-mediated death.⁵²

LAPT4M4B gene and autophagy initiation

Autophagy is a salvage pathway that damages organelles to lysosomes for recycling energy and nutrients. A series of studies have demonstrated that autophagy has a dual function in tumorigenesis.⁵³ On one hand, increased autophagy can make cancer cells tolerant to metabolic stress to promote tumorigenesis,⁵⁴ and on the other hand defective autophagy can also drive tumorigenesis by the accumulation of genotoxic cellular waste, which boosts the acquisition of gene mutations resulting in the transformation of precancerous cells.⁵⁵ Owing to the functions of lysosomes in the completion of autophagy, the effect of LAPT4M4B was also examined on the process of autophagy. Under the condition of starvation stress, breast cancer cells with LAPT4M4B knockdown fail to undergo autophagosome-lysosome fusion and autolysosome formation. In addition, depletion of LAPT4M4B in breast cancer cells leads to increased autophagosome but decreased autophagy flux, which suggests that LAPT4M4B has a key role in later stages of autophagy maturation.⁵⁶ For a further step, these findings were also explored in multiple kinds of cancer patients. A survey including 211 human autophagy-associated genes was conducted for tumor-related alterations to DNA sequence and RNA expression levels and their association with patient survival outcomes in multiple cancer types. In the survey, researchers found that the *LAPT4M4B* gene was a positive modulator in autophagy progress but was differentially expressed in various kinds of cancer. Compared with normal tissue, LAPT4M4B was highly expressed in lung adenocarcinoma and lung squamous cell carcinoma, but the expression was low in kidney renal clear cell carcinoma. Besides, mRNA of LAPT4M4B was found to be increased or decreased in patients with disease-specific molecular alterations or clinical phenotypes, compared with patients not harboring those alteration or phenotypes in three cancers (Table 1).⁵⁷ Recently, researchers found that LAPT4M4B had functions in autophagy progress related with inactive epidermal growth factor receptor (EGFR) or active EGFR. The inactive EGFR was shown to initiate autophagy independent of its kinase activity, and serum starvation led to the accumulation of unphosphorylated EGFR at LAPT4M4B-positive endosomes. Further, a series of assays showed that inactive EGFR that localized at endosomes interacted with LAPT4M4B and stabilized each other. Regardless of whether EGFR or LAPT4M4B was knocked down, the other one would be decreased accordingly, which finally led to the inhibition of autophagy. Investigating the mechanism of this, researchers suggested that inactive EGFR and LAPT4M4B recruited exocyst subcomplex containing Sec5 to promote EGFR association with autophagy inhibitor Rubicon, which in turn dissociated Beclin1 to start autophagy.⁵⁸ On the other side, LAPT4M4B could promote active EGFR signaling by blocking EGF-stimulated EGFR intraluminal sorting and lysosomal degradation to inhibit

Table 1. Autophagy-associated genes found to have significantly increased or decreased mRNA in patients with disease-specific molecular alterations or clinical phenotypes compared with patients not harboring those alterations or phenotypes in three cancer types

Gene	BRCA	HNSC	LUAD
	AKT1 mut↓ Basal-like↑ BRCA1 mut↑BRCA2 mut↑		CDKN2A homdel ↓
LAPT4M4B	CDH1 mut↓ GF1R amp↑ KMT2C mut↓ Luminal A↓ MAP2K4 mut↓MAP3K1 mut↓	HRAS mut↓	Lymphnode spread N0↑
	MYC amp↑ NBN amp↑ TP53 mut↑		

Abbreviations: Amp, amplification; BRCA, invasive breast carcinoma; HNSC, head and neck squamous cell carcinoma; homdel, homozygous deletion; LUAD, lung adenocarcinoma; mut, mutant; up arrows, significantly increased AA mRNA; down arrows, significantly decreased mRNA.

autophagy. By enhancing the ubiquitination of Hrs by the E3 ubiquitin ligase Nedd4, LAPT4M4B could inhibit the function of Hrs so that EGF-stimulated EGFR signaling could be prolonged.⁵⁹ Although LAPT4M4B may have different roles in the EGFR-related autophagy progression, LAPT4M4B facilitates the prosurvival functions of EGFR in cancer cells in both conditions.

