Research Article

Clinical Significance and Prognostic Value of the Expression of LAMP3 in Oral Squamous Cell Carcinoma

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Recent studies demonstrated high expression of lysosome-associated membrane protein 3 (LAMP3) in a variety of malignancies including esophageal squamous cell carcinoma, gastrointestinal cancer, breast cancer, and cervical cancer and its involvement in several biological activities of tumor cells. However, the expression of LAMP3 and its value in oral squamous cell carcinoma (OSCC) remain unclear. In this study, we examined the expression of LAMP3 in OSCC tissue samples and investigated the relationship between LAMP3 and clinical characteristics of patients with OSCC. We examined mRNA and protein levels of LAMP3 in OSCC tissues and neighboring normal tissues using quantitative real-time polymerase chain reaction and immunohistochemistry analyses, respectively. Both the mRNA and protein levels of LAMP3 were significantly higher in OSCC tissues than in adjacent normal tissues. Chi-square analysis showed that the high LAMP3 expression was notably linked to the degree of tumor differentiation and advanced TNM stage. Univariate and multivariate analyses showed that the high LAMP3 expression was an independent prognostic marker in OSCC. Our results suggest that LAMP3 might act as a potential anticancer target and a prognostic marker in patients with OSCC.

1. Introduction

Oral squamous cell carcinoma (OSCC) constitutes a large subgroup of head and neck squamous cell carcinoma and occupies more than 90% of malignancies in the oral cavity [1]. OSCC is a tobacco- and alcohol-related cancer; however, it also can develop in the absence of tobacco and alcohol consumption [2]. More than 300,000 new cases of OSCC are diagnosed annually worldwide [3], and the incidence rate of OSCC is continuously increasing in many countries [4].

The conventional treatment strategy for OSCC includes surgery, radiation therapy, or both surgery and radiation therapy. Treatment methods of advanced OSCC include surgical resection with postoperative adjuvant radiotherapy. A primary aim of oncologic surgery is to achieve curative resection with histological tumor-free margins [5]. Adequate surgical resection is crucial for local control and personalized postoperational management [6–8]. This disease frequently appears along with metastasis, high recurrence, and poor prognosis due to late detection or diagnosis at advanced stages [9]. Despite improvement in early diagnosis and progress in therapy, the outcomes of the disease have remained high, with 30% local or regional recurrence and 25% distant metastasis, leading to an unfavorable 5-year survival rate (about 50%) [10]. Therefore, exploring new methods of diagnosis and seeking novel molecular markers that can predict the prognosis of patients with OSCC for management of OSCC are urgently needed.

Lysosome-associated membrane protein 3 (LAMP3) belongs to the LAMP protein family, which was initially indicated as a molecular marker of mature dendritic cells (CD208, DC-LAMP) [11]. Recent studies have demonstrated that increased expression of LAMP3 correlated with unfavorable prognosis of patients with esophageal squamous cell carcinoma [12], gastrointestinal stromal tumor (GIST) [13], breast cancers [14, 15], cervical cancer [16], and head and
2. Material and Methods

2.1. Tissue Samples and Patient Characteristics. A total of 248 OSCC tissue samples (including 107 buccal squamous cell carcinoma (BSCC) and 141 tongue squamous cell carcinoma (TSCC)) and 55 control samples (including 32 normal oral mucosas and 23 chronic inflammations) were collected for IHC analysis. Additional 25 frozen OSCC tissues and 25 normal oral tissues as controls were collected for mRNA determination using qPCR. All clinical characteristics, such as gender, age, habits (including tobacco and alcohol consumption), differentiation, tumor location, and stages of T, N, and TNM, were obtained from the medical records of patients in Affiliated Hospital of Nantong University, Nantong, Jiangsu, China. All the patients did not receive preoperative radiotherapy, immunotherapy, or chemotherapy. The research protocol was authorized by the Human Research Ethics Committee of the local hospital.

