



Prognostic value of LAMP1 in surgically resected esophageal squamous cell carcinoma

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Background: The 5-year survival rate of esophageal squamous cell carcinoma (ESCC) is very low. It has been confirmed that lysosomal associated membrane protein 1 (LAMP1) plays an important role in the invasion and metastasis of some tumors. Our previous study showed that the LAMP1 expression levels were significantly different between TNM stages and histological differentiation of ESCC.

Methods: We followed up the 584 ESCC patients enrolled in our previous study and analyzed the relationship between LAMP1 and patients' outcome.

Results: Binary logistic regression analysis showed that the degrees of differentiation showed medium negative correlation with LAMP1 expression ($OR_{\text{differentiation}} = 0.502$, $P < 0.001$). Kaplan-Meier survival curves indicated that patients with a higher LAMP1 expression level showed worse prognosis ($P < 0.05$). The hazard plots showed that organ metastasis, high TNM classification, and high LAMP1 expression level were risk factors for survival in ESCC patients ($P < 0.05$).

Conclusions: LAMP1 expression levels correlated with tumor histological differentiation and patient prognosis. High expression of LAMP1 predicts poor prognosis in ESCC patients.

Keywords: Esophageal tumor; logistic regression analysis; lysosomal associated membrane protein 1 (LAMP1); prognosis; survival curves

Submitted Nov 17, 2017. Accepted for publication Mar 06, 2018.

doi: 10.21037/tcr.2018.03.16

View this article at: <http://dx.doi.org/10.21037/tcr.2018.03.16>

Introduction

Esophageal cancer (EC) is a common malignant tumor. And esophageal squamous cell carcinoma (ESCC) accounted for 90% in China (1,2). The 5-year survival rate of ESCC remains very low (3), which was negatively related to tumor invasion and metastasis (4). And less than 30% of ESCC patients can achieve early diagnosis and treatment.

Lysosomal associated membrane proteins (LAMPs), including LAMP1 and LAMP2, are major protein components of lysosome (5-7), which plays an important

role in tumor evolution (8,9). Previous studies confirmed that LAMP1 showed high expression in astrocytoma (10). Furthermore, LAMP1 has also been found expressing on the cell surface of highly metastatic tumor cells, suggesting a role for LAMP1 in tumor cells migration (11-13).

In our previous study, we detected the LAMP1 expression in the surgically resected tissues of 584 patients with ESCC (14), which has been published on the "*Pathology - Research and Practice*" journal. The results showed that the LAMP1 expression levels were significantly different between TNM stages and degrees of histological differentiation, and that

