# LAMP1 is more sensitive than LAMP2 in predicting prognosis of esophageal squamous cell carcinoma

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**Background:** Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers, especially in China. Its 5-year survival rate remains very low. Lysosomal associated membrane proteins (LAMPs), including LAMP1 and LAMP2, are major protein components of the lysosome, which also plays an important role in tumor evolution. Our previous studies have confirmed that LAMP1 and LAMP2 play an important role in the evolution and prognosis of ESCC. To further explore their roles in ESCC, we compared the roles of LAMP1 and LAMP2 in predicting the prognosis of ESCC.

**Methods:** Five hundred and seventy-nine ESCC patients were enrolled in this study. The clinical information, address and telephone number of patients were collected from the medical records.

**Results:** The 3-year survival rate was 51.91% (231/445), and 5-year survival rate was 34.58% (83/240). Kaplan-Meier survival curves indicated that the higher the expression level of LAMP1, the worse the prognosis of patients, compared with LAMP2 ($P<0.05$). Additionally, we used the receiver operating characteristic (ROC) curves to compare the sensitivity and specificity of LAMP1 and LAMP2 in predicting prognosis of ESCC. The area under the curve (AUC) of LAMP1 was significantly higher than that of LAMP2 ($AUC_{LAMP1} =0.593$, $AUC_{LAMP2} =0.534$, $P<0.05$), which indicated that the LAMP1 is a more sensitive marker than LAMP2.

**Conclusions:** LAMP1 is more sensitive than LAMP2 in predicting the prognosis of ESCC, which indicated that LAMP1 may play a more important role in the evolution and metastasis of ESCC.

**Keywords:** Esophageal tumor; LAMP1; LAMP2; survival curves; ROC curves; area under the curve (AUC)

## Introduction

Esophageal squamous cell carcinoma (ESCC), the fourth most common cause of cancer-related deaths, is one of the most common cancers, especially in China (1,2). The 5-year survival rate of ESCC remains very low (3), which was closely related to tumor invasion and metastasis (4). At present, only less than 30% of ESCC patients can achieve early diagnosis and treatment. To improve the clinical outcome of ESCC, novel molecular biomarkers used for early diagnosis and predicting prognosis have been widely investigated (5,6).

Lysosomes contain more than 60 kinds of acid hydrolases and various lysosomal specific membrane proteins, whose

**Table 1** Clinicopathological data of ESCC patients

| Item | LW | LE | LH |
|---|---|---|---|
| Gender | | | |
| Female | 30 | 55 | 45 |
| Male | 101 | 196 | 152 |
| Age | | | |
| <60 | 53 | 109 | 81 |
| ≥60 | 78 | 142 | 116 |
| TNM | | | |
| I | 1 | 6 | 7 |
| II | 85 | 145 | 122 |
| III | 45 | 100 | 68 |
| Metastasis* | | | |
| No | 46 | 87 | 50 |
| Yes | 26 | 46 | 41 |
| Differentiation** | | | |
| Well | 1 | 1 | 7 |
| Well-moderate | 28 | 33 | 25 |
| Moderate | 29 | 65 | 83 |
| Moderate-poor | 58 | 105 | 68 |
| Poor | 15 | 47 | 14 |

*, some cases were not sure whether metastasis occurred; **, $\chi^2$=40.074, P<0.001. LW: LAMP1 < LAMP2; LE: LAMP1 = LAMP2; LH: LAMP1 > LAMP2. ESCC, esophageal squamous cell carcinoma.

main function is to receive and degrade macromolecules, including cellular components derived from autophagy, such as damaged organelles, aging and misfolded macromolecules (7). Lysosomal associated membrane proteins (LAMPs), including LAMP1 and LAMP2, are the major protein components of lysosomes (8-10), which also play an important role in tumor evolution (11,12). It has been confirmed that the LAMP1 gene showed high expression in astrocytoma (13). 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 (14-16).

