


LC3B in Malignant Cells Correlates With Immune Infiltrate in Hypopharyngeal Squamous Cell Carcinoma

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Abstract

The objective of this study was to investigate the between autophagy activity and local immune response in hypopharyngeal squamous cell carcinoma (HSCC). Herein, we observed the expression of autophagy marker microtubule-associated protein light chain 3B (MAP1LC3B), CD8 cytotoxic T lymphocytes (CTLs), CD39 (regulatory T cells Tregs) and CD163 (tumor-associated macrophages TAMs) in HSCC, and determined the prognostic roles of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ in patients with HSCC. The expression of light chain 3B (LC3B) and CD8⁺/CD39⁺ was found to be significantly lower in HSCC tissues than in adjacent non-tumor mucosa tissue samples; LC3B expression was positively correlated with the infiltration rate of CD8⁺/CD39⁺ in HSCC. Further studies revealed that the ratio of CD8⁺/CD39⁺ immune cells was negatively correlated with tumor lymph node metastasis and TNM classification, while the ratio of CD8⁺/CD163⁺ immune cells was negatively correlated with TNM classification. Moreover, the expression of LC3B was analyzed and the patients were grouped according to their immune infiltration characteristics. The 5-year cumulative survival rates of LC3B⁺, CD8⁺/CD39⁺, and CD8⁺/CD163⁺ patients were significantly higher than those of other group patients. Collectively, our studies indicated that the expression of LC3B in HSCC was correlated with the infiltration ratio of immune cells, and a change in autophagy activity may affect the cellular immunity in HSCC. The ratios of tCD8⁺/CD39⁺ and tCD8⁺/CD163⁺ may serve as prognostic factors for HSCC.

Keywords

hypopharyngeal squamous cell carcinoma, autophagy, LC3B, immune cell, CD8, CD39, CD163

Abbreviations

ATP, Adenosine Triphosphate; CTL, Cytotoxic effector cell; ENTPD1, Ectonucleoside triphosphate diphosphohydrolase-1; HNSCC, Head and neck squamous carcinoma; HSCC, Hypopharyngeal squamous cell carcinoma; LC3, Light chain 3; MHC-I, Major histocompatibility complex I; OSCC, Oral squamous cell carcinoma; OS, Overall survival; RFS, Relapse-free survival; TIL, Tumor infiltrating lymphocyte; Tregs Regulatory, T cells; TAM, Tumor-associated macrophage

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Introduction

Hypopharyngeal malignancies, primarily categorized as squamous cell carcinomas, account for approximately 5% of head and neck malignancies.^{1,2} According to GLOBOCAN's statistics in 2018 estimates, there were about 80,608 new confirmed cases of hypopharyngeal cancer and 34,984 deaths reported globally, accounting for approximately 0.4% of the total sites cancers.³ Although the low incidence of hypopharyngeal squamous cell carcinoma (HSCC) is low, most patients present at advanced stages because early symptoms are non-specific. Approximately 70-85% of HSCCs are diagnosed at stage III or IV, with a 5-year overall survival rate of 15-45% for those

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patients.⁴ Therefore, it is imperative to explore the molecular mechanisms regulating HSCC and discover new diagnostic and prognostic biomarkers.

Autophagy is a highly conserved and ubiquitous catabolic process characterized by the degradation of intracellular components in lysosomes, an evolutionarily conserved process of self-depletion that involves maintaining organelle function to remove cellular waste and provide metabolic substrates.⁵ In normal cells, autophagy protects against excessive reactive oxygen species, DNA damage, and genomic instability.⁶ In some cases, autophagy may play a role in inhibiting cancer in the early stage of tumorigenesis,⁷ however, in the latter stages, autophagy maintains tumor metabolism and promotes tumor cell survival.^{8,9} Microtubule-associated protein light chain 3 (MAP1LC3), referred to as light chain 3 (LC3), is currently recognized as a marker of autophagy. LC3 in mammals can be divided into 3 types: LC3A, LC3B and LC3C. LC3B, as a type of LC3, can also be used as a molecular marker of autophagy.¹⁰

Immunogenicity plays an important role in the control of tumorigenesis. The interaction between the host's immune response and tumor cells often results in immune cell infiltration.¹¹ Studies have shown that tumor infiltrating lymphocytes (TILs) are important prognostic factors for a variety of tumors, such as esophageal cancer, renal cancer, breast cancer, colon cancer, and so on.¹²⁻¹⁵ Therefore, immune factors may play a crucial role in assessing the prognosis of HSCC patients. The factors affecting the immune function of tumors are relatively complex. Recent studies have found that changes in autophagy activity can affect the function of immune cells.¹⁶

Regulatory T cells (Tregs), an important subset of TILs, mediate self-antigen immune responses and inhibit abnormal immune responses. Tumor-infiltrating Tregs play an important role by suppressing antitumor immune responses, and promoting tumor immune escape.¹⁷ Regulatory T cells express a variety of surface antigens, including CD25, CD103, and CTLA-4, which are commonly used Treg immunology markers.¹⁸ Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1/CD39) is an adenosine generated enzyme, expressed at the cell surface of Tregs. It is a novel surface marker of Tregs,¹⁹ and plays a role in nucleotide hydrolysis promoting the secretion of adenosine, which affects both A2A receptors on the surface of T cells, inhibiting T-cell proliferation, migration, and antitumor effect.^{20,21}

Macrophages are important components of the body's natural immune response and can be divided into different subtypes in different tissues. In the tumor microenvironment, macrophages are primarily divided into M1 classically activated macrophages and M2 substitutively activated macrophages.²² The markers CD16/32, CD11b, and CD11c are highly expressed in M1-type macrophages, whereas markers CD163 and CD206 are highly expressed in M2-type macrophages.²³ Multiple studies have shown that M2 macrophage invasion of the tumor microenvironment could lead to tumor progression and is associated with angiogenesis, metastasis,

and immunosuppression. This macrophage phenotype is called tumor-associated macrophage (TAM).^{24,25}

CD8⁺ cytotoxic effector cells (CTLs) and Tregs, as well as the balance between CTLs and TAM have been shown to be important in tumor progression and prognosis. Tumor invasive CD8⁺T lymphocytes play an important role in the anti-tumor immune response and have prognostic value for various tumor types.²⁶ At present, no such studies have been reported in HSCC. Thus, to discover a better prognostic indicator of HSCC, the relationship between autophagy and immune infiltration was investigated

This study observed the autophagy marker LC3B²⁷ and the expression of cytotoxic T lymphocytes (CTL, expression of CD8²⁸), immunosuppressive factors of regulatory T cells (Treg expression of CD39¹⁹) and tumor-associated macrophage (TAM, expression of CD163²⁹) infiltration in HSCC and adjacent non-tumor mucosa tissue.

