INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 4, p. 295-301, 2017

RESEARCH PAPER OPEN ACCESS

Characterization of LPTM4B: A Computational Approach

Zaira Rehman, Hajra Sadia*, Ammad Fahim

Healthcare Biotechnology Department, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences & Technology (NUST), Islamabad, Pakistan

Key words: Lysosomal-associated protein transmembrane-4 beta, Extra cellular domain, Multi drug resistance

http://dx.doi.org/10.12692/ijb/10.4.295-301 Article published on April 30, 2017

Abstract

Lysosomal-associated protein transmembrane-4 beta (LAPTM4B) is a putative novel oncogene. Elevated expression has been observed in many solid tumors as HCC, lung, breast, colon and ovarian cancer. The expression of LAPTM4B is also associated with MDR1 expression in solid tumors. Despite the importance of LAPTM4B in cancer progression and chemotherapy resistance, the structure of LAPTM4B is not known. The current study aimed to identify the three dimensional structure of LAPTM4B and its interactions with MDR1. Protscale server was used to predict the hydrophilicity, accessibility, polarity, flexibility, mutability, bulkiness and refractivity of LAPTM4B and results showed that it is a stable protein. There is no signal peptide in LAPTM4B as predicted through Signal Server. One acetylation site present in LAPTM4B at Ser3 as predicted through Net Acet. One O-linked glycosylation site present at position 62. According to Netphos 3.1 server predictions ten threonine phosphorylation sites, fourteen serine phosphorylation sites, four tyrosine specific phosphorylation sites might present in LAPTM4B. PKC and PKA are kinases do phosphorylation of LAPTM4B. Different physiological parameter of LAPTM4B was predicted trough Protparam server. Secondary structure showed that LAPTM4B is structured protein with alpha helices, coils and one beta sheet. Due to absence of any structural template, ab-initio modeling was used to predict three dimensional structure of LAPTM4B. The structure was stable and it may accord with the rule of stereochemistry. LAPTM4B was predicted to interact with ABCB1, PIK3R1 and so on. These results will help to understand protein structure and how it involved in multidrug resistance.

* **Corresponding Author:** Hajra Sadia ✉ hajrasadia@asab.nust.edu.pk

Introduction

Lysosomal associated protein transmembrane-4 beta (LAPTM4B) is a putative novel oncogene. It is member of the mammalian 4- tetra transmembrane spanning protein superfamily, mainly localized on plasma membrane and membranous organelles including endosomes and lysosomes (Zhou *et al*., 2007). It is mapped to chromosome 8q22.1 with seven exons and encodes a 35-kDa protein. It contain four transmembrane domains, two extracellular domains (EC1 and EC2), and two cytoplasmic tails consisting of the N-terminus and C-terminus of the protein, the latter of which contains typical lysosome-targeting motifs. It is expressed in normal human tissues such as high level of expression in heart, uterus, skeletal muscle, and testis while low level of expression in lungs and liver (Shao *et al*., 2003).

LAPTM4B-35 having elevated expression in various solid tumors as 87.3% (48 / 55) of HCC, lung cancer (88%, 23 / 26), colon cancer (67%, 18 / 27), uterus cancer (68%, 30/44), breast cancer (51%, 27/53), and ovarian cancer (69%, 11 / 16), and significantly over expressed in adreno corticotrophin-secreting adenomas and non-functioning pituitary adenomas (Kasper *et al*., 2005; Morris *et al*., 2005).

Previous reports indicated that LAPTM4B-35 over-expression increased cell growth and proliferation, and promoted the progression of cancer cells towards highly invasive and metastatic stages (He *et al*., 2003; Liu *et al*., 2009; Zhou *et al*., 2010; Yang *et al*., 2010). 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 (Li *et al*., 2010). By sequestering drug in cytoplasmic compartment and enhancing efflux of drugs from cancer cells, overexpression of LAPTM4B decreases nuclear localization of drug and drug-induced DNA damage and thereby reduces drug effectiveness. Overexpression of LAPTM4B has been associated with enhancement of efflux from cancer cells of various cytotoxic drugs.

LAPTM4B participates in multidrug resistance by increasing the efflux of chemotherapeutic drugs. This in turn due to the overexpression of P-glycoproteins- a member of ATP binding cassette (ABC) family of proteins. LAPTM4B regulates the expression of Pgp through activation of PI3K/AKT signalling pathway (Li *et al*., 2010). But this is not the only mechanism involved in chemotherapy resistance and cancer progression through LAPTM4B. In order to fully understand the role of LAPTM4B in cancer progression and chemo resistance, its three dimensional structure must be known. The current study aimed to fully characterize the LAPTM4B protein and determines its three dimensional structure which will further helps to understand how LAPTM4B involve in multidrug resistance.