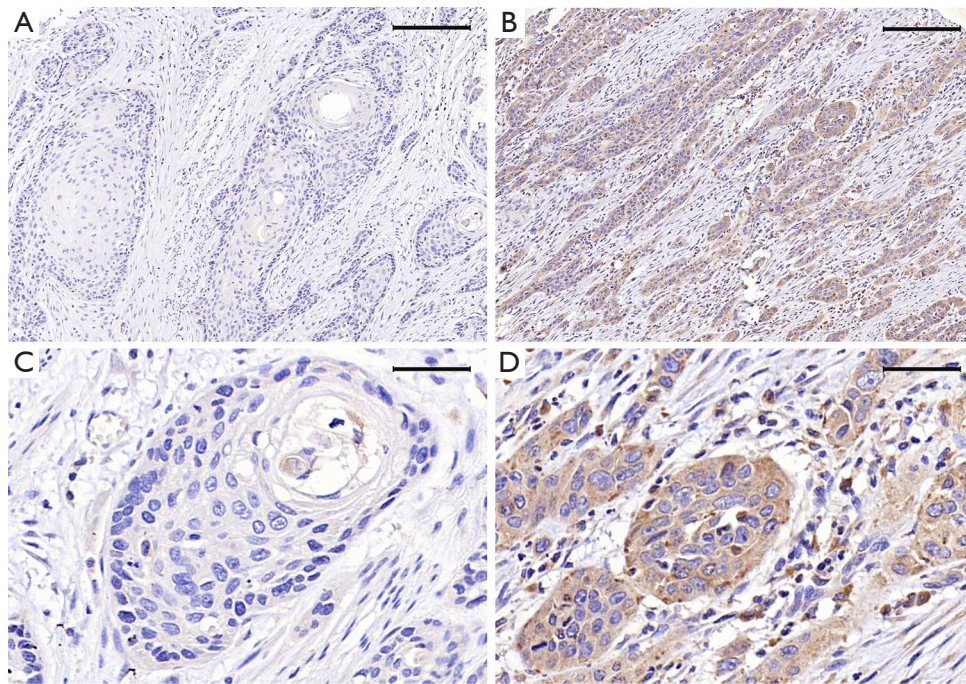


Figure 1 Immunohistochemical staining of LAMP1 antibody in ESCC tissue. (A) Low expression case (10×); (B) high expression case (10×); (C) low expression case (40×); (D) high expression case (40×). Scale bars: (A,B) 200 μm; (C,D) 50 μm. LAMP1, lysosomal associated membrane protein 1; ESCC, esophageal squamous cell carcinoma.

LAMP1 expression levels were negatively correlated with degrees of histological differentiation. But, one flaw is that our previous research is only limited to staging and histopathological classification without patient's prognosis. In present study, we conducted a telephone follow-up of all the patients, and statistical analysis was carried out.

Methods

Patients' information

Five hundred and eighty-four ESCC patients, included in our previous study (14), were followed up by the Follow-up Center of Affiliated Hospital of Jining Medical University. Patients' clinical information and telephone number were collected from medical records. This study was reviewed and approved by the Medical Ethics Committee of Jining Medical University (2017-FY-007). Informed consent was obtained from all patients.

Immunohistochemistry (IHC)

Experimental procedures and criteria for determining results

have been reported in our previous study. And the results of IHC were divided into four grades: “–”, “+”, “++”, and “+++”, which has been reported in our previous study (14). Except for the IHC results, all data in this study did not appear in our previous study. That is to say, we supplemented the patient's prognostic information on the basis of existing immunohistochemical staining results (*Figure 1*).

Statistical analysis

Logistic regression analysis and Kaplan-Meier survival curves were performed using SPSS software 22.0. $P < 0.05$ was considered as statistically significant.

Results

Clinical data

After telephone follow-up, we obtained prognostic information for only 298 ESCC patients. In addition, 7 cases of the 298 ESCC patients were only known to have died, but there was no accurate survival time. The remaining patients failed to get in touch. We calculated

Table 1 LAMP1 expression and clinicopathological characteristics

Characteristic data	Category	IHC				χ^2	P value
		–	+	++	+++		
Gender	Female	13	25	24	8	1.036	0.793
	Male	49	85	76	18		
Age groups	<60	19	45	41	11	2.276	0.517
	≥60	43	65	59	15		
TNM stage	I	1	1	5	0	14.79	0.022*
	II	45	66	57	10		
	III	16	43	38	16		
Differentiation	Well	0	3	0	0	90.878	<0.001*
	Well–moderate	32	11	9	0		
	Moderate	14	35	38	5		
	Moderate–poor	14	50	30	11		
	Poor	2	11	23	10		
Tumor size	≤4.0	37	72	64	13	2.447	0.485
	>4.0	25	38	36	13		
Tumor location	Upper	1	1	0	0	3.229	0.78
	Middle	32	52	44	11		
	Lower	19	36	22	10		
Metastasis [#]	No	44	73	54	13	7.798	0.034*
	Yes	18	37	46	13		

*, the difference was statistically significant; [#], the patient had liver, lung, or bone metastasis. LAMP1, lysosomal associated membrane protein 1; IHC, immunohistochemistry.

the patient's survival rate simply according to the patient's survival, on the basis of the deletion of a later collection of specimens. The 3-year survival rate was 54.34% (119/219), and the 5-year survival rate was 38.94% (44/113). The patients' information and IHC results are listed in *Table 1*.

Data consistency

Before the new data analysis, we first compared the previous data with the existing data. In the existing data, the correlation analysis between the expression levels of LAMP1 and the degrees of differentiation was consistent with our previous paper. Similar results were also found in the analysis of TNM staging and other clinicopathological factors (data not shown).