Materials and Methods

Patients and Tissue Specimens

Human tissue specimens were collected from the Department of Otolaryngology of The First Affiliated Hospital of Anhui Medical University from 2011 to 2014. A total of 70 surgical specimens of HSCC and 22 adjacent non-tumor mucosa tissue samples were collected. Sample use was approved by the Biomedical and written informed consent was provided by all patients. All selected cases were first identified and did not receive neoadjuvant chemotherapy or radiotherapy. Diagnosis and histology classification were performed according to the World Health Organization's classification system, by 2 experienced pathologists. TNM staging was performed according to the American Joint Committee on Cancer (Eighth Edition AJCC). Formalin-fixed paraffin specimens with complete clinicopathological and follow-up data were selected.

Reagents

Rabbit anti-human LC3B (SAB5600101) was purchased from Sigma-Aldrich (St Louis, MO, USA). Mouse anti-human CD39 (TAB804559) and 3,3-diaminobenzidine (DAB) were purchased from Beijing Chinese Zhongshan Golden Bridge. Ready-to-use antibody CD8 (RMA-0514), CD163 (MAB-0206), sheep rabbit/rat secondary antibody (ab5878) were purchased from Fuzhou Maxim Biotech (China).

Immunohistochemistry

For immunohistochemistry, 4- μ m-thick sections of 70 HSCC tissues and 22 adjacent normal mucosal tissues were deparaffinized and rehydrated. Antigen retrieval was carried out by microwave treatment of slides in 50 mM Tris-EDTA (pH 9.0) for 20 min. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 10 min at 37°C. Slides were incubated with rabbit polyclonal anti-LC3B antibody (1:75), mouse anti-human CD39 (1:200), and ready-to-use antibody CD8,

Table 1. Expression Patterns of LC3B and CD8⁺/CD39⁺ CD8⁺/CD163⁺ in Hypopharyngeal Squamous Cell Carcinoma (HSCC) and Adjacent Non-Tumor Tissues.

Tissues	N	CD8 ⁺ /CD39 ⁺	P	CD8 ⁺ /CD163 ⁺	P	LC3B ⁺	P
HSCC	70	31 (44.3%)		30 (42.9%)		25 (35.71%)	
Normal tissues	22	16 (72.7%)	0.0199*	16 (72.7%)	0.0145*	15 (68.2%)	0.0074**

* $p < 0.05$, ** $p < 0.01$.

CD163 overnight at 4°C. Thereafter, sections were incubated with sheep rabbit/rat secondary antibody at 37°C for 15 min. The chromogenic reaction was carried out using DAB. Slides were counterstained with hematoxylin. For negative controls, the primary antibody was PBS.

Quantification Methods

Diagnoses were performed by viewing of radiographs by 2 senior double-blinded pathologists. All samples were anonymous and the pathologists had no knowledge of the clinicopathological data before diagnosis by double-blind radiographs by 2 senior pathologists. LC3B was expressed in the cytoplasm of tumor cells, showing a brown-yellow color as a positive marker. In at least 5 high-power microscope fields (200×), the experimental results were determined according to cell staining intensity. The LC3B proportion of positive cells were divided into 4 categories: 0 (5%), 1 (6%–25%), 2 (26%–50%), and 3 (>50%). A score of 0 to 1 was defined as negative expression, and a score >2 was defined as positive.³⁰ CD8 and CD39 were expressed in the cell membrane of lymphocytes with corresponding immunophenotypes, and CD163 was expressed in the cell membrane of antigen-presenting cells with corresponding immunophenotypes, all of which were brown. Positive staining of CD39 in 5 fields was detected under a microscope (200×) by the optical density method.³¹ At low magnification, 10 independent fields were observed. The 5 regions with the highest numbers of CD8- and CD163-positive cells were selected, counted at high magnification, and averaged.³¹ We selected the median intratumoral CD8/CD39, CD8/CD163 cell count as the cutoff for possible risk factors.³¹

Overall Survival and Relapse-Free Survival

Overall survival (OS) rate and relapse-free survival (RFS) were calculated from data received telephonically and hospital follow-up in which patient status, time of death, immediate cause of death, and time of recurrence was recorded.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software Inc. USA) and SPSS 25.0 (SPSS Inc. USA). The chi-square test was used for enumeration data, the independent sample t-test was used for comparison between 2 groups, and 1-way analysis of variance was used for comparison between multiple groups. Spearman's correlation analysis

was performed on protein expression, and $P < 0.05$ was considered statistically significant.

Results

LC3B Expression in HSCC and Adjacent Non-Tumor Mucosa Tissues, and the Ratio of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ Immune Cells.

Immunohistochemical results showed that LC3B was expressed in HSCC tissues and adjacent non-tumor mucosa tissues to different degrees, and the positive rate was higher in adjacent non-tumor mucosa tissues (68.18%) than that in HSCC tissues (35.71%). The difference between both the 2 groups was statistically significant ($P < 0.05$, Table 1, Figure 1). The positive ratio of CD8⁺/CD39⁺ in HSCC tissues and adjacent non-tumor mucosa tissues was 44.29% and 72.73%, respectively. The positive ratios of CD8⁺/CD163⁺ in HSCC tissues and adjacent non-tumor mucosa tissues was 42.86% and 72.73%, respectively. ($P < 0.05$, Table 1, Figure 1).

Correlation Between LC3B Expression, and the Ratio of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ Immune Cells in HSCC.