Material and methods

The FASTA sequence of LAPTM4B was retrieved from NCBI (Accession No: 30409982). The protein sequence was further used for analysis of protein.

Primary sequence analysis of PTM4B

Primary sequence of LAPTM4B was used to predict the hydrophilicity, accessibility, polarity, flexibility, mutability, bulkiness and refractivity of LAPTM4B using Protscale Server in expasy platform (www.web .expasy.org/protscale). All the default parametrs were used except normalization of scale. The signal peptide of LAPTM4B was predicted by SignaIP-4.1 (www.cbs.dtu.dk/services/SignalP). It predicts the cleavage sites and signal peptides on basis of artificial neural network.

Acetylation sites of LAPTM4B were predicted through Net Acet web server (www.cbs.dtu.dk/services/ NetAcet). Mannosylation sites in LAPTM4B were predicted through Net CGlyc web server (www.cbs.dtu.dk/services/NetCGlyc).

Glycosylation sites were predicted through Net OGlyc and Net NGlyc web server (www.cbs.dtu.dk/ services). Phosphorylation sites were predicted through Netphos 3.1 server (www.cbs.dtu.dk/services/ NetPhos) for each Thr, Ser and Tyr residues with minimum threshold value of 0.5. Net Phos K 1.0 server (www.cbs.dtu.dk/services/NetPhosK) was used for the prediction of kinase-specific phosphorylation sites in human LAPTM4B.

Net SurfP program (www.cbs.dtu.dk/services/NetSurfP) was used to determine the surface accessibility of predicted phosphorylation sites. Then various physico-chemical parameters like molecular weight, amino acid composition, estimated half-life, instability index etc. of LAPTM4B were validated by Protparam Server (www.web.expasy. org/protparam). The secondary structure of LAPTM4B (Accession No: 30409982) was predicted through GOR4 (Garnier *et al.*, 1996), Psi Pred (Jones, 1999) and JPred3 (Cole *et al.*, 2008).

Tertiary structure prediction

LAPTM4B (Accession No: 30409982) was subjected to BLAST search (Altschul *et al.*, 1995) and no suitable structural homologue were available. In the absence of structural homologue the abinitio modeling technique was used for structure prediction. I-TASSER (www.zhanglab.ccmb.med.umich.edu/I-TASSER) was used for three dimensional structure prediction of human LAPTM4B. Best structure was selected on basis of C-score. The model was analyzed through py Mol.

Results

Primary Sequence Analysis

LAPTM4B-35 (Accession No: 30409982) is 317 amino acid long protein. Protscale server was used to predict the hydrophilicity, accessibility, polarity, flexibility, mutability, bulkiness and refractivity of LAPTM4B.

The results are shown in Fig. 1. The higher score means the higher probability of each parameter of LAPTM4B. The hydrophilicity values (Fig.1A) lie between 0.243 (position 249 aa) and 0.901 (position 59 aa). The free energy of transfer from inside to outside of a globular protein is predicted through Janin score (Fig.1B) and the values are between 0.259 (position 63 aa) and 0.889 (position 248 aa). Polarity is the dipole-dipole intermolecular interactions between the positively and negatively charged residues, is predicted through Zimmerman score (Fig. 1C) and the values lie between 0.001 (position 24 aa) and 0.776 (position 59 aa).

The probability that amino acid will change over a small evolutionary time period is predicted through relative mutability score (Fig.1D) and the values lie between 46.889 (position 110 aa) and 99.778 (position 278 aa). How much flexibility is present in LAPTM4B is measured through average flexibility index (Fig.1E) and the values lie between 0.292 (position 176 aa) and 0.921 (position 65 aa). Bulkiness is the ratio of the side chain volume to the length of an amino acid and may affect the local structure of a protein. The bulkiness values of LAPTM4B (Fig.1F) are between 10.320 (position 50 aa) and 21.241 (position 249 aa).