LAPT4M4B motivates multidrug resistance

Chemotherapy resistance is always a main obstacle in the progression of treatment. Some genes located at the 8q22 chromosome have been shown to be associated with chemotherapy resistance in breast cancer cells.⁶⁰ The chemotherapy-resistant function of *LAPT4M4B* has attracted the attention of researchers, as this gene is located in the same region. By using small interfering RNAs against LAPT4M4B in BT549 cells, researchers found that cell lines without LAPT4M4B increased the sensitivity to anthracyclines, doxorubicin and daunorubicin. Following the autofluorescence of doxorubicin, researchers showed that breast cancer cell lines with *LAPT4M4B* gene knockdown resulted in a significant increase in nuclear localization of doxorubicin.⁵⁶ Besides, LAPT4M4B overexpression can also increase the efflux of chemodrugs such as paclitaxel and cisplatin, and LAPT4M4B knockdown increases the intention of these drugs in HeLa cells. However, the LAPT4M4B protein does not contain ATP-binding cassette, which means that the LAPT4M4B protein cannot function as an efflux pump itself to be responsible for multidrug resistance. The other ATP-dependent membrane efflux transporter, P-glycoprotein, was demonstrated to be the assistant of LAPT4M4B-35 protein in the progression of chemotherapy resistance. Besides drug efflux, other molecular mechanisms of LAPT4M4B for multidrug resistance were explored. It is suggested that overexpression of LAPT4M4B-35 also motivates chemotherapy resistance by the activation of the PI3K/AKT signaling pathway through interaction of PPRP motif contained in the N terminus of LAPT4M4B-35 with the p85α regulatory subunit of PI3K.⁶¹ Recently, a study showed that LAPT4M4B can promote AKT signaling by blocking EGFR degradation specifically, and this would be one mechanism for the role of LAPT4M4B in chemotherapy resistance. LAPT4M4B interacts with E3 ubiquitin ligase Nedd4 to promote ubiquitination of Hrs (an ESCRT-0 subunit), which inhibits the Hrs association with ubiquitinated EGFR and therefore inhibits EGFR intraluminal sorting and lysosomal degradation. At the same time, a PIP kinase, PIPKIγ5, directly binds to LAPT4M4B and antagonizes the function of LAPT4M4B in EGFR sorting by generating PtdIns(4,5)P₂ signals and recruiting SNX5 (Figure 5).⁵⁹