2.2. qRT-PCR. Total RNA was isolated from fresh frozen tissues using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) and converted to cDNA using a High Capacity RNA-to-cDNA Kit (Life Technologies, Carlsbad, CA, USA). Next, qRT-PCR was performed using the Power SYBR Green PCR Master Mix (Life Technologies, Carlsbad, CA, USA). Next, qRT-PCR was performed using the Power

2.3. IHC Staining. We used IHC staining to determine the protein expression of LAMP3 in 303 documented paraffin-fixed tissue specimens (including 107 BSCC, 141 TSCC, and 55 matched noncancerous oral tissues). The whole tissue blocks were constructed to tissue microarrays (TMA) in preparation. TMAs were then deparaffinized with 100% xylene and rehydrated in a graded alcohol. Antigen retrieval was performed by boiling in citrate buffer (pH 6.0) for 10 min in an autoclave, followed by quenching in 3% hydrogen peroxide to block endogenous peroxidase activity. After washing with phosphate-buffered saline (PBS), slides were incubated with rabbit polyclonal anti-LAMP3 antibody (ab111090; 1:100 dilution; Abcam, Cambridge, MA, USA) at 4°C overnight. The next day, sections were incubated with biotinylated goat anti-rabbit secondary antibody (SN135; 1:1000 dilution; Beyotime Institute of Biotechnology, Haimen, China) at room temperature for 30 min. Following washing with PBS, the slides were processed using horseradish peroxidase and dyed with 3,3-diaminobenzidine (DAB) chromogen solution. Finally, the sections were counterstained with hematoxylin, dehydrated, and coverslipped.

Two independent pathologists evaluated the results of IHC staining in a double-blind manner. The expression of LAMP3 was scored as described in a previous study [21]. Briefly, we scored the percentage of LAMP3-positive cells as follows: 0 for 0% staining, 1 for 1%–33%, 2 for 34%–66%, and 3 for 67%–100%. The intensity of LAMP3 staining was scored as follows: 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. The product of the percentage and intensity scores was taken as the final staining score (range from 0% to 300%) [22]. The X-tile software program (The Rimm Lab, Yale University; http://www.tissuearray.org/rimmlab) was used to determine the cutoff point for the IHC staining score of the expression of LAMP3 to analyze the overall survival [21]. We defined 140% as the cutoff point in LAMP3 level in cancer cells (P = 0.013). Samples higher than 140% were categorized as high expression, and samples lower than 140% were categorized as low expression.

2.4. Statistical Analysis. The SPSS20.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis as described previously [21]. A nonparametric test, Mann-Whitney U test, was performed for comparing the mRNA expression of LAMP3 between OSCC tissues and normal tissues. Chi-square analysis was used to compare the differences between the expression of LAMP3 and clinical characteristics of patients with OSCC. The Cox proportional hazards model was used to calculate the univariate and multivariate analyses. Kaplan-Meier method was used to draw survival curves. P value <0.05 was deemed to be statistically significant for all analyses.

3. Results

3.1. The mRNA Level of LAMP3 Was Increased in OSCC Tissues Compared with That in Adjacent Normal Samples. We performed qPCR to measure expression level of LAMP3 mRNA in OSCC tissues and normalized LAMP3 mRNA...
levels to mRNA levels of the housekeeping gene β-actin [23, 24]. LAMP3 mRNA levels were significantly elevated in cancerous tissues compared with adjacent normal control samples by the Mann-Whitney U test \( (P < 0.0001) \) (Figure 1).

### 3.2. LAMP3 Protein Level Was Significantly Increased in OSCC Tissues Compared with That in Adjacent Normal Tissues.

IHC staining showed that LAMP3 was detectable primarily in the cytoplasm of cancer cells and presented as brown particles (Figure 2), consistent with previous studies [25]. The frequency of high LAMP3 protein expression in the cytoplasm of cancer cells was significantly higher in OSCC tissues than in the normal oral mucosa \( (P < 0.001) \) (Table 1). Low or no positive staining was found in the nuclei of cancer cells or stromal cells, while no positive signals were found in the normal oral mucosa. We classified samples based on high or low LAMP3 expression, as described in Methods. A total of 62 (25%) cases were categorized as high LAMP3 expression, and 186 (75%) cases were categorized as low LAMP3 expression.