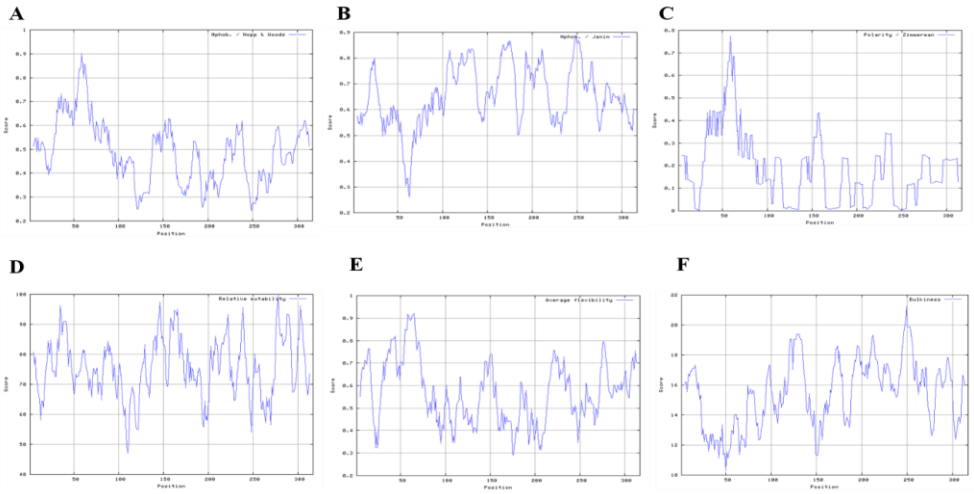

Fig. 1. Prediction of hydrophilicity, accessibility, polarity, mutability, flexibility and bulkiness of LAPTM4B. X-axis shows amino acid sequence from N- to C-terminal while Y-axis show scores computed by each algorithm. (A) hydrophilicity; (B) accessibility; (C) polarity; (E) relative mutability; (D) flexibility; (F) bulkiness.

The signal peptide of TIPE1 was predicted using SignaIP Server and the results were shown as Fig. 2. The presence of signal peptide was measured through C- (raw cleavage site score), S- (signal peptide score) and Y-score (combined cleavage site score). S-score is the estimation of possible signal peptide while D-score is the average of mean S and the max Y-score and it discriminate signal peptide from non-signal peptides. In case of LAPTM4B the D-score is 0.381 which is less than the cut off value of 0.5 which shows there is no signal peptide in LAPTM4B protein.

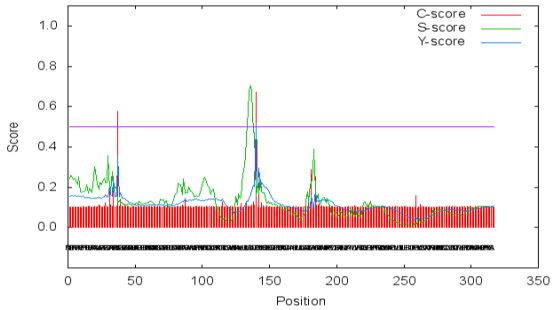

Fig. 2. Prediction of signal peptide of LAPTM4B by SignalP server. X-axis represents amino acid sequence from N- to C- terminal while Y-axis represents scores.

Post translation modification sites

Acetylation sites were predicted through Net Acet web server. One acetylation site present in LAPTM4B at Ser3 (1-MTSRTR-6) with score of 0.503 while cut off is 0.5. There is no mannosylation sites present in LAPTM4B as predicted through Net CGlyc wed server. Glycosylation sites were predicted.

Through Net OGlyc and Net NGlyc web server. Only one potential N-glycosylation site (Fig.3) may be present in LAPTM4B at position 145 (NFSS). One O-linked glycosylation site present at position 62. According to Netphos 3.1 server predictions ten threonine phosphorylation sites (position 2, 8, 31, 80, 87, 99, 114, 116, 181, and 258 aa).

fourteen serine phosphorylation sites (position 11, 38, 39, 62, 75, 76, 103, 147, 149, 170, 239, 279, 280 and 316 aa), four tyrosine specific phosphorylation sites (position 144, 273, 285 and 314 aa) might present in LAPTM4B (Fig. 4). Kinase specific phosphorylation sites in LAPTM4B are shown in Table 1.