The 70 cases of HSCC were divided into LC3B positive and LC3B negative groups according to LC3B expression. The positive rate of infiltration of CD8⁺/CD39⁺ was (15/25, 60%) in the LC3B positive group and (16/45, 35.6%, $P < 0.05$) in the negative group. The positive rate of infiltration of CD8⁺/CD163⁺ was (14/25 56%) in the LC3B positive group and (16/45, 35.6%) in the negative group. Spearman's correlation analysis showed that there was a positive and significant correlation between LC3B expression and the infiltration of CD8⁺/CD39⁺ immune cells (Table 2)

The Relationship Between LC3B Expression, the Ratio of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ Immune Cells, and the Clinicopathological Features of HSCC

No significant correlation was observed between LC3B expression and the clinicopathological features of HSCC; and the infiltration amount of CD8⁺/CD39⁺ negatively correlated with lymph node metastasis and clinical stage ($P < 0.05$). Additionally, the infiltration amount of CD8⁺/CD163⁺ negatively correlated with lymph node metastasis ($P < 0.05$, Table 3).

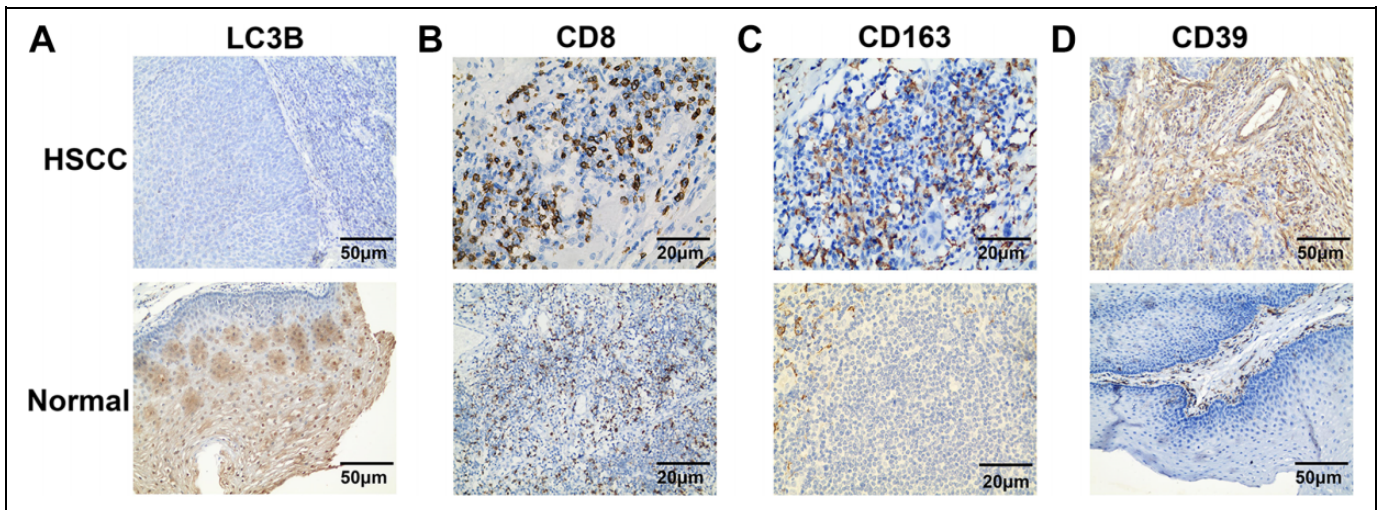


Figure 1. Representative immunohistochemical staining patterns. (A) Hypopharyngeal squamous cell carcinoma (HSCC) specimen and paracancer normal tissues were stained for the detection of LC3B (200 \times), (B) CD8+ cytotoxic T lymphocytes (400 \times), (C) CD163+ tumor-associated macrophages (400 \times), (D) CD39+ regulatory T cells (200 \times).

Table 2. The Correlation Between LC3B and the Infiltration of CD8⁺/CD39⁺, CD8⁺/CD163⁺ Immune Cells in Hypopharyngeal Squamous Cell Carcinoma (HSCC).

	CD8 ⁺ /CD39 ⁺		CD8 ⁺ /CD163 ⁺	
	High		High	
LC3B ⁺	15 (68.2%)	P = 0.0494*	14 (63.6%)	P = 0.1005
LC3B ⁻	16 (72.7%)	r = 0.2358	16 (72.7%)	r = 0.1980

* $p < 0.05$.

Relationship Between LC3B Expression, Immune Cell Ratio in HSCC, and Overall Survival Rate of HSCC Patients

According to Kaplan Meier curves and the Log-rank test, we found that patients with HSCC tissue LC3B⁺ overall survival rate is higher than that of negative patients ($P < 0.05$, Figure 2A), and the CD8⁺/CD39⁺ and CD8⁺/CD163⁺ overall survival rate of patients was higher than other groups ($P < 0.05$ and $P < 0.05$, respectively; Figure 2B and 2C, respectively). Moreover, in adjacent non-tumor mucosa tissue patients, the LC3B⁺ overall survival rate was better than that of negative patients. The CD8⁺/CD39⁺ and CD8⁺/CD163⁺ overall survival rate of patients was higher than the other groups; however, the difference was not statistically significant ($P > 0.05$, Figure 2D–F). Further analysis showed that the overall survival rate of LC3B⁺ and CD8⁺/CD39⁺, LC3B⁺, and CD8⁺/CD163⁺ tumor tissue patients was significantly higher than those of other groups ($P < 0.05$, Figure 3A and 3B), and the overall survival rate of patients in the above 2 groups, in adjacent non-tumor mucosa tissues, was also significantly higher than that of other groups; however the difference was not statistically significant ($P > 0.05$, Figure 3C and 3D).