In our previous studies, we detected the expression of LAMP1 and LAMP2 in the surgically resected tissues of nearly 600 patients with ESCC, the results of which have also confirmed that LAMP1 and LAMP2 play an important

role in the evolution and prognosis of ESCC (17-19). To further explore their roles in ESCC, we compared the roles of LAMP1 and LAMP2 in predicting the prognosis of ESCC in the present study.

## Methods

### Patients' information

Five hundred seventy nine ESCC patients with complete IHC results and prognosis data were enrolled in this study. The clinical information, address and telephone number of patients were collected from the medical records. The prognosis data were provided by the Follow-up Center of Affiliated Hospital of Jining Medical University. This study was reviewed and approved by the Medical Ethics Committee of Affiliated Hospital of Jining Medical University (No. 2017-FY-007). Informed consent was obtained from all patients.

### Immunohistochemistry staining (IHC)

In our previous studies, IHC of LAMP1 and LAMP2 was performed simultaneously under the same conditions. The experimental protocols and criteria for determining results have been reported. And the results of IHC were divided into four grades: "−", "+", "++", and "+++", which has also been reported in our previous studies (17,18). It should be noted that all results in this study are the first to be reported.

### Statistical analysis

Kaplan-Meier survival curves and receiver operating characteristic (ROC) curves were performed using SPSS software 22.0. P<0.05 was considered as statistically significant.

## Results

### Clinical data

After summarizing our previous research data, 579 patients with complete IHC results and prognosis data were enrolled in this study. The patients' information and IHC results are listed in *Table 1* and *Figure 1*. The patient's survival rates were calculated simply according to the patient's survival, based on the deletion of some recent cases. The 3-year survival rate was 51.91% (231/445), and the 5-year survival rate was 34.58% (83/240).
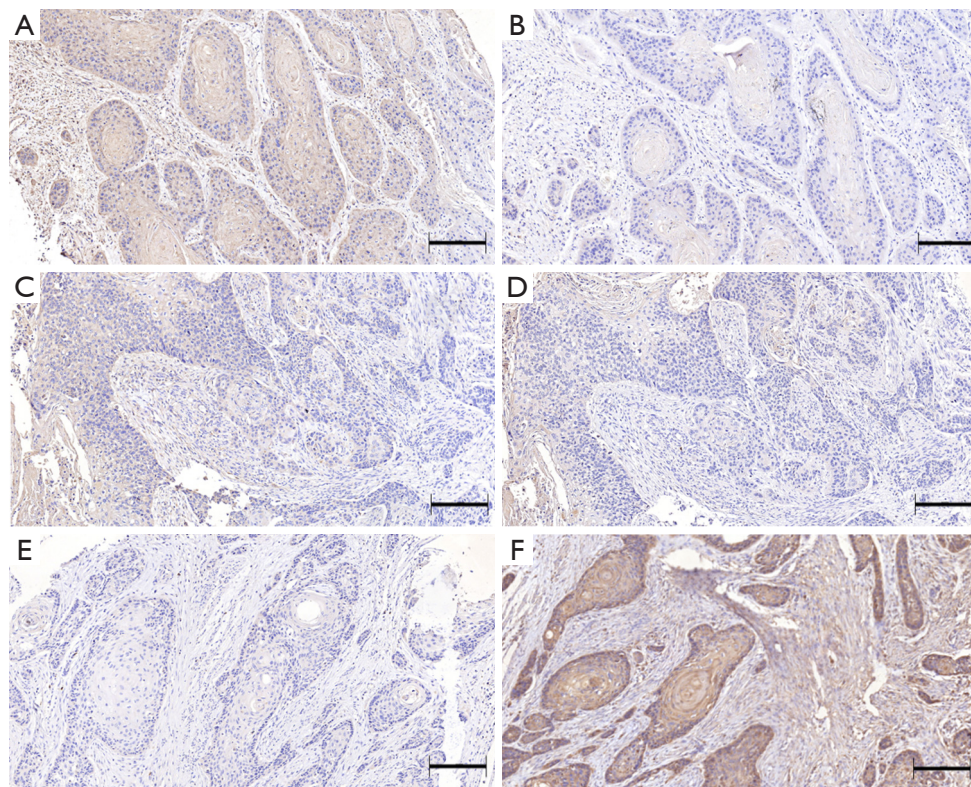
**Figure 1** Immunohistochemical staining of LAMP1 and LAMP2 in ESCC tissues. (A) The patient with high LAMP1 expression (LH); (B) the patient with low LAMP2 expression (LH); (C) LAMP1 expression in LE; (D) LAMP2 expression in LE; (E) the patient with low LAMP1 expression (LW); (F) the patient with high LAMP2 expression (LW). The same case was used in (A) and (B), as shown in (C) and (D), as well as in (E) and (F). The magnification of all pictures is 10×. Scale bars: 200 µm. LH: LAMP1 > LAMP2; LE: LAMP1 = LAMP2; LW: LAMP1 < LAMP2. ESCC, esophageal squamous cell carcinoma.