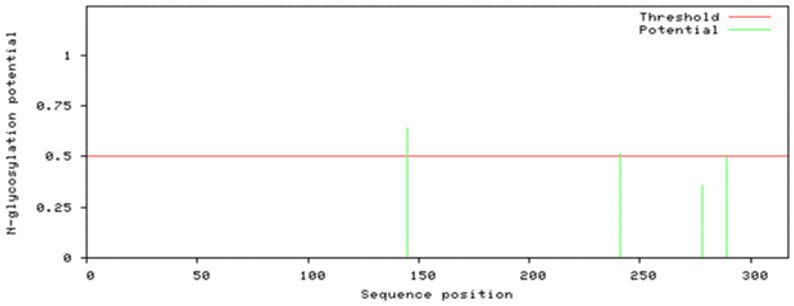

Fig. 3. N-linked glycosylation sites of LAPTM4B predicted through Net NGlyc. X axis represents amino acid sequence fromN- to C- terminal while Y axis represents scores. 0.5 is the threshold, the amino acid positions above threshold level means having potential of glycosylation.

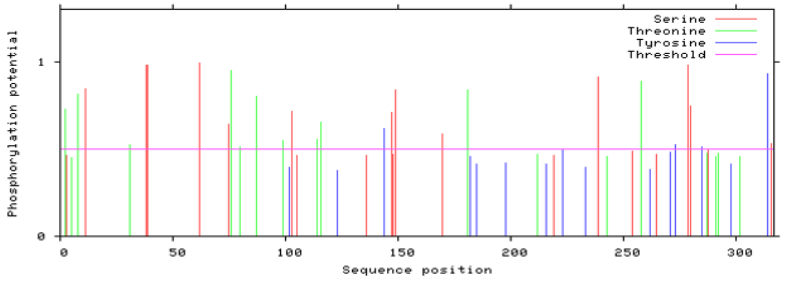

Fig. 4. Potential phosphorylation sites of LAPTM4B predicted through Net Phos. X axis represents amino acid sequence from N- to C- terminal while Y axis represents scores. 0.5 is the threshold, the amino acid positions above threshold level means having potential of phosphorylation.

NetsurfP predicts the surface accessibility of predicted phosphorylation sites and results showed that Thr2, Thr8, Ser11, Ser39, Ser62, Thr99, Ser103, Ser279, and Ser280 are exposed for phosphorylation while Ser76, Thr87, Thr114, Thr116, Ser170, Thr181, and Thr258 are buried inside and not exposed for phosphorylation. Different physiological parameters of LAPTM4B were predicted trough Protparam server (Table. 2).

Table 1. Kinase specific phosphorylation sites.

Site	Kinase	Score
T-2	PKC	0.598
T-8	PKC	0.810
S-11	Cdk5, p38MAPK	0.677
S-39	PKA	0.575
S-62	PKA	0.877
S-76	PKA	0.826
T-87	PKC	0.634
T-99	PKC	0.549
S-103	PKA	0.718
T-114	PKA	0.557
T-116	PKA	0.654
S-170	PKA	0.584
T-181	PKC	0.839
T-258	PKC	0.888
S-279	PKA	0.735
S-280	PKA	0.749

Table 2. Physiological parameters of LAPTM4B.

Molecular Weight	35122.9
Theoretical PI	9.05
Formula	$C_{1581}H_{2486}N_{428}O_{437}S_{20}$
Total no.of Atoms	4952
Estimated half life	30 hours
Instability Index	49.30

Secondary structure of LAPTM4B

GOR4, Psi Pred and JPred3 were used in order to get the secondary structure of LAPTM4B and all of them gave the same results (Fig.5). The result showed that LAPTM4B is structured protein with alpha helices, coils and one beta sheet. Therefore in order to verify the predicted secondary structure, tertiary structure of LAPTM4B was built.

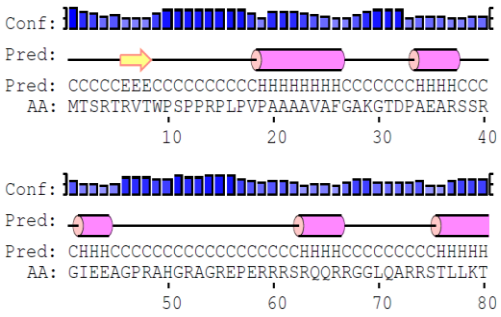

Fig. 5. Secondary structure of LAPTM4B.

Tertiary structure of LAPTM4B

Tertiary structure was predicted through I-Tasser. It generates 5 models and best model is selected on basis of high C-score value. The structure consists of helices and colis (Fig. 6).