Logistic regression analysis

Binary logistic regression analysis between LAMP1 expression and patients' information (TNM stage, degrees of differentiation, gender, age, tumor location, metastasis, and tumor size) were performed. In this analysis, the positive degrees of LAMP1 were redefined. “–” and “+” were defined as the low expression. “++” and “+++” were defined as high expression (15). The results showed that the expression levels of LAMP1 were only correlated with degrees of differentiation. Further analysis showed that the degrees of differentiation showed medium negative correlation with LAMP1 expression with statistical significance ($OR_{\text{differentiation}} = 0.502$, $P < 0.001$), while the correlation between another patients' information (TNM stage, gender, age, tumor

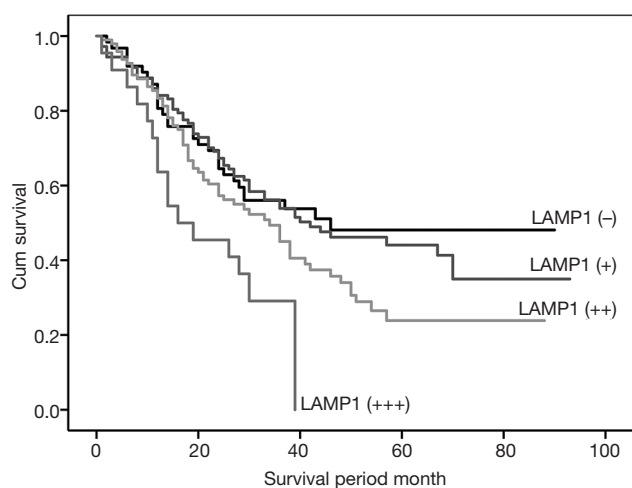


Figure 2 Kaplan-Meier survival curves for ESCC patients. Kaplan-Meier survival curves for ESCC patients between the different IHC positive levels were made. Patients with a higher expression of LAMP1 showed worse prognosis ($P < 0.05$). LAMP1, lysosomal associated membrane protein 1; ESCC, esophageal squamous cell carcinoma.

location, metastasis, and tumor size) and LAMP1 expression were not statistically significant ($P > 0.05$).

Survival analysis

We made the Kaplan-Meier survival curves to analyze the correlations between LAMP1 expression levels and patients' prognosis. Kaplan-Meier survival curves indicated that patients with a higher LAMP1 expression level showed worse prognosis (Figure 2, $P < 0.05$). Additionally, we also analyzed the prognostic risk of LAMP1 expression levels and clinical features (age, gender, tumor location, organ metastasis, and TNM stage). The hazard plots showed that organ metastasis, high TNM classification, and high LAMP1 expression level were risk factors for survival in patients with ESCC (Figure 3, $P < 0.05$), but age, gender, and tumor location not.

Discussion

LAMP1 is localized at the lysosomal membrane under physiological conditions (16,17). Previous studies showed that LAMP1 has been identified expressing higher in many

cancers, especially in the metastatic cancer cells (11,13), which suggested that LAMP1 may be involved in the tumor invasion and metastasis.

In the present study, binary logistic regression analysis showed that LAMP1 expression was negatively correlated with degrees of tumor histological differentiation. The relationship between LAMP1 and tissue differentiation has been reported previously, which may be related to the high glycosylation of LAMP1 (18,19). Kaplan-Meier survival curves and hazard plots showed that high expression of LAMP1 is one of the risk factors for survival in patients with ESCC. The higher the expression of LAMP1, the worse the prognosis of ESCC patients. We will continue to study its roles and molecular mechanism in the following research.

The correlation between LAMP1 expression levels and TNM staging is inconsistent with our previous study. To test this inconsistency, we also made a Spearman Correlation Coefficient of the data for this study, which is consistent with our previous papers (data not shown). Further, we also performed a logistics regression analysis of the IHC results and TNM stages in all 584 patients, which is also inconsistent with Spearman Correlation Coefficient analysis (data not shown). In short, logistics regression analysis is inconsistent with Spearman Correlation Coefficient analysis in this study. We think it may be caused by the following reasons: (I) sensitivity differences between the two analysis methods. Although Spearman correlation coefficient has statistical significance, but its value is relatively small; (II) the small number of some subgroups (Table 1). We planned to follow up 584 patients, but a large portion of the follow-up (nearly 50%) failed to be reached. This leads to the fact that our sample size is greatly reduced, and the accuracy of statistical analysis is affected to some extent. This limited sample number may influence the statistical analysis. On the other hand, many patients who were not contacted were likely to have died, which led to the discontinuation of the telephone number. We speculate that it could bias our final results. Perhaps there were more death patients in the population we couldn't contact. So, our results still need to be further validated.