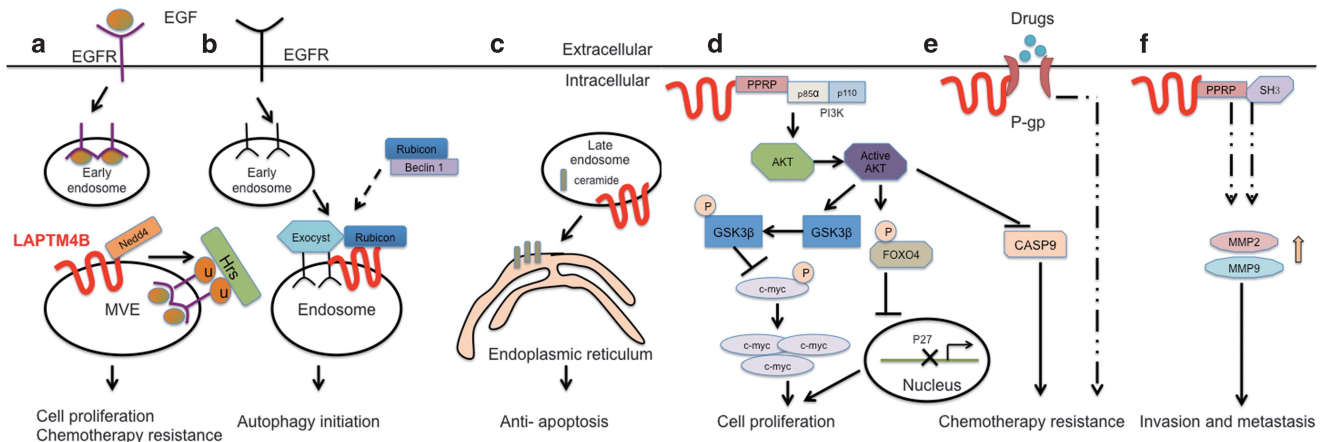


Figure 5. Summary of the mechanisms for LAPT M4B promoting cancer development. (a) LAPT M4B inhibits the function of Hrs so that EGF-stimulated EGFR signaling can be prolonged and finally promotes cell proliferation and chemotherapy resistance, by enhancing the ubiquitination of Hrs by the E3 ubiquitin ligase Nedd4. (b) Inactive EGFR and LAPT M4B recruited exocyst subcomplex containing Sec5 to promote the association of EGFR with autophagy inhibitor Rubicon, which in turn dissociated Beclin1 to start autophagy. (c) Cells with high levels of LAPT M4B expression displayed increased clearance of ceramide from late endosome, which makes late endosome membranes stable and cells insensitive to lysosome-mediated death. (d) LAPT M4B activates the PI3K/AKT signaling pathway by its PPRP motif. The active AKT makes the glycogen synthase kinase-3 β phosphorylated, which attenuates the phosphorylation of c-Myc and results in the accumulation of c-Myc. The active AKT also phosphorylates FOXO4, resulting in transcription failure of p27 gene. (e) LAPT M4B motivates multidrug resistance through efflux by interaction with P-glycoprotein and activating PI3K/AKT signaling. However, LAPT M4B-overexpressing cells do not have a high expression of P-glycoprotein, and hence further research is needed regarding this mechanism. (f) Overexpression of LAPT M4B leads to overexpression of MMP-2 or MMP-9, and the PPRP motif of LAPT M4B can interact with SH3 domain-containing signaling proteins related with invasion and metastasis; however, the specific mechanism should be explored in a further step.

LAPT M4B may be a novel therapeutic target for cancer treatment. Although many kinds of molecular targeting drugs have been developed, some targeting therapies may be ineffective and require additional targets such as LAPT M4B for cancer treatment. Although EGFR TKIs block the cellular functions mediated by EGFR kinase signaling in non-small cell lung cancers, they also activate a role for inactive EGFR in autophagy at the mean while, which could potentially provide a survival advantage and TKI resistance in cancers. Thus, cotargeting EGFR and other molecular may be a promising strategy to overcome TKI resistance in cancers. LAPT M4B as an oncogene, which promotes active EGFR signaling in cancer cells and be necessary in the progression of autophagy initiated by inactive EGFR, could be a cotargeting molecular in cancer treatment.⁶² In addition, restraining LAPT M4B from activating AKT signaling to suppress cell proliferation, breaking the interaction between LAPT M4B and SH3 domain-containing proteins to control cancer invasion and metastasis, inhibiting LAPT M4B to decrease late endosome ceramide export to ameliorate antiapoptosis condition and dissociating the LAPT M4B from the efflux pump P-glycoprotein to attenuate chemotherapy resistance can be potential cancer treatments.

Outstanding questions about LAPT M4B in cancer research

As we all know, LAPT M4B allele *2 contains an extra tandemly arranged 19-bp sequence at the 5'-UTR, which results in a 40-kDa protein. However, the function of the 40-kDa protein in cancer cells and the relation of the 40-kDa protein with the disease risk are not clear now. In addition, whether there are some different translocation modifications between two alleles affecting their function in tumor susceptibility is also unknown.

CONCLUSION AND PROSPECTIVE

LAPT M4B as a gene has been demonstrated to be a positive modulator in the progression of carcinogenesis. It is suggested that LAPT M4B has a key role in tumor proliferation, invasion and

metastasis, anti-apoptosis, autophagy promotion and multidrug resistance. The protein of LAPT M4B gene, LAPT M4B-35, has been certified to be a poor prognostic factor in many kinds of solid tumors.