### Kaplan-Meier survival curves

Firstly, the positive degrees were redefined. "–" and "+" were defined as the low expression (–). "++" and "+++" were defined as high expression (+). Then, we divided all cases into four groups according to the results of IHC: LAMP1(–)LAMP2(–), LAMP1(–)LAMP2(+), LAMP1(+) LAMP2(–), LAMP1(+)LAMP2(+). Kaplan-Meier survival curves indicated that there was no statistical difference between the four groups. However, it is interesting that the survival curve of patients with similar expression of LAMP1 and LAMP2 tended to be consistent, and that the difference between the other two groups was very obvious (*Figure 2A*).

Based on the above results, we adjusted the grouping method. We divided all cases into three groups: LAMP1 high expression group (LH, LAMP1>LAMP2, *Figure 1A,B*), expression equivalent group (LE, LAMP1 = LAMP2, *Figure 1C,D*), and LAMP1 low expression group (LW, LAMP1 < LAMP2, *Figure 1E,F*). Then, we used Kaplan-Meier survival curves again to analyze the prognosis difference of the three groups. The Kaplan-Meier survival curves indicated that the prognosis of group LH was the worst, followed by group LE and group LW. In other words, compared with LAMP2, the higher the expression level of LAMP1, the worse the prognosis of patients (*Figure 2B*, P<0.05).

### ROC curves

Additionally, we used the ROC curve to compare the sensitivity and specificity of LAMP1 and LAMP2 in predicting prognosis of ESCC patients. The area under the curve (AUC) of LAMP1 was significantly higher than that of LAMP2 (*Figure 3*, $AUC_{LAMP1}$ =0.593, $AUC_{LAMP2}$ =0.534, P<0.05), which indicated that LAMP1 is a more sensitive marker than LAMP2 in the prognosis of ESCC.