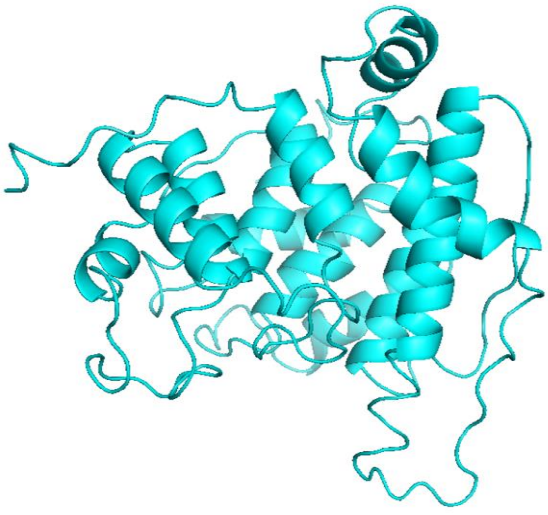

Fig. 6. Tertiary structure of LAPTM4B.

Protein-protein interaction analysis of LAPTM4B

String database is used to identify the interactions of LAPTM4B with other cellular proteins and results (Fig. 7) showed that it interacts with N-acetylated alpha-linked acidic dipeptidase-like 2 (NAALADL2), neural precursor cell expressed, developmentally down-regulated 4 (NEDD4), tyrosine-3 monooxygenase/tryptophan 5-monooxygenase activation protein (YWHAZ), phosphoinositide-3-kinase, regulatory subunit 1 (PIK3R1), transferrin receptor (TFRC), retionalsaturase (RETSAT), chromosome 8 open reading frame 47 (C8orf47), aralkylamine N-acetyltransferase (AANAT) and most importantly ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1).

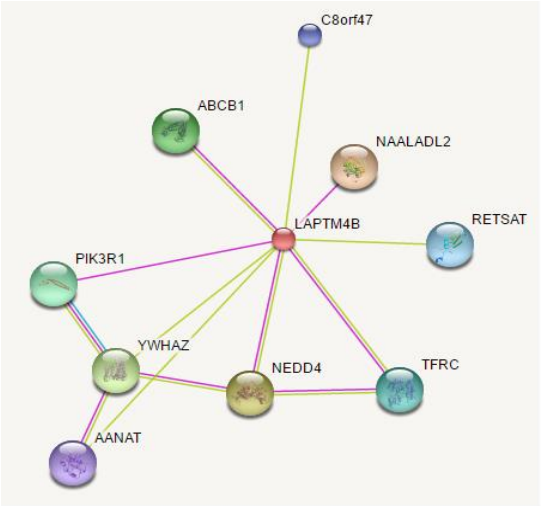

Fig. 7. Interactions of LAPTM4B with cellular proteins. NAALADL2 (N-acetylated alpha-linked acidic dipeptidase-like 2); NEDD4 (neural precursor cell expressed, developmentally down-regulated 4); YWHAZ (tyrosine-3 monooxygenase/tryptophan 5-monooxygenase activation protein); PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1); TFRC (transferring receptor); RETSAT (retionalsaturase); C8orf47 (chromosome 8 open reading frame 47); AANAT (aralkylamine N-acetyltransferase); ABCB1 (ATP-binding cassette, sub-family B (MDR/TAP), member 1).

Discussion

LAPTM4B is a transcription factor and known ontogeny of mammalian 4- tetra Tran membrane spanning protein super family. It was originally identified as HCC associated gene but studies have shown that it is associated with cancer progression and malignant transformation in many solid tumors. It is known to be cancer biomarker in cell cycle progression, DNA damage/repair, differentiation and angiogenesis (Kasper *et al*., 2005). LAPTM4B not only have role in cancer progression and metastasis but also have role in chemotherapy resistance. Li and colleagues have reported that overexpression of LAPTM4B in solid tumors has also been associated with increase efflux of variety of chemo drugs (Li *et al*., 2010). Keeping in view the importance of LAPTM4B in solid tumors, current study conducted to understand the structure of LAPTM4B that will be helpful in designing new therapies against cancer.

The LAPTM4B protein contain four transmembrane domains, two extracellular domains (EC1 and EC2), and two cytoplasmic tails consisting of the N-terminus and C-terminus of the protein, the latter of which contains typical lysosome-targeting motifs. LAPTM4B is transmembrane protein with no signal peptide. Post translation modifications showed that one acetylation site present in protein at Ser3 position. No mannosylation not any N-linked glycosylation sites present inLAPTM4B. The proteins with no signal peptide don't have any N-linked glycosylation sites. One O-linked glycosylation site present at position 62. 28 potential phosphorylation sites present within LAPTM4B. But all of these sites are not available for phosphorylation, only nine residues are exposed for phosphorylation (Thr2, Thr8, Ser11, Ser39, Ser62, Thr99, Ser103, Ser279, and Ser280) while seven residues (Ser76, Thr87, Thr114, Thr116, Ser170, Thr181, and Thr258) are buried inside and not exposed for phosphorylation. Three dimensional structure of LAPTM4B was predicted through ab-initio modeling approach and resulting model have helices and colis which shows that LAPTM4B is structured protein. The PPRP motif at N-terminal known to be involved in binding with SH3 domains of many proteins.