LAPT M4B may become the new target of cancer therapy. In particular, in recent researches, the relationship between EGFR signaling and LAPT M4B indicated that LAPT M4B can facilitate prosurvival functions of EGFR in cancer cells and be regarded as a therapeutic target for EGFR-positive cancers or a combined target for anti-EGFR therapies.

ABBREVIATIONS

MMP-2, matrix metalloprotein 2; MMP-9, matrix metalloprotein 9.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all subjects who participated in this study. This work was supported by the National Natural Science Foundation of China (No. 81572910).

SEARCH STRATEGY AND SELECTION CRITERIA

References for this Review were identified through searches of PubMed with the search terms 'LAPT M4B' and 'Neoplasms'. Articles were also identified through searches of the authors' own files. Papers published in English or Chinese were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

AUTHOR CONTRIBUTIONS

YM acquired, analyzed and interpreted the data and drafted the manuscript. LW acquired, analyzed and interpreted the data and drafted the manuscript. DC acquired, analyzed and interpreted the data and drafted part of the manuscript. YC acquired, analyzed and interpreted the data. MZ acquired, analyzed and interpreted the data. J-JX acquired, analyzed and interpreted

the data. RZ contributed to data interpretation, and revised the manuscript for important intellectual content. Q-YZ performed the study design, interpreted the data and revised the manuscript for important intellectual content.