Relationship Between LC3B Expression, Immune Cell Ratio, and Relapse-Free Survival Rate in HSCC

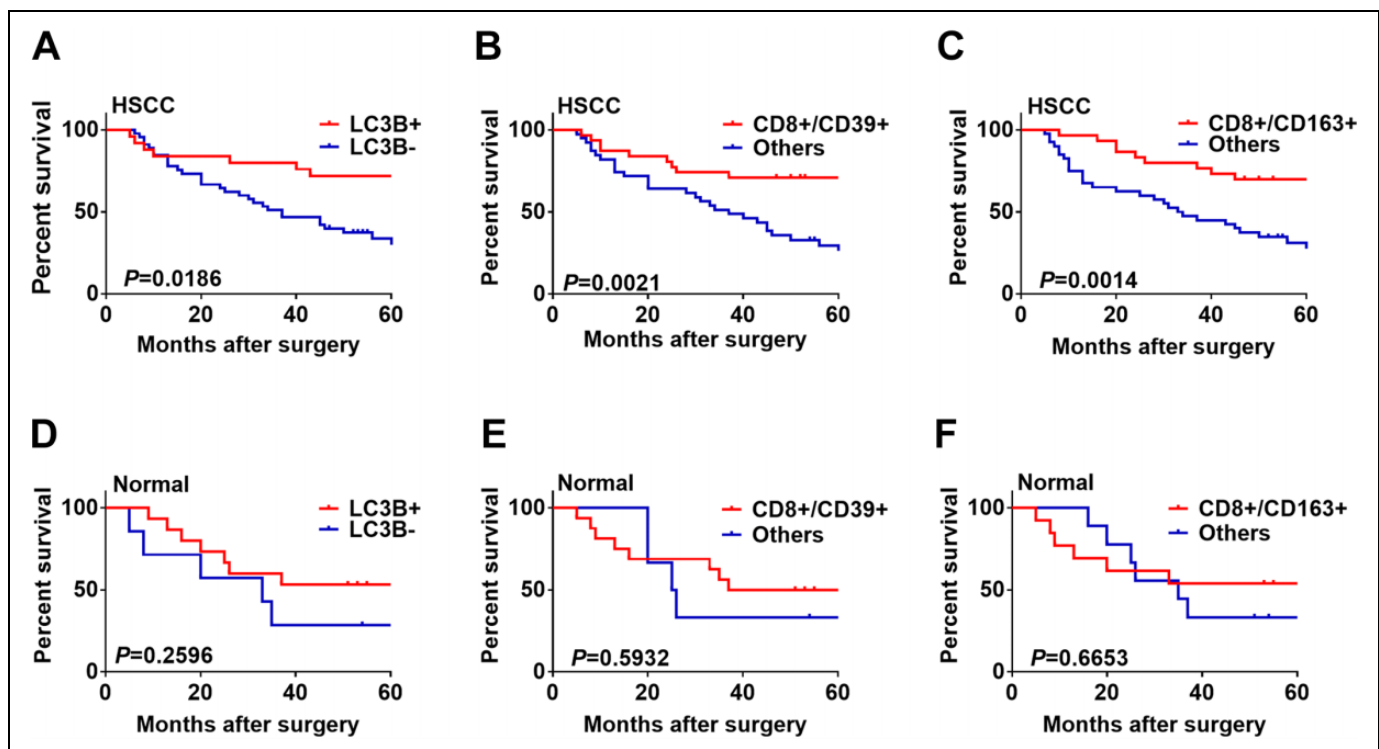
Kaplan Meier curves and the Log-rank test showed that with LC3B⁺, CD8⁺/CD39⁺, and CD8⁺/CD163⁺ patients, with co-positive expression of LC3B and CD8⁺/CD39⁺, CD8⁺/CD163⁺, regardless of in HSCC tissues or adjacent non-tumor mucosa tissues all had a higher relapse-free survival rate than other patient groups; however, the difference was not statistically significant ($P > 0.05$ Figures 4 and 5).

Discussion

HSCC is a highly malignant squamous cell carcinoma of the head and neck with poor clinical prognosis.³² The molecular mechanism regulating the development of hypopharyngeal cancer remains unclear. Currently, surgery combined with radiotherapy is the most effective treatment, and its efficacy is markedly better than radiotherapy alone and surgery alone; however, immunotherapy has not been fully applied.³³ In adaptive immunity, the autophagy pathway has been shown necessary for antigen presentation, and downregulation of the major histocompatibility complex I (MHC-I) has been reported to be directly or indirectly involved in the mechanism of immune escape.³⁴ Autophagy is involved in cancer-related regulation, and its particular in cancer is complex and most likely associated with the internal environment.³⁵ In other types of tumors, CD8 infiltration is functionally considered cytotoxic/inhibits T lymphocytes and is typically associated with a good prognosis.³⁶ Distel et al.³⁷ reported 62 patients with low-risk head and neck squamous carcinoma (HNSCC) and high CD8 expression, who had a higher survival rate after first stage surgery and external radiotherapy. Additionally, Wang et al. reported that patients with high CD8⁺ T cell infiltration had a better prognosis than those with low CD8⁺ T cell infiltration. Moreover,

Table 3. Association of LC3B Expression and Infiltration of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ Immune Cells in hypopharyngeal Squamous Cell Carcinoma (HSCC) With Clinicopathological Parameters From HSCC Patients.

Variable	N	LC3B ⁺	P	CD8 ⁺ /CD39 ⁺	P	CD8 ⁺ /CD163 ⁺	P
Gender							
Male	65	23(35.4%)		29(44.6%)		29(44.6%)	
Female	5	2(40%)	0.9999	2(40%)	0.9999	1(20%)	0.3832
Age/year							
≥63years	31	11(35.5%)		17(54.8%)		13(41.9%)	
< 63 years	29	14(48.3%)	0.1414	14(48.3%)	0.1130	17(58.6%)	0.8895
Histologic differentiation							
Well	12	3(25%)		3(25%)		3(25%)	
Moderate	48	17(35.4%)	0.4745	23(47.9%)	0.3332	21(43.8%)	0.2493
Poor	10	5(50%)		5(50%)		6(60%)	
Node metastasis							
N0	30	11(36.7%)		18(60%)		17(56.7%)	
N1	40	14(35%)	0.8855	13(32.5%)	0.0219*	13(32.5%)	0.0432*
Clinical Stage							
I, II, III	41	15(36.6%)		23(56.1%)		21(51.2%)	
IV	29	10(34.5%)	0.8565	8(27.6%)	0.0180*	9(31%)	0.0928

p* < 0.05.Figure 2.** Overall survival analyses based on LC3B and related immune cell ratio. Kaplan-Meier curves of overall survival in relation to LC3B (A and D), CD8⁺/CD39⁺ (B and E) CD8⁺/CD163⁺ (C and F).