This motif also exhibit proper tertiary structure. The interaction studies of LAPTM4B showed that it interact with MDR1 but there is no physical interactions of LAPTM4B with MDR1 have been reported so far. Li *et al.*, 2010 reported that LAPTM4B activates MDR1 by activating PI3K/AKT pathway (Li *et al.*, 2010).

In conclusion LAPTM4B is stable protein with no signal peptide. It has important role in multidrug resistance. Current study will help to explore how LAPTM4B modulates MDR1 expression.

Competing interests

The authors declare that they have no competing interest.

References

Altschul SF, Gish W, Miller W. 1995. Basic local alignment search tool. Journal of molecular biology **215(3),** 403-410.

Cole C, Barber JD, Barton GJ. 2008. The Jpred 3 secondary structure prediction server. Nucleic acids research **36(suppl 2),** W197-W201.

Garnier J, Gibrat JF, Robson B. 1996. GOR method for predicting protein secondary structure from amino acid sequence. Methods in enzymology **266,** 540-553.

He J, Shao G, Zhou R. 2003. Effects of the novel gene, LAPTM4B, highly expression in hepatocellular carcinoma on cell proliferation and tumor genesis of NIH3T3 cells. Beijing Da Xue Xue Bao **35,** 348-352.

Jones DT. 1999. Protein secondary structure prediction based on position-specific scoring matrices. Journal of molecular biology **292(2),** 195-202.

Kasper G, Vogel A, Klaman I, Gröne J, Petersen I, Weber B, Castaños-Vélez E, Staub E, Mennerich D. 2005. The human LAPTM4b transcript is upregulated in various types of solid tumors and seems to play a dual functional role during tumor progression. Cancer Letter **224,** 93-103.

Li L, Wei XH, Pan YP, Li HC, Yang H, He QH, Pang Y, Shan Y, Xiong FX, Shao GZ, Zhou RL. 2010. LAPTM4B: a novel cancer-associated gene motivates multidrug resistance through efflux and activating PI3K/AKT signaling. Oncogene **29,** 5785-5795.

Li Y, Zou L, Li Q, Haibe-Kains B, Tian R, Li Y, Desmedt C, Sotiriou C, Szallasi Z, Iglehart JD, Richardson AL, Wang ZC. 2010. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. Natural Medicine **16,** 214-218.

Liu X, Xiong F, Wei X, Yang H, Zhou R. 2009. LAPTM4B-35, a novel tetratransmembrane protein and its PPRP motif play critical roles in proliferation and metastatic potential of hepatocellular carcinoma cells. Cancer Science **100,** 2335-2340.

Morris DG, Musat M, Czirják S, Hanzély Z, Lillington DM, Korbonits M, Grossman AB. 2005. Differential gene expression in pituitary adenomas by oligonucleotide array analysis. European Journal of Endocrinology **153,** 143-51.

Shao GZ, Zhou RL, Zhang QY, Zhang Y, Liu JJ, Rui JA, Wei X, Ye DX. 2003. Molecular cloning and characterization of LAPTM4B, a novel gene unregulated in hepatocellular carcinoma. Oncogene **22,** 5060-9.

Yang H, Xiong F, Wei X, Yang Y, McNutt MA, Zhou R. 2010. Overexpression of LAPTM4B-35 promotes growth and metastasis of hepatocellular carcinoma in vitro and in vivo. Cancer Letter **294,** 236-244.

Zhou L, He XD, Chen J, Cui QC, Qu Q, Rui JA, Zhao YP. 2007. Overexpression of LAPTM4B-35 closely correlated with clinic pathological features and post-resectional survival of gallbladder carcinoma. European Journal of **Cancer 43(4),** 809-815.

Zhou L, He XD, Yu JC, Zhou RL, Yang H, Qu Q, Rui JA. 2010. Overexpression of LAPTM4B promotes growth of gallbladder carcinoma cells *in vitro*. American journal of Surgery **199,** 515-521.