### 3.3. Relationship Between the Expression of LAMP3 And Clinical Characteristics of OSCC Patients.

We next examined the correlation of LAMP3 expression with clinical features of patients with OSCC. We found that high protein level of LAMP3 was significantly linked to the degree of differentiation \( (P = 0.011) \) and advanced TNM stage \( (P = 0.043) \) (Table 2).

### 3.4. High Expression of LAMP3 Predicts Poor Overall Survival in Patients with OSCC.

LAMP3 was significantly elevated in cancerous tissues compared with adjacent normal tissues by qPCR. The mRNA expression of LAMP3 (normalized to that of β-actin) was significantly higher in cancerous tissues compared with adjacent normal tissues by Mann-Whitney U test \( (P < 0.0001) \).

Previous studies reported that LAMPs are involved in human malignancies. Carlsson et al. report that LAMPs can help predict poor outcome in patients with laryngeal cancer and breast, and ovary cancers [36] and correlated with node metastasis in colon cancer cell lines [35]. Some molecular markers are even enable to significantly influence survival [32, 33].

### 4. Discussion

With increasing incidence and mortality, cancer is the leading cause of death in China and is a major public health problem. Because of the massive population in China, which constitutes approximately one-fifth of the world population, cancer cases in China significantly contribute to the global burden of cancer: almost 22% of new global cancer cases and close to 27% of global cancer deaths occur in China [26]. The incidence of OSCC also continues to grow. OSCC is a highly invasive and metastatic malignancy, and despite advances in surgery, radiation, and chemotherapy, the 5-year disease-free survival (DFS) is about 50% [27], with frequent local recurrence and metastasis. To date, studies seeking potential predictive molecular markers for OSCC have been extensively performed and some markers were identified as significantly correlating with tumor biological response and clinical features in vitro and in vivo [28–32]. Some molecular markers are even enable to significantly influence survival [32, 33].

Previous studies reported that LAMPs are involved in human malignancies. Carlsson et al. report that LAMPs can relocate to the plasma membrane in several types of cancer cells [34]. Saitoh et al. found that the elevated expression of LAMP1 and LAMP2 predicts high metastatic viability in colon cancer cell lines [35]. These observations indicate that LAMPs might promote the metastasis of malignant tumors.

LAMP3 was first identified as a lung-specific gene [28] and now represents a well-established cell surface marker of mature dendritic cells. Overexpression of LAMP3 has been found in several human cancers such as lung, colon, esophage, breast, and ovary cancers [36] and correlated with node metastasis by affecting cell migration [16, 19]. Dominguez-Bautista et al. demonstrated that LAMP3 is required for cell survival during proteasomal inhibition in vitro [37]. In addition, the protein expression of LAMP3 contributes to locoregional recurrence in breast cancer [15, 18]. Nagelkerke et al. reported that LAMP3 is also involved in tamoxifen resistance in MCF7 breast cancer cells through the modulation of autophagy, while knockdown of this gene presents an increased sensitivity toward tamoxifen [38]. In addition, high LAMP3 expression predicts poor survival in patients with cervix cancer and esophageal squamous cell carcinoma [12, 16]. Moreover, LAMP3 combined with TP53 determination can help predict poor outcome in patients with laryngeal
squamous cell carcinoma (LSCC) and GIST [13, 17]. However, the expression of LAMP3 and its relationship with the clinical features of OSCC patients have remained unclear. Here, we investigated the potential role of LAMP3 in OSCC development.