CD8⁺ T cell infiltration was closely related to adenocarcinoma of the esophagogastric junction (AEG) patient TNM stage.³⁸ Recent studies have shown that CD39 is a member of the ex-nucleoside triphosphate bisphosphonolytic enzyme (e-ntpdase) family and plays an important role in regulating cancer progression in several human cancers. For example, Magis et al.

showed that CD39⁺ Treg frequency and adenosine mediated inhibition were significantly increased (*P* < 0.05) in HNSCC patients, and that CD39 expression on Treg was significantly correlated (*P* < 0.05) with HNSCC disease stage.³⁹ Mouse experiments have shown that autophagy can promote the release of immune nonirritating chemokine adenosine

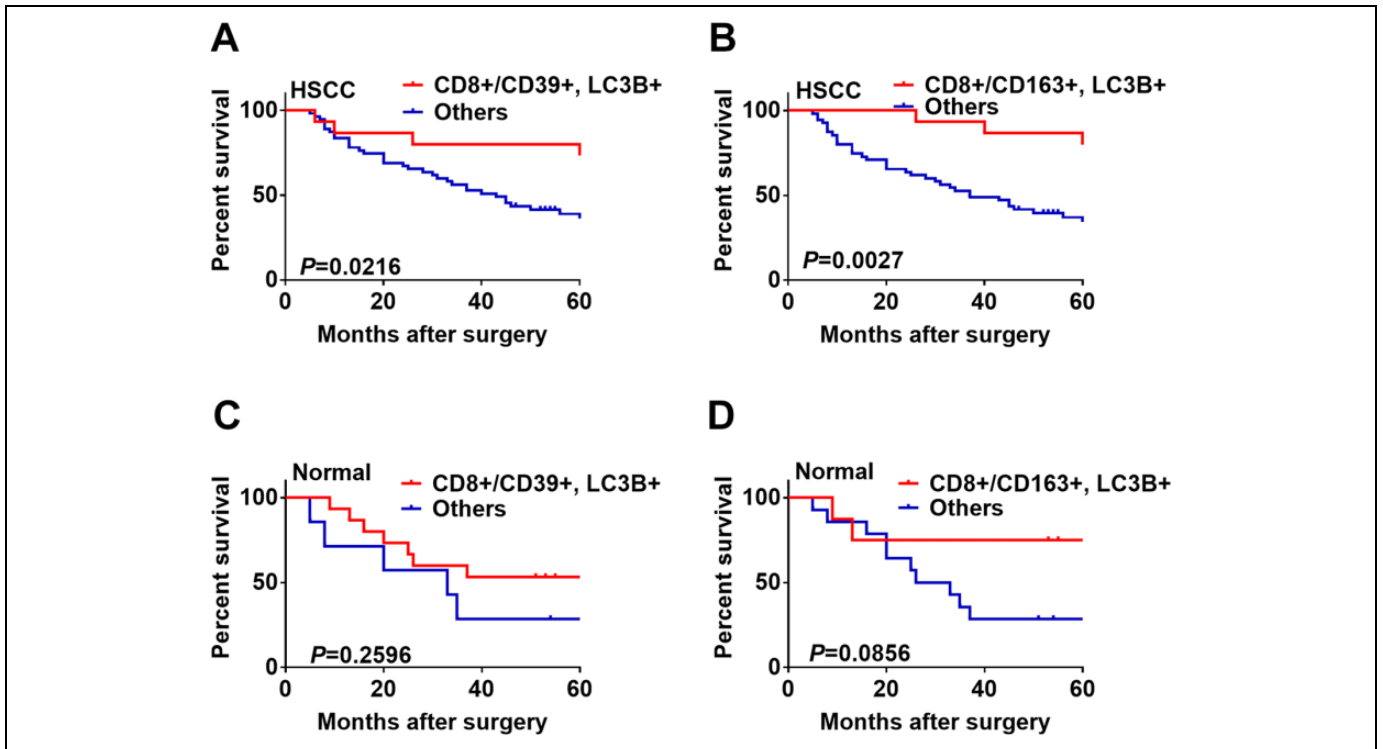


Figure 3. Kaplan-Meier curves of overall survival in relationship to the LC3B+ and CD8+/CD39+ co-positive, (A and C) LC3B+ and CD8+/CD163+ co-positive (B and D).

