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Original article

Altered expression of lysosomal associated membrane protein 1 in esophageal squamous cell carcinoma

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ABSTRACT

Background: Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers. LAMP1, major protein components of lysosome, is primarily located on the lysosomal membrane and rarely expressed on the surface of normal cells, playing an important role in the lysosome-mediated physiological processes. Previous studies confirmed that LAMP1 showed high expression in astrocytoma. The purpose of this study was to investigate the expression levels of LAMP1 and to discuss its roles in ESCC.
Methods: We collected 610 tissue samples of ESCC patients to construct tissue microarrays, which were subsequently stained by immunohistochemistry with LAMP1 antibody.
Results: After immunohistochemical staining, a total of 584 patients, including 453 men and 131 women, were analysed. The positive immunostaining was mainly located at the cytoplasm. The LAMP1 expression levels were significantly different between different T status ($P < 0.001$), TNM stages ($P < 0.01$) and degrees of tumor histological differentiation ($P < 0.001$). Besides, LAMP1 expression levels were positively correlated with TNM stages ($P < 0.05$). The higher the TNM stages, the higher the LAMP1 expression levels. Similar results also appeared in degrees of tumor histological differentiation ($P < 0.01$), but not in ages, genders, tumor size, T status, lymphatic metastasis and tumor locations ($P > 0.05$).
Conclusion: LAMP1 is involved in the TNM stages and histological differentiation of the ESCC. Targeted therapy for LAMP1 may be a promising novel therapeutic strategy against poorly differentiated ESCC.
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1. Introduction

Esophageal squamous cell carcinoma (ESCC), the fourth most common cause of cancer-related deaths, is a common malignant tumor, especially in China [1,2]. Although surgery, radiotherapy and chemotherapy have been widely used, the five-year survival rate of patients with advanced ESCC is still very low [3]. ESCC progresses rapidly and remains poor prognosis with a five-year overall survival rate ranging from 15% to 25%. The survival period was negatively correlated with tumor invasion and metastasis [4]. However, less than 30% of ESCC patients can achieve early diagnosis and treatment. To improve the clinical outcome of ESCC patients, novel molecular biomarkers used for early diagnosis, molecular targeted therapy, and prognostic prediction have been widely investigated [5,6].

Lysosomal associated membrane proteins (LAMPs), including LAMP1 and LAMP2, are major protein components of the lysosome [7,8], which plays an important role in tumor evolution [9]. LAMPs are type I transmembrane proteins with a C-terminal cytoplasmic tail, a transmembrane domain and a large luminal domain [10]. The conserved cytoplasmic tail consists of 11 amino acid residues and contains necessary information for their intracellular targeting after biosynthesis. Despite their 37% amino acid sequence homology, LAMP1 and LAMP2 are distinct proteins, which is evidenced by their localisation on different chromosomes. The heavily glycosylation creates a sugar coat or glycocalyx on the inner side of the lysosomal membrane protecting the membrane from the hydrolytic enzymes and degradation [8]. Besides, LAMPs also play an important role in lysosomal trafficking, exocytosis, chaperone-mediated autophagy, autophagosome-lysosome fusion

Abbreviations: LAMP1, lysosomal associated membrane protein 1; ESCC, esophageal squamous cell carcinoma; IHC, immunohistochemistry; TMAs, tissue microarrays.

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and cholesterol transport. Mice deficient in both of them have an embryonic lethal phenotype, but viable and fertile upon loss of any one of them, suggesting that LAMP1 and LAMP2 share some common functions. Additionally, LAMP2 seems to have more specific functions, because LAMP2 single deficiency has a more severe phenotype than LAMP1 loss [11].

LAMP1 is primarily located on the lysosomal membrane and rarely expressed on the surface of normal cells, playing an important role in the lysosome-mediated physiological processes. Previous studies confirmed that LAMP1 showed high expression in astrocytoma [12]. Furthermore, it has also been found expressing on the cell surface of highly metastatic tumor cells, suggesting a role for the LAMP1 in cell–cell adhesion and migration [13–15].

In this study, we detected the LAMP1 expression through immunohistochemistry in tumor tissues to analysing the correlation between the LAMP1 expression levels and the clinicopathological characteristics of ESCC patients, and evaluated its possibility as a cancer biomarker for ESCC.

2. Materials and methods

2.1. ESCC patients and samples

A total of 610 ESCC patients, who underwent surgery in Thoracic Surgery of the Affiliated Hospital of Jining Medical University, were enrolled in this study from 2008 to 2014. The criteria for included patients was as follows: (1) All cases were diagnosed with ulcerative squamous cell carcinoma by pathologists; (2) All patients did not receive radiotherapy or chemotherapy before surgery; (3) Patients' clinical information was collected from medical records, and follow-up data was collected through telephone interview. The clinical and histologic grades were defined according to the 7th edition of TNM classification of the International Union Against Cancer (2010) [16]. This study was reviewed and approved by the Medical Ethics Committee of the Affiliated Hospital of Jining Medical University. Informed consent was obtained from all enrolled patients.