We first examined LAMP3 mRNA expression in OSCC tissues and surrounding normal oral tissues using qPCR and found a significantly higher level of LAMP3 mRNA in malignant tissues. Consistent with the qPCR result, IHC staining on 248 paraffin-embedded OSCC and 55 matched noncancerous specimens showed that LAMP3 protein expression was higher in OSCC compared with normal tissues. Chi-square analysis revealed a significantly positive correlation of high expression of LAMP3 with a low degree of tumor differentiation \( (P = 0.011) \) and an advanced TNM stage \( (P = 0.043) \). Univariate and multivariate analyses exhibited that positive LAMP3 expression, degree of differentiation, and TNM stage were independent prognostic factors affecting the survival of patients with OSCC, and Kaplan-Meier analysis showed that the lifespan of patients with positive LAMP3 expression was shorter than that of patients with negative expression.

More than half of OSCCs evolve from oral precancerous lesions (OPLs) in the oral cavity. Thus, early diagnosis and intervention of OSCCs can increasingly prolong the survival of patients and improve their quality of life. Furthermore, the recognition of squamous cell hyperplasia or dysplasia in the oral cavity might be helpful in ameliorating strategies during the process of oral oncogenesis at the precancerous stage; however, the relevant knowledge is lacking [39–41]. In this study, the expression of LAMP3 was compared between cancerous tissues of OSCC and normal oral mucosa, and we exhibited that positive LAMP3 expression, degree of differentiation, and TNM stage were independent prognostic factors affecting the survival of patients with OSCC, and Kaplan-Meier analysis showed that the lifespan of patients with positive LAMP3 expression was shorter than that of patients with negative expression.

Table 1: LAMP3 expression in cancerous and noncancerous oral tissues.

| Characteristics          | n    | Low LAMP3 expression (%) | High LAMP3 expression (%) | Pearson \( \chi^2 \) | \( P \) |
|--------------------------|------|--------------------------|---------------------------|----------------------|------|
| OSCC                     | 248  | 186 (75.00)              | 62 (25.00)                | 12.332               | <0.001* |
| Noncancerous oral tissues| 55   | 53 (96.36)               | 2 (3.64)                  |                      |      |

\( *P < 0.05 \).
Table 2: Relationship between the expression of LAMP3 and clinicopathological characteristics in OSCC.

| Characteristic | n   | Low expression (%) | High expression (%) | Pearson χ² | P   |
|---------------|-----|--------------------|---------------------|------------|-----|
| Total         | 248 | 186 (75.00)        | 62 (25.00)          |            |     |
| Gender        |     |                    |                     |            |     |
| Male          | 115 | 88 (76.52)         | 27 (23.48)          | 0.265      | 0.607|
| Female        | 133 | 98 (73.68)         | 35 (26.32)          |            |     |
| Age           |     |                    |                     |            |     |
| <60           | 110 | 79 (71.82)         | 31 (28.18)          | 1.067      | 0.302|
| ≥60           | 138 | 107 (77.54)        | 31 (22.46)          |            |     |
| Tobacco consumption |     |                    |                     |            |     |
| No            | 134 | 98 (73.13)         | 36 (26.87)          |            |     |
| Yes           | 114 | 88 (77.19)         | 26 (22.81)          | 0.541      | 0.462|
| Alcohol consumption |     |                    |                     |            |     |
| No            | 132 | 97 (73.48)         | 35 (26.52)          |            |     |
| Yes           | 116 | 89 (76.72)         | 27 (23.28)          | 0.346      | 0.557|
| Tumor location|     |                    |                     |            |     |
| Buccal        | 107 | 77 (71.96)         | 30 (28.04)          |            |     |
| Tongue        | 141 | 109 (77.30)        | 32 (22.70)          | 0.926      | 0.336|
| Differentiation |     |                    |                     |            |     |
| Poor          | 102 | 85 (83.33)         | 17 (16.67)          | 6.417      | 0.011*|
| Moderate and well | 146 | 101 (69.18) | 45 (30.28) | 6.417 | 0.011* |
| 0-1           | 95  | 76 (80.00)         | 19 (20.00)          |            |     |
| TNM stage     |     |                    |                     |            |     |
| II-III        | 73  | 58 (79.45)         | 15 (20.55)          | 6.305      | 0.043*|
| IV            | 80  | 52 (65.00)         | 28 (35.00)          |            |     |
| Tis-T1        | 110 | 82 (74.55)         | 28 (25.45)          |            |     |
| T             |     |                    |                     |            |     |
| T2            | 58  | 42 (72.41)         | 16 (27.59)          | 0.486      | 0.784|
| T3-T4         | 80  | 62 (77.50)         | 18 (22.50)          |            |     |
| Node metastasis|     |                    |                     |            |     |
| No            | 193 | 150 (77.72)        | 43 (22.28)          | 3.434      | 0.064|
| Yes           | 55  | 36 (65.45)         | 19 (34.55)          |            |     |