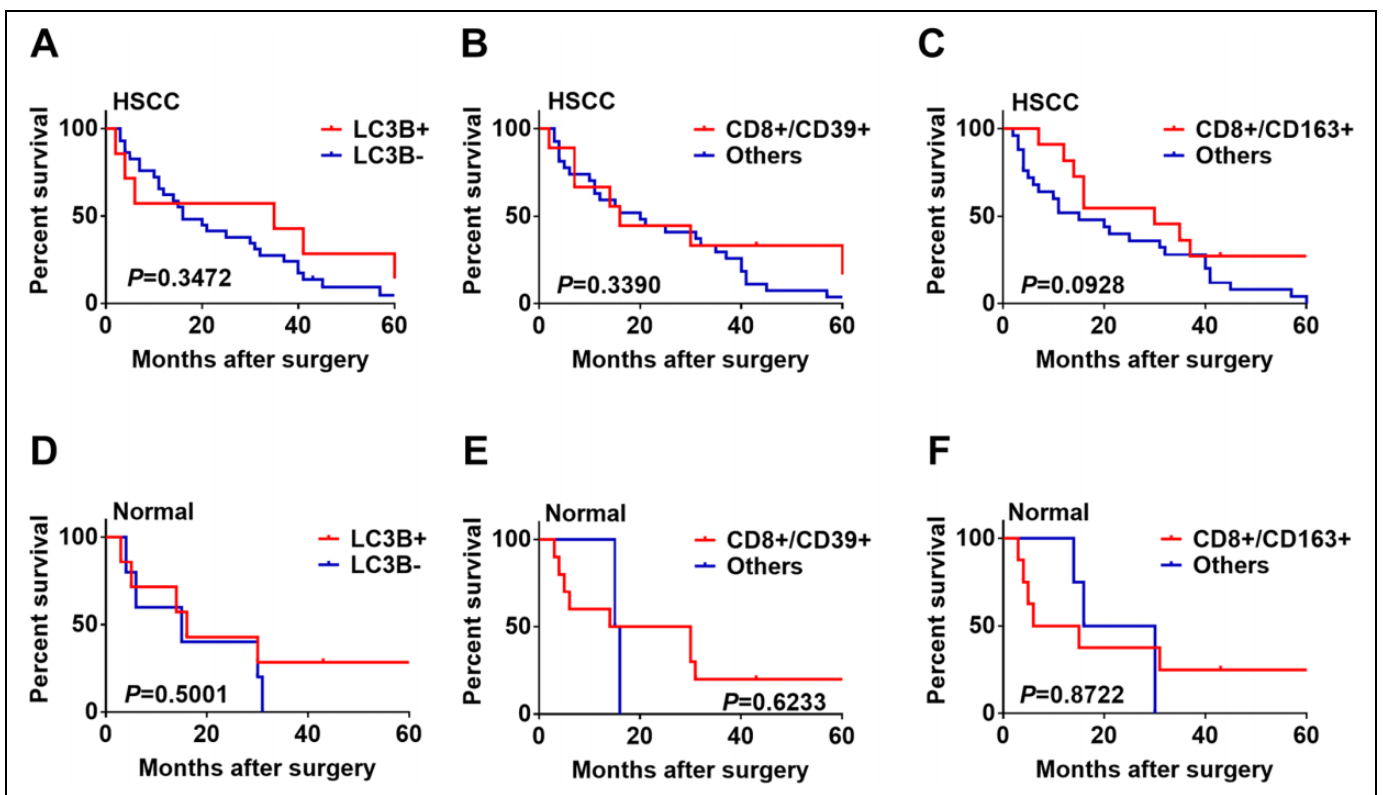


Figure 4. Relapse-free survival analyses based on LC3B and related immune cells ratio. Kaplan-Meier curves of overall survival in relationship to the LC3B (A and D), CD8+/CD39+ (B and E) CD8+/CD163+ (C and F).

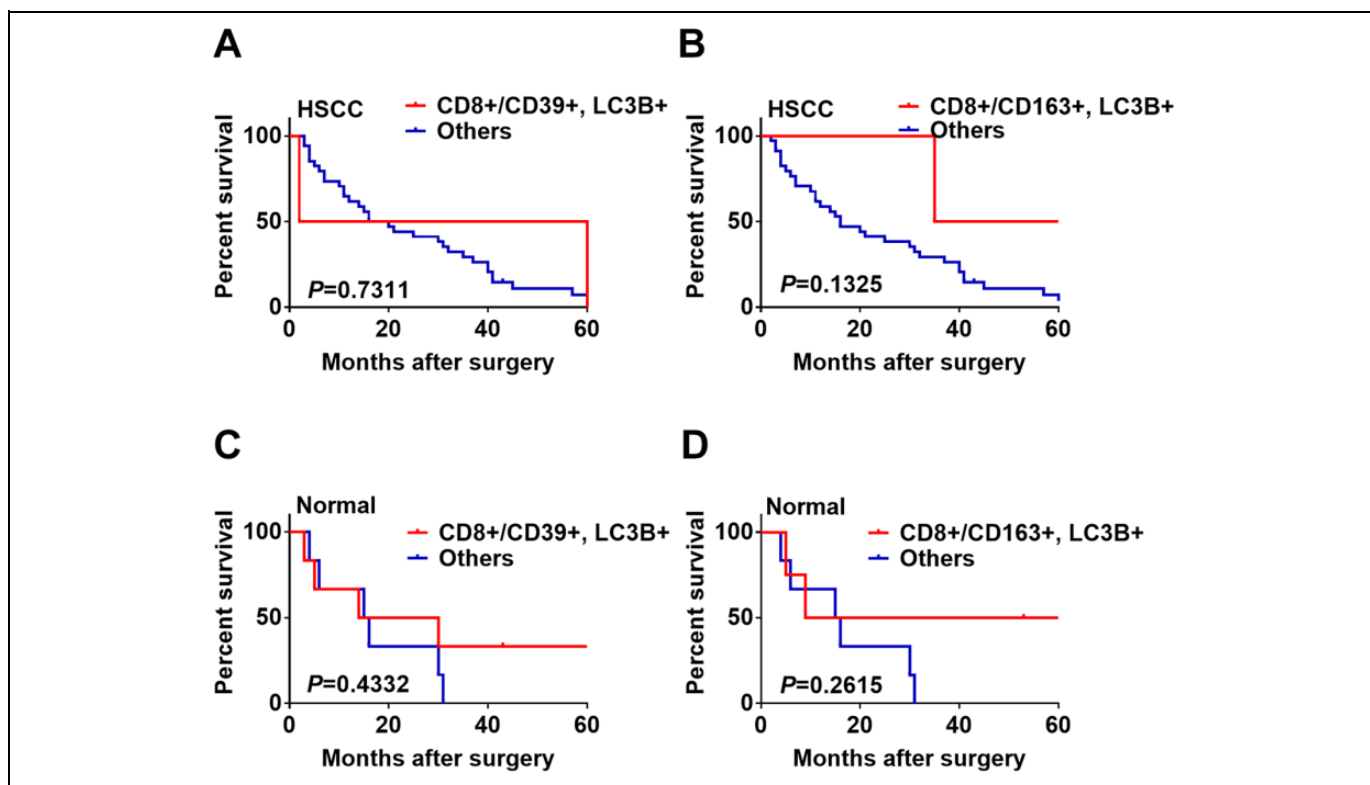


Figure 5. Kaplan-Meier curves of relapse-free survival in relationship to the LC3B⁺ and CD8⁺/CD39⁺ copositive (A and C), LC3B⁺ and CD8⁺/CD163⁺ co-positive (B and D).