REFERENCES

- Liu JJ, Zhang J, Zhang N, Rui JA, Jin C, Zhou RL. Identification of new hepatocellular carcinoma related genes by fluorescent differential display. *J Beijing Med Univ* 2000; **05**: 411–414.
- Shao GZ, Zhou RL, Zhang QY, Zhang Y, Liu JJ, Rui JA et al. Molecular cloning and characterization of LAPTM4B, a novel gene upregulated in hepatocellular carcinoma. *Oncogene* 2003; **22**: 5060–5069.
- Liu X, Xiong F, Wei X, Yang H, Zhou R. LAPTM4B-35, a novel tetratransmembrane protein and its PPRP motif play critical roles in proliferation and metastatic potential of hepatocellular carcinoma cells. *Cancer Sci* 2009; **100**: 2335–2340.
- Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu Rev Biochem* 2003; **72**: 395–447.
- Milkereit R, Rotin D. A role for the ubiquitin ligase Nedd4 in membrane sorting of LAPTM4 proteins. *PLoS One* 2011; **6**: e27478.
- Kasper G, Vogel A, Klamann I, Groner J, Petersen I, Weber B et al. The human LAPTM4B transcript is upregulated in various types of solid tumours and seems to play a dual functional role during tumour progression. *Cancer Lett* 2005; **224**: 93–103.
- Zhou L, He XD, Yu JC, Zhou RL, Yang H, Qu Q et al. Overexpression of LAPTM4B promotes growth of gallbladder carcinoma cells *in vitro*. *Am J Surg* 2010; **199**: 515–521.
- Zhou L, He XD, Cui QC, Zhou WX, Qu Q, Zhou RL et al. Expression of LAPTM4B-35: a novel marker of progression, invasiveness and poor prognosis of extrahepatic cholangiocarcinoma. *Cancer Lett* 2008; **264**: 209–217.
- Yang Y, Yang H, McNutt MA, Xiong F, Nie X, Li L et al. LAPTM4B overexpression is an independent prognostic marker in ovarian carcinoma. *Oncol Rep* 2008; **20**: 1077–1083.
- Yang H, Xiong FX, Lin M, Yang Y, Nie X, Zhou RL. LAPTM4B-35 overexpression is a risk factor for tumor recurrence and poor prognosis in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2010; **136**: 275–281.
- Zhang H, Tian B, Fau - Yu H, Yu H Fau - Yao H, Yao H Fau - Gao Z, Gao Z. LAPTM4B-35 protein as a potential therapeutic target in gastric cancer. (1423-0380 (Electronic)).
- Meng F, Tan S, Liu T, Song H, Lou G. Predictive significance of combined LAPTM4B and VEGF expression in patients with cervical cancer. *Tumour Biol* 2015; **37**: 4849–4855.
- Xue SL, Zhang QY, Zhou RL. Structural and SP1-binding Analysis of Promoter of LAPTM4 β , A Novel Gene Associated with Hepatocellular Carcinoma. *Chinese J Biochem Mol Biol* 2007; **1**: 45–50.
- Zhang M, Xu JJ, Zhou RL, Zhang QY. cAMP responsive element binding protein-1 is a transcription factor of lysosomal-associated protein transmembrane-4 Beta in human breast cancer cells. *PLoS One* 2013; **8**: e57520.
- Zhang H, Qi S, Zhang T, Wang A, Liu R, Guo J et al. miR-188-5p inhibits tumour growth and metastasis in prostate cancer by repressing LAPTM4B expression. *Oncotarget* 2015; **6**: 6092–6104.
- Lee HM, Zhang H, Schulz V, Tuck DP, Forget BG. Downstream targets of HOXB4 in a cell line model of primitive hematopoietic progenitor cells. *Blood* 2010; **116**: 720–730.
- Liu X, Zhou R, Zhang Q, Zhang Y, Shao G, Jin Y et al. [Identification and characterization of LAPTM4B encoded by a human hepatocellular carcinoma-associated novel gene]. *Beijing Da Xue Xue Bao* 2003; **35**: 340–347.
- Zhai G, Yang H, Ji X, Xiong F, Su J, McNutt MA et al. Correlation of LAPTM4B polymorphisms with hepatocellular carcinoma in Chinese patients. *Med Oncol* 2012; **29**: 2744–2749.
- Wang S, Zhang QY, Zhou RL. Relationship between LAPTM4B gene polymorphism and susceptibility of primary liver cancer. *Ann Oncol* 2012; **23**: 1864–1869.
- Fan M, Liu Y, Zhou R, Zhang Q. Association of LAPTM4B gene polymorphism with breast cancer susceptibility. *Cancer Epidemiol* 2012; **36**: 364–368.
- Li X, Kong X, Chen X, Zhang N, Jiang L, Ma T et al. LAPTM4B allele *2 is associated with breast cancer susceptibility and prognosis. *PLoS One* 2012; **7**: e44916.
- Shaker O, Taha F, Salah M, El-Marzouky M. LAPTM4B gene expression and polymorphism as diagnostic markers of breast cancer In Egyptian patients. *J Med Biochem* 2015; **34**: 393.
- Tang H, Tian H, Yue W, Li L, Li S, Gao C et al. LAPTM4B polymorphism is associated with nonsmall cell lung cancer susceptibility and prognosis. *Oncol Rep* 2014; **31**: 2454–2460.
- Liu Y, Zhang QY, Qian N, Zhou RL. Relationship between LAPTM4B gene polymorphism and susceptibility of gastric cancer. *Ann Oncol* 2007; **18**: 311–316.
- Meng F, Song H, Luo C, Yin M, Xu Y, Liu H et al. Correlation of LAPTM4B polymorphisms with cervical carcinoma. *Cancer* 2011; **117**: 2652–2658.