2.2. Tissue microarrays (TMAs)

Available formalin-fixed paraffin-embedded tissue blocks of the ESCC patients from Pathology Department of the Affiliated Hospital of Jining Medical University were used to construct TMAs [17,18]. The pathologists microscopically examined hematoxylin and eosin stained sections of the tumors and selected representative areas, excluding espohageal adenocarcinoma and tumor necrosis. Cores (0.6 mm) of malignant tissues were spotted in duplicates. After pathologists' review, we constructed 9 TMAs blocks containing a total of 610 cores of ESCC tumor tissues. 5-micrometer sections were cut from TMAs paraffin blocks and transferred to adhesive slides. Sections were stained with haematoxylin to assess for adequate tumor representation.

2.3. Immunohistochemistry (IHC)

TMAs were stained using the rabbit anti-LAMP1 monoclonal antibody (Proteintech, 21997-1-AP, 1:50). The immunostainings were performed by manufacturer's instructions. Firstly, TMAs were deparaffinised and endogenous peroxidase activity was quenched by 1.5% hydrogen peroxide. And the microwave was used to perform the antigen retrieval. Subsequently, TMAs were incubated for 60 min with the LAMP1 antibody. The detection of the antigen-antibody complex was performed using horseradish peroxidase conjugated secondary antibodies (goat anti-rabbit IgG-HRP) followed by visualization with diaminobenzidine (DAB) as chromogen. Then, TMAs were counterstained with haematoxylin. LAMP1 antibody omission was used as a negative control. Finally, the TMAs were scanned and analysed with CaseViewer 1.4.

The immunohistochemical scores were based on the average percentage of positive cells and their average staining intensity for the whole tissue section. Scoring criteria was based on the existing references[19,20]. All patients were divided into four groups according to total scores. ÷ïcorresponds to 0–1 scores, ÷ïto 2–3 scores, ÷ï+ïto 4–6 scores, and ÷ï+ï+ïto 8–12 scores.

2.4. Statistical analysis

Comparison between subgroups was performed using the Pearson chi-squared (χ^2) test, and correlation analysis was performed using Spearman Correlation Coefficient (r_s) [17,21]. $P < 0.05$ was taken as statistically significant. And all statistical analysis was performed using SPSS software 22.0.

3. Results

3.1. Clinical data

All 610 cases were diagnosed with ulcerative squamous cell carcinoma. Lymphatic metastasis was present in 287 cases, but no distant metastasis was present. After the immunostainings were performed, 26 patients' tissues failed to stain due to the tissue removal. The remaining 584 patients were continued to be analysed subsequently. The 584 patients comprised of 453 men and 131 women, ageing from 34 to 83 years (mean age: 60.9 years). The clinicopathological characteristics and IHC results are listed in Table 1.

3.2. Expression levels of LAMP1 in ESCC

LAMP1 expression levels in ESCC tissues, detected by IHC, were significantly different among the various subgroups. In ESCC tumor cells, the positive immunostaining was mainly located at the cytoplasm (Fig. 1D and E).

As listed in Table 1, the LAMP1 expression levels were significantly different between different T status ($\chi^2 = 31.494$, $P < 0.001$). The similar results also appeared in TNM stages ($\chi^2 = 19.531$, $P < 0.01$) and degrees of tumor histological differentiation ($\chi^2 = 111.410$, $P < 0.001$), but not in ages, genders, tumor size, lymphatic metastasis and tumor locations ($P > 0.05$).

Besides, the correlations between LAMP1 expression levels and clinical data were analysed using Spearman Correlation Coefficient (r_s). LAMP1 expression levels were positively correlated with TNM stages (Fig. 2E, $P < 0.05$). The higher the TNM stages, the higher the LAMP1 expression levels. Similar results also appeared in degrees of tumor histological differentiation (Fig. 2D, $P < 0.01$), but not in ages, genders, tumor size, T status, lymphatic metastasis and tumor locations ($P > 0.05$).

4. Discussion

In this study, the results showed that the LAMP1 expression levels were significantly different between different T status, TNM stages and degrees of tumor histological differentiation, and that LAMP1 expression was significantly correlated with TNM stages and degrees of tumor histological differentiation (Table 1, Fig. 2E and F). These results suggested that LAMP1 may be involved in the progression and histological differentiation of ESCC.

LAMP1 is localized at the lysosomal membrane under physiological conditions and is characterized by heavily glycosylation [22,23]. Previous studies showed that LAMP1 has been identified expressing higher in many cancers, especially in the metastatic

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