triphosphate (ATP) by stressed cancer cells, and inhibit local production of adenosine, conducive to the recruitment of immunosuppressive Treg.⁴⁰⁻⁴² CD163 positive macrophages positively correlated with the histological malignancy of human ovarian tumors, thus, CD163 is a useful marker for human TAM activation.⁴³ In addition, in malignant lymphoma, glioma, and renal cancer, the higher the expression of CD163 in TAMs, the poorer the clinical prognosis.⁴⁴ Studies have demonstrated that CD163⁺ TAMs promote T cell apoptosis and immunosuppression via IL-10 and PD-L1, and predicts poor prognosis in oral squamous cell carcinoma (OSCC) patients.⁴⁵ Moreover some studies have investigated autophagy and immune infiltration. Autophagy induced MHC-I expression and increased CD8⁺ T cell infiltration in non-small cell lung cancer⁴⁶; autophagy inhibition upregulated PD-L1 expression in gastric cancer cells in vitro and in vivo.⁴⁷ Ladoire S et al. showed that autophagy deficiency had poor prognostic value in breast cancer patients because of its negative impact on the immune system's ability to suppress tumor lesions.⁴⁸

Here, we detected the expression of LC3B, CD8, CD39 and CD163 in the tumors and para-cancer tissues of hypopharyngeal cancer patients. The expression levels of LC3B in para-cancer tissues were positive, and the ratios of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ in tumor tissues was lower than that in para-cancer tissues. The ratio of CD8⁺/CD39⁺ was negatively correlated with the lymph node metastasis and clinical stage of patients, and the ratio of CD8⁺/CD163⁺ was negatively

correlated with the lymph node metastasis in patients. Moreover, the ratios of CD8⁺/CD39⁺ and CD8⁺/CD163⁺ was associated with prognosis. We found that in patients with intra-tumor co-positive LC3B⁺ and CD8⁺/CD39⁺ and co-positive LC3B⁺ and CD8⁺/CD163⁺, the overall 5-year survival rate was higher than that of the other groups. Our study confirmed the influence of tLC3B⁺ and tCD8⁺/CD39⁺ ratio, tLC3B⁺ and tCD8⁺/CD163⁺ ratio, but not the pLC3B⁺ and pCD8⁺/CD39⁺ ratios and pLC3B⁺ and pCD8⁺/CD163⁺ ratio as prognostic factors in HSCC.

Furthermore, autophagy affected the relapse-free survival rate of patients with HSCC affected by autophagy and autophagy through immunity was also detected. We found that LC3B⁺, CD8⁺/CD39⁺, CD8⁺/CD163⁺, LC3B⁺, CD8⁺/CD39⁺, and LC3B⁺ and CD8⁺/CD163⁺ co-positive patients had higher RFS than other groups in both intra and peritumor immune infiltrates, but the effect was not significant ($P > 0.05$), possibly due to an insufficient number of cases.

Several mechanisms by which LC3B induced autophagy can prevent cancer by influencing the immune microenvironment. Firstly, autophagy leads to the death of tumor cells.⁴⁹ Secondly, autophagy can eliminate damaged organelles, remove the source of genotoxic substances including damaged DNA products, and prevent oxidative stress from damaging organelles.⁵⁰ The tumor inhibitory effects of autophagy also stems from the negative regulation of cell proliferation ability to quench excessive activation of autophagy in cancer and may

strengthen metabolism protein.⁵¹ The protein degradation process is likely to produce new and different molecular substances such as protein or antigen peptide conformations, including MHC-I and MHC-II molecules and the activity of these antigens affect different immune cell subsets. It is suggested that the change in autophagy level may be an important factor affecting the immune function of tumor cells.

In summary, the changes in autophagy activity of HSCC are closely associated with the degree of infiltration and prognosis of immune cells. The enhancement of autophagy activity of tumor cells is of great importance in improving the immune function of HSCC patients, and could potentially be a new implementation in research and development of tumor immunotherapy. However, the biological mechanism of autophagy is complex, and a variety of factors affect the immune function of tumors. In this study, the relationship between autophagy and known immune cells in HSCC was preliminarily discussed. However, the molecular biological mechanisms regarding the metabolism of autophagy proteins to produce new antigens, and changes in the corresponding immune response require further discussion.

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Author Contributions

C.G. and Z.W. conceived and designed the experiments, the data analysis; S.G., J.C., X.H., D.W., performed the experiments and the data analysis; S.G. wrote the paper; C.G. and Z.W. revised the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Statement

The use of the group was approved by the Biomedical Ethics Committee of Anhui Medical University with informed consent was signed by all relevant patients (approval No. 20200148.)