- Meng F, Li H, Zhou R, Luo C, Hu Y, Lou G. LAPTM4B gene polymorphism and endometrial carcinoma risk and prognosis. *Biomarkers* 2013; **18**: 136–143.
- Cheng XJ, Xu W, Zhang QY, Zhou RL. Relationship between LAPTM4B gene polymorphism and susceptibility of colorectal and esophageal cancers. *Ann Oncol* 2008; **19**: 527–532.
- SUN L, ZHANG QY, LIU Y, QIAN. NRelationship between human novel gene LAPTM4B polymorphism and susceptibility of lymphoma. *Zhong Liu Fang Zhi Yan Jiu* 2007; **4**: 245–248.
- Yang H, Zhai G, Ji X, Xiong F, Su J, McNutt MA. Correlation of LAPTM4B polymorphisms with gallbladder carcinoma susceptibility in Chinese patients. *Med Oncol* 2012; **29**: 2809–2813.
- Xu Y, Liu Y, Zhou R, Meng F, Gao Y, Yang S et al. LAPTM4B polymorphisms is associated with ovarian cancer susceptibility and its prognosis. *Jpn J Clin Oncol* 2012; **42**: 413–419.
- Zhang M, Zhou R, Xu J, Zhang Q. Relationship Between LAPTM4B Gene Polymorphism and Susceptibility of Malignant Melanoma in Chinese Patients. *Transl Oncol* 2014; **7**: 638–643.
- Wang B, Xu J, Zhou R, Zhang Q. Association of LAPTM4B gene polymorphism with nasopharyngeal carcinoma susceptibility in a Chinese population. *Med Oncol* 2013; **30**: 470.
- Xia LZ, Yin ZH, Ren YW, Shen L, Wu W, Li XL et al. The relationship between LAPTM4B polymorphisms and cancer risk in Chinese Han population: a meta-analysis. *Springerplus* 2015; **4**: 179.
- Yang H, Xiong F, Wei X, Yang Y, McNutt MA, Zhou R. Overexpression of LAPTM4B-35 promotes growth and metastasis of hepatocellular carcinoma *in vitro* and *in vivo*. *Cancer Lett* 2010; **294**: 236–244.
- Meng F, Chen X, Song H, Lou G. LAPTM4B down regulation inhibits the proliferation, invasion and angiogenesis of HeLa cells *in vitro*. *Cell Physiol Biochem* 2015; **37**: 890–900.
- Kadara H, Fujimoto J, Yoo SY, Maki Y, Gower AC, Kabbout M et al. Transcriptomic architecture of the adjacent airway field cancerization in non-small cell lung cancer. *J Natl Cancer Inst* 2014; **106**: dju004.
- Milkereit R, Persaud A, Vanoaica L, Guetg A, Verrey F, Rotin D. LAPTM4b recruits the LAT1-4F2hc Leu transporter to lysosomes and promotes mTORC1 activation. *Nat Commun* 2015; **6**: 7250.
- Liu L, Xu X, Jing L, Zhou G, Cao Z, Han Y et al. Lysosomal-associated protein transmembrane 4 Beta-35 overexpression is a novel independent prognostic marker for gastric carcinoma. *PLoS One* 2015; **10**: e0118026.
- Xiao M, Jia S, Wang H, Wang J, Huang Y, Li Z. Overexpression of LAPTM4B: an independent prognostic marker in breast cancer. *J Cancer Res Clin Oncol* 2013; **139**: 661–667.
- Kang Y, Yin M, Jiang W, Zhang H, Xia B, Xue Y et al. Overexpression of LAPTM4B-35 is associated with poor prognosis in colorectal carcinoma. *Am J Surg* 2012; **204**: 677–683.
- Yin M, Lou C, Zhang W, Meng F, Zhang H, Ning X et al. LAPTM4B overexpression is a novel independent prognostic marker for metastatic ovarian tumors. *Int J Gynecol Cancer* 2012; **22**: 54–62.
- Yin M, Xu Y, Lou G, Hou Y, Meng F, Zhang H et al. LAPTM4B overexpression is a novel predictor of epithelial ovarian carcinoma metastasis. *Int J Cancer* 2011; **129**: 629–635.
- Yang H, Xiong F, Qi R, Liu Z, Lin M, Rui J et al. LAPTM4B-35 is a novel prognostic factor of hepatocellular carcinoma. *J Surg Oncol* 2010; **101**: 363–369.
- Zhang G, Liang Y, Huang Y, Chen Y, Zhou R. Elevated lysosome-associated protein transmembrane-4beta-35 is an independent prognostic marker in pancreatic carcinoma. *J Int Med Res* 2012; **40**: 1275–1283.
- Meng F, Luo C, Hu Y, Yin M, Lin M, Lou G et al. Overexpression of LAPTM4B-35 in cervical carcinoma: a clinicopathologic study. *Int J Gynecol Pathol* 2010; **29**: 587–593.
- Zhang H, Wei Q, Liu R, Qi S, Liang P, Qi C et al. Overexpression of LAPTM4B-35: a novel marker of poor prognosis of prostate cancer. *PLoS One* 2014; **9**: e91069.
- Tang H, Tian H, Yue W, Li L, Li S, Gao C et al. Overexpression of LAPTM4B is correlated with tumor angiogenesis and poor prognosis in non-small cell lung cancer. *Med Oncol* 2014; **31**: 974.
- Meng FL, Yin MZ, Song HT, Yang H, Lou G, Zhou RL. LAPTM4B-35 overexpression is an independent prognostic marker in endometrial carcinoma. *Int J Gynecol Cancer* 2010; **20**: 745–750.
- Zhou L, He XD, Chen J, Cui QC, Qu Q, Rui JA et al. Overexpression of LAPTM4B-35 closely correlated with clinicopathological features and post-resectional survival of gallbladder carcinoma. *Eur J Cancer* 2007; **43**: 809–815.
- Zhou L, He XD, Yu JC, Zhou RL, Shan Y, Rui JA. Overexpression of LAPTM4B-35 attenuates epirubicin-induced apoptosis of gallbladder carcinoma GBC-SD cells. *Surgery* 2011; **150**: 25–31.