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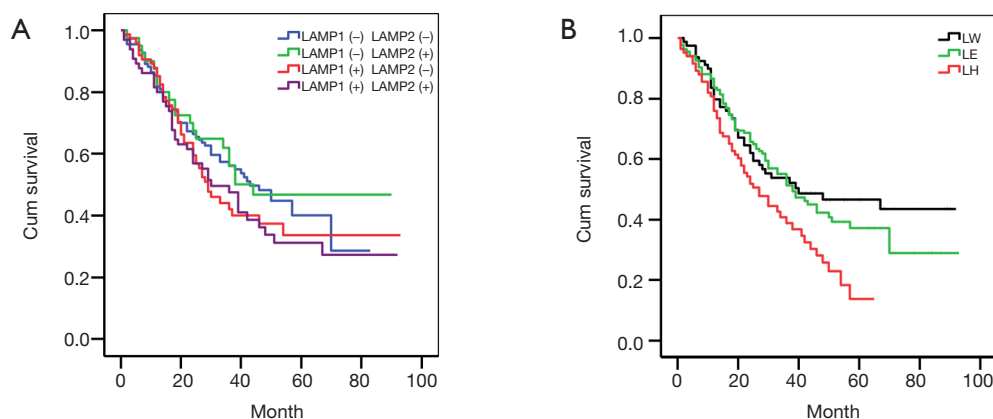
**Figure 2** Kaplan-Meier survival curves for ESCC patients. (A) All patients were divided into four groups: LAMP1(–)LAMP2(–), LAMP1(–)LAMP2(+), LAMP1(+)LAMP2(–), LAMP1(+)LAMP2(+). Kaplan-Meier survival curves showed that there was no significant difference in survival rate between the four groups (P>0.05). But, the survival curves of LAMP1(–)LAMP2(–) group and LAMP1(+)LAMP2(+) group were getting closer and closer, the difference between the other two groups is obvious. (B) Kaplan-Meier survival curves for ESCC patients between the three groups indicated that the prognosis of group LH was the worst, followed by group LE and group LW. The higher the expression level of LAMP1, the worse the prognosis of patients, compared with LAMP2 (P<0.05). LH: LAMP1 > LAMP2; LE: LAMP1 = LAMP2; LW: LAMP1 < LAMP2. ESCC, esophageal squamous cell carcinoma.
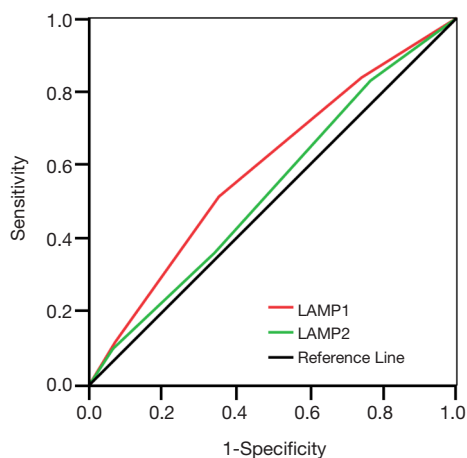


**Figure 3** ROC curves of the effect of IHC results on prognosis of ESCC. The area under the curve (AUC) of LAMP1 was significantly higher than LAMP2 (AUC$_{LAMP1}$ =0.593, AUC$_{LAMP2}$ =0.534, P<0.05). ESCC, esophageal squamous cell carcinoma.

## Discussion

LAMP1 and LAMP2, markers of the lysosomes, are localized at the lysosomal membrane under physiological conditions, playing an important role in the lysosomal physiological process (20,21). LAMP1 and LAMP2 share most common functions, but they also play different roles in some other aspects, such as lysosome transport, exocytosis, chaperone-mediated autophagy, autophagy-lysosome fusion and cholesterol transport (22). In terms of malignant tumors, LAMP1 has been identified expressing higher in many cancers, especially in metastatic cancer cells, playing an important role in tumor invasion and metastasis, but LAMP2 cannot (14,16,23).

In the present study, the Kaplan-Meier survival curves showed that the sensitivity of LAMP1 was significantly higher than that of LAMP2 in the prognosis of ESCC. Compared to LAMP2, the higher the expression of LAMP1, the worse the prognosis of ESCC patients. To further confirm this result, we performed a ROC curve analysis of the sensitivity. The AUC of LAMP1 was significantly higher than that of the LAMP2, indicating that the LAMP1 is more sensitive than LAMP2 in predicting prognosis of ESCC. We will continue to study its molecular mechanism in the following studies.

Additionally, it needs to be pointed out that there are still some other defects in the present study. Firstly, the small sample size of some subgroups (*Table 1*) makes it impossible for us to conduct more in-depth analysis between subgroups; meanwhile, the defects of data may affect, to a certain extent, the accuracy of our statistical analysis. Secondly, this study only focused on the statistical analysis of clinical data and IHC results, but failed to explore the molecular mechanisms of LAMP1 and LAMP2 associated with the prognosis of ESCC. So, our results still need to be

further validated.

## Conclusions

LAMP1 is more sensitive than LAMP2 in predicting the prognosis of ESCC, which indicated that LAMP1 may play a more important role in the evolution and metastasis of ESCC.

## Acknowledgments

## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.03.27). 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 reviewed and approved by the Medical Ethics Committee of Affiliated Hospital of Jining Medical University (No. 2017-FY-007). Informed consent was obtained from all patients.

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