In conclusion, we have demonstrated that LAMP1 expression levels correlated with tumor histological differentiation and patients' prognosis. High expression of LAMP1 predicts poor prognosis in ESCC patients.

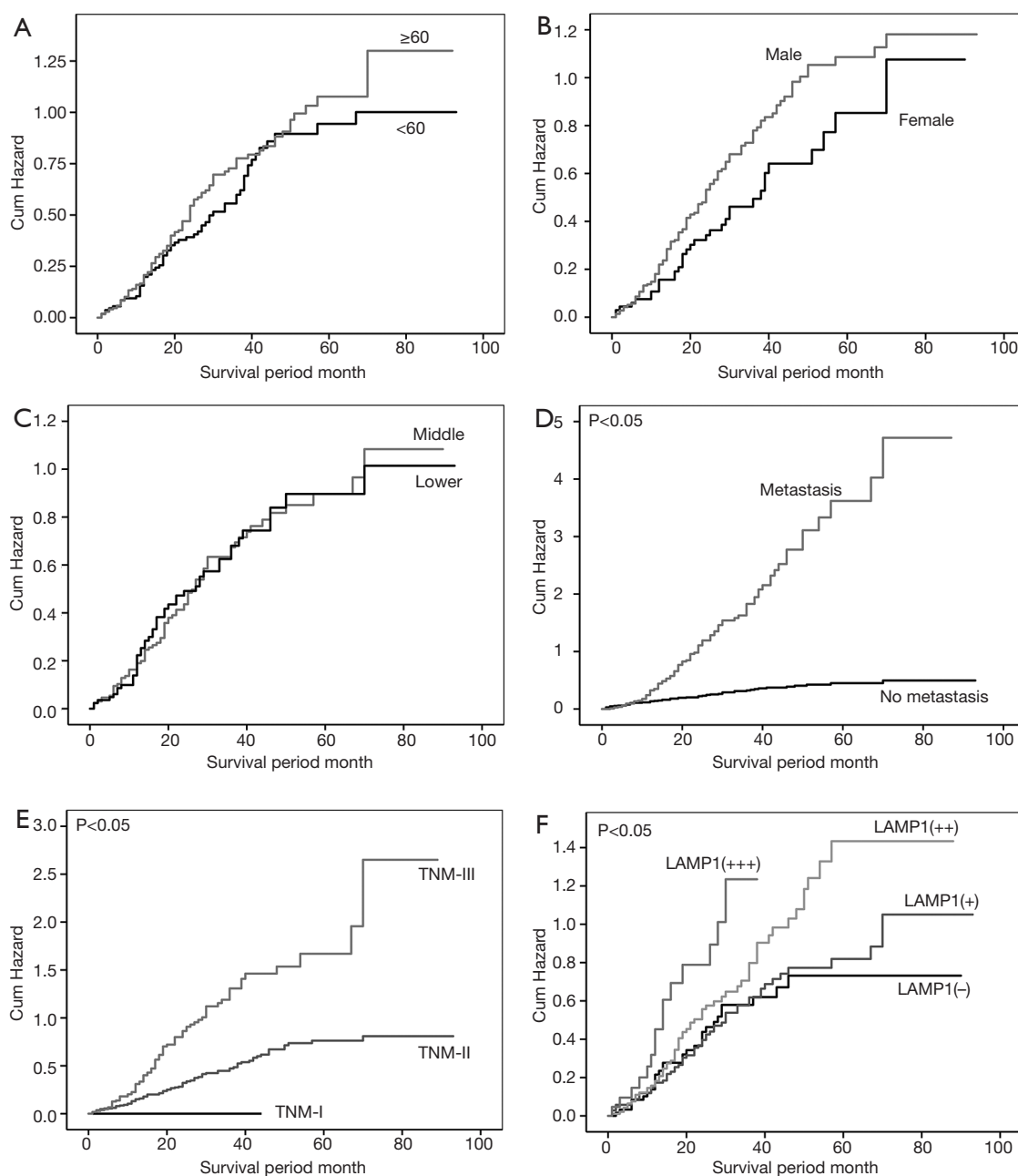


Figure 3 Hazard plots for ESCC patients. The prognostic risk of LAMP1 expression levels and clinical features were analyzed. The hazard plots showed that age (A), gender (B) and tumor location (C) were not risk factors for survival in patients with ESCC. However, organ metastasis (D), high TNM classification (E) and high LAMP1 expression level (F) were risk factors for survival in ESCC patients. LAMP1, lysosomal associated membrane protein 1; ESCC, esophageal squamous cell carcinoma.