*P < 0.05.

Table 3: Univariate and multivariate analyses of prognostic factors in OSCC for 5-year survival.

| Variable                        | HR  | Univariate analysis | Multivariate analysis |
|---------------------------------|-----|---------------------|-----------------------|
|                                 |     | P value             | 95% CI                | P value       | 95% CI        |
| LAMP3 expression                | 1.722| 0.013*              | 1.119–2.648           | 1.546         | 0.048*        | 1.003–2.383   |
| High versus low                 |     |                     |                       |               |               |
| Gender                          | 1.040| 0.847               | 0.696–1.556           | 1.003         | 0.847         | 0.696–1.556   |
| Female versus male              |     |                     |                       |               |               |
| Age (years)                     | 1.259| 0.273               | 0.834–1.898           | 1.259         | 0.273         | 0.834–1.898   |
| <60 versus ≥60                  |     |                     |                       |               |               |
| Tobacco consumption             | 0.847| 0.420               | 0.565–1.268           | 0.847         | 0.420         | 0.565–1.268   |
| Yes versus no                   | 0.889| 0.569               | 0.592–1.334           | 0.889         | 0.569         | 0.592–1.334   |
| Alcohol consumption             |     |                     |                       |               |               |
| Yes versus no                   | 0.889| 0.569               | 0.592–1.334           | 0.889         | 0.569         | 0.592–1.334   |
| Tumor location                  |     |                     |                       |               |               |
| Buccal versus tongue            | 0.983| 0.934               | 0.655–1.474           | 0.983         | 0.934         | 0.655–1.474   |
| Differentiation                 |     |                     |                       |               |               |
| Well versus moderate versus poor| 1.715| 0.018*              | 1.097–2.682           | 1.715         | 0.018*        | 1.097–2.682   |
| T stage                         |     |                     |                       |               |               |
| Tis-1 versus T2 versus T3-4     | 1.257| 0.056               | 0.994–1.589           | 1.257         | 0.056         | 0.994–1.589   |
| Node metastasis                 | 3.009| <0.001*             | 1.976–4.582           | 3.009         | <0.001*       | 1.976–4.582   |
| No versus yes                   |     |                     |                       |               |               |
| TNM stage                       | 1.562| 0.001*              | 1.213–2.011           | 1.562         | 0.001*        | 1.213–2.011   |
| Stages 0-1 versus stages II-III versus stage IV |     |                     |                       |               |               |

*P < 0.05.
found that LAMP3 was highly expressed in tumor cells. Furthermore, high expression of LAMP3 was linked to poor prognosis. Future research with a large number of OPL cases is urgently needed to determine the expression of LAMP3 and analyze the differences between OPL and OSCC.

5. Conclusion

This study investigated the potential role of LAMP3 in OSCC. The expression of LAMP3 was determined in cancerous OSCC tissues and matched normal tissues using qPCR and IHC methods, respectively. A comparison between the expression of LAMP3 and clinical characteristics of patients with OSCC was performed, and the survival curves were drawn. In the current study, we found that higher expression of LAMP3 in OSCC tissues than in control samples may be a novel prognostic marker and a potential anticancer target for OSCC patients.

Conflicts of Interest

All the authors declare no competing financial interests.


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Authors’ Contributions

Jun Lu, Hengcheng Ma, Jianfei Huang, and Xingmei Feng contributed equally to this paper.
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