- 51 Li Y, Zou L, Li Q, Haibe-Kains B, Tian R, Li Y *et al*. Amplification of LPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. *Nat Med* 2010; **16**: 214–218.
- 52 Blom T, Li S, Dichlberger A, Back N, Kim YA, Loizides-Mangold U *et al*. LPTM4B facilitates late endosomal ceramide export to control cell death pathways. *Nat Chem Biol* 2015; **11**: 799–806.
- 53 Goldsmith J, Levine B, Debnath J. Autophagy and cancer metabolism. *Methods Enzymol* 2014; **542**: 25–57.
- 54 Leone RD, Amaravadi RK. Autophagy: a targetable linchpin of cancer cell metabolism. *Trends Endocrinol Metab* 2013; **24**: 209–217.
- 55 Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. *Mol Cancer Ther* 2011; **10**: 1533–1541.
- 56 Li Y, Zhang Q, Tian R, Wang Q, Zhao JJ, Iglehart JD *et al*. Lysosomal transmembrane protein LPTM4B promotes autophagy and tolerance to metabolic stress in cancer cells. *Cancer Res* 2011; **71**: 7481–7489.
- 57 Lebovitz CB, Robertson AG, Goya R, Jones SJ, Morin RD, Marra MA *et al*. Cross-cancer profiling of molecular alterations within the human autophagy interaction network. *Autophagy* 2015; **11**: 1668–1687.
- 58 Tan X, Thapa N, Sun Y, Anderson RA. A kinase-independent role for EGF receptor in autophagy initiation. *Cell* 2015; **160**: 145–160.
- 59 Tan X, Sun Y, Thapa N, Liao Y, Hedman AC, Anderson RA. LPTM4B is a PtdIns(4,5)P2 effector that regulates EGFR signaling, lysosomal sorting, and degradation. *EMBO J* 2015; **34**: 475–490.
- 60 Hu G, Chong RA, Yang Q, Wei Y, Blanco MA, Li F *et al*. MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. *Cancer Cell* 2009; **15**: 9–20.
- 61 Li L, Wei XH, Pan YP, Li HC, Yang H, He QH *et al*. LPTM4B: a novel cancer-associated gene motivates multidrug resistance through efflux and activating PI3K/AKT signaling. *Oncogene* 2010; **29**: 5785–5795.
- 62 Tan X, Lambert PF, Rapraeger AC, Anderson RA. Stress-Induced EGFR. Trafficking: mechanisms, functions, and therapeutic implications. *Trends Cell Biol* 2016; **26**: 352–366.



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