Acknowledgments

Funding: This study was supported by the Medical Science and Technology Development Project of Shandong

Province (2016WS0173) and Science and Technology Research Project of Shandong Higher Education Institutions (J13LK140), China.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was reviewed and approved by the Medical Ethics Committee of Jining Medical University (2017-FY-007). Informed consent was obtained from all patients.

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References

- Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016;66:115-32.
- Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
- Bao Y, Liu S, Zhou Q, et al. Three-dimensional conformal radiotherapy with concurrent chemotherapy for postoperative recurrence of esophageal squamous cell carcinoma: clinical efficacy and failure pattern. *Radiat Oncol* 2013;8:241.
- Rice TW, Rusch VW, Apperson-Hansen C, et al. Worldwide esophageal cancer collaboration. *Dis Esophagus* 2009;22:1-8.
- Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat Rev Mol Cell Biol* 2009;10:623-35.
- Schroder BA, Wrocklage C, Hasilik A, et al. The proteome of lysosomes. *Proteomics* 2010;10:4053-76.
- Hook G, Jacobsen JS, Grabstein K, et al. Cathepsin B is a New Drug Target for Traumatic Brain Injury Therapeutics: Evidence for E64d as a Promising Lead Drug Candidate. *Front Neurol* 2015;6:178.
- Hamalisto S, Jaattela M. Lysosomes in cancer-living on the edge (of the cell). *Curr Opin Cell Biol* 2016;39:69-76.
- Liao X, Chen Y, Liu D, et al. High Expression of LAMP3 Is a Novel Biomarker of Poor Prognosis in Patients with Esophageal Squamous Cell Carcinoma. *Int J Mol Sci* 2015;16:17655-67.
- Jensen SS, Aaberg-Jessen C, Christensen KG, et al. Expression of the lysosomal-associated membrane protein-1 (LAMP-1) in astrocytomas. *Int J Clin Exp Pathol* 2013;6:1294-305.
- Agarwal AK, Srinivasan N, Godbole R, et al. Role of tumor cell surface lysosome-associated membrane protein-1 (LAMP1) and its associated carbohydrates in lung metastasis. *J Cancer Res Clin Oncol* 2015;141:1563-74.
- Saitoh O, Wang WC, Lotan R, et al. Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. *J Biol Chem* 1992;267:5700-11.
- Agarwal AK, Gude RP, Kalraiya RD. Regulation of melanoma metastasis to lungs by cell surface Lysosome Associated Membrane Protein-1 (LAMP1) via galectin-3. *Biochem Biophys Res Commun* 2014;449:332-7.
- Huang J, Li L, Liu J, et al. Altered expression of lysosomal associated membrane protein 1 in esophageal squamous cell carcinoma. *Pathol Res Pract* 2017;213:938-42.
- Li L, Wang W, Zhang R, et al. High expression of LAMP2 predicts poor prognosis in patients with esophageal squamous cell carcinoma. *Cancer Biomark* 2017;19:305-11.
- Hasilik A, Wrocklage C, Schroder B. Intracellular trafficking of lysosomal proteins and lysosomes. *Int J Clin Pharmacol Ther* 2009;47 Suppl 1:S18-33.
- Xu H, Ren D. Lysosomal physiology. *Annu Rev Physiol* 2015;77:57-80.
- Sarafian V, Jans R, Poumay Y. Expression of lysosome-associated membrane protein 1 (Lamp-1) and galectins in human keratinocytes is regulated by differentiation. *Arch Dermatol Res* 2006;298:73-81.
- Tyleckova J, Valekova I, Zizkova M, et al. Surface N-glycoproteome patterns reveal key proteins of neuronal differentiation. *J Proteomics* 2016;132:13-20.

Cite this article as: Wang W, Zhang Y, Xu Y, Zhang X, Ma M, Zhang G, Huang J. Prognostic value of LAMP1 in surgically resected esophageal squamous cell carcinoma. *Transl Cancer Res* 2018;7(2):347-352. doi: 10.21037/tcr.2018.03.16