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References

- Qian Y, Liu D, Cao S, et al. Upregulation of the long noncoding RNA UCA1 affects the proliferation, invasion, and survival of hypopharyngeal carcinoma. *Mol Cancer*. 2017;16(1):68.
- Zhou J, Li Y, Wei D, et al. Overall survival with and without laryngeal function preservation in 580 patients with hypopharyngeal squamous cell carcinoma. *Oncol Rep*. 2015;34(6):3196-3202.
- Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018; 68(6):394-424.
- Jang JY, Kim EH, Cho J, et al. Comparison of oncological and functional outcomes between initial surgical versus non-surgical treatments for hypopharyngeal cancer. *Ann Surg Oncol*. 2016; 23(6):2054-2061.
- Green DR, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. *Cell*. 2014;157(1):65-75.
- Jin S.p53, Autophagy and tumor suppression. *Autophagy*. 2005; 1(3):171-173.
- Meng L, Liu S, Ding P, Chang S, Sang M. Circular RNA ciRS-7 inhibits autophagy of ESCC cells by functioning as miR-1299 sponge to target EGFR signaling. *J Cell Biochem*. 2020;121(2): 1039-1049.
- Rosenfeldt MT, O'Prey J, Morton JP et al. p53 status determines the role of autophagy in pancreatic tumour development. *Nature*. 2013;504(7479):296-300.
- Sha J, Han Q, Chi C, et al. Upregulated KDM4B promotes prostate cancer cell proliferation by activating autophagy. *J Cell Physiol*. 2020;235(3):2129-2138.
- Liu X, Wang B, Jiang X, et al. Heterozygous disruption of Beclin 1 alleviates zinc oxide nanoparticles-induced disturbance of cholesterol biosynthesis in mouse liver. *Int J Nanomedicine*. 2019;14: 9865-9875.
- Sokratous G, Polyzoidis S, Ashkan K. Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme. *Hum Vaccin Immunother*. 2017;13(11): 2575-2582.
- Okadome K, Baba Y, Yagi T, et al. Prognostic nutritional index, tumor-infiltrating lymphocytes, and prognosis in patients with esophageal cancer. *Ann Surg*. 2020;271(4):693-700.
- Ghatalia P, Gordetsky J, Kuo F, et al. Prognostic impact of immune gene expression signature and tumor infiltrating immune cells in localized clear cell renal cell carcinoma. *J Immunother Cancer*. 2019;7(1):139.
- Baxevanis CN, Sofopoulos M, Fortis SP, Perez SA. The role of immune infiltrates as prognostic biomarkers in patients with breast cancer. *Cancer Immunol Immunother*. 2019;68(10):1671-1680.
- Cao Y, Nishihara R, Qian ZR, et al. Regular aspirin use associates with lower risk of colorectal cancers with low numbers of tumor-infiltrating lymphocytes. *Gastroenterology*. 2016;151(5): 879-892.e4.
- Carleton G, Lum JJ. Autophagy metabolically suppresses CD8(+) T cell antitumor immunity. *Autophagy*. 2019;15(9): 1648-1649.
- Deaglio S, Dwyer KM, Gao W, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med*. 2007;204(6):1257-1265.
- Piccirillo CA, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring CD4+CD25+ regulatory T cells. *Trends Immunol*. 2004;25(7):374-380.

19. Gu J, Ni X, Pan X, et al. Human CD39(hi) regulatory T cells present stronger stability and function under inflammatory conditions. *Cell Mol Immunol.* 2017;14(6):521-528.
20. Sundstro MP, Stenstad H, Langenes V, et al. Regulatory T Cells from colon cancer patients inhibit effector T-cell migration through an adenosine-dependent mechanism. *Cancer Immunol Res.* 2016;4(3):183-193.
21. Ma S, Deng W, Liu J, et al. Blockade of adenosine A2A receptor enhances CD8+ T cells response and decreases regulatory T cells in head and neck squamous cell carcinoma. *Mol Cancer.* 2017;16(1):99.
22. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 2002;23(11):549-555.
23. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest.* 2012;122(3):787-795.
24. Jacques A, Bleau C, Turbide C, Beauchemin N, Lamontagne L. Macrophage interleukin-6 and tumour necrosis factor- α are induced by coronavirus fixation to Toll-like receptor 2/heparan sulphate receptors but not carcinoembryonic cell adhesion antigen 1a. *Immunology.* 2009;128(1pt2):e181-e192.
25. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol.* 2003;3(1):23-35.
26. Liu X, Xu D, Huang C, et al. Regulatory T cells and M2 macrophages present diverse prognostic value in gastric cancer patients with different clinicopathologic characteristics and chemotherapy strategies. *J Transl Med.* 2019;17(1):192.
27. Klionsky DJ, Abeliovich H, Agostinis P, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy.* 2008;4(2):151-175.
28. Ladoire S, Senovilla L, Enot D, et al. Biomarkers of immunogenic stress in metastases from melanoma patients: correlations with the immune infiltrate. *Oncoimmunology.* 2016;5(6):e1160193.
29. Weber M, Iliopoulos C, Moebius P, et al. Prognostic significance of macrophage polarization in early stage oral squamous cell carcinomas. *Oral Oncol.* 2016;52:75-84.
30. Wang J, Pan XL, Ding LJ, Liu DYDa-Peng Lei, Jin T. Aberrant expression of Beclin-1 and LC3 correlates with poor prognosis of human hypopharyngeal squamous cell carcinoma. *Plos One.* 2013;8(7):e69038.
31. Cai XY, Wang XF, Li J, et al. Overexpression of CD39 and high tumoral CD39(+)/CD8(+) ratio are associated with adverse prognosis in resectable gastric cancer. *Int J Clin Exp Pathol.* 2015;8(11):14757-14764.
32. Hall SF, Groome PA, Irish J, O'Sullivan B. The natural history of patients with squamous cell carcinoma of the hypopharynx. *Laryngoscope.* 2008;118(8):1362-1371.
33. Mura F, Bertino G, Occhini A, Benazzo M. Surgical treatment of hypopharyngeal cancer: a review of the literature and proposal for a decisional flow-chart. *Acta Otorhinolaryngol Ital.* 2013;33(5):299-306.
34. Park IA, Hwang S, Song IH, et al. Expression of the MHC class II in triple-negative breast cancer is associated with tumor-infiltrating lymphocytes and interferon signaling. *Plos One.* 2017;12(8):e0182786.
35. Ren J, Taegtmeier H. Too much or not enough of a good thing—The Janus faces of autophagy in cardiac fuel and protein homeostasis. *J Mol Cell Cardiol.* 2015;84:223-226.
36. Anitei MG, Zeitoun G, Mlecnik B, et al. Prognostic and predictive values of the immunoscore in patients with rectal cancer. *Clin Cancer Res.* 2014;20(7):1891-1899.
37. Distel LV, Fickenscher R, Dietel K, et al. Tumour infiltrating lymphocytes in squamous cell carcinoma of the oro- and hypopharynx: prognostic impact may depend on type of treatment and stage of disease. *Oral Oncol.* 2009;45(10):e167-e174.
38. Wang Y, Wang S, Zhu C, Cao H, Zhang Z, Zhao E. The association between immune characteristic and clinical pathology in Chinese patients with adenocarcinoma of esophagogastric junction. *Cancer Manag Res.* 2020;12:3259-3269.
39. Mandapathil M, Szczepanski MJ, Szajnik M, et al. Increased ectonucleotidase expression and activity in regulatory T cells of patients with head and neck cancer. *Clin Cancer Res.* 2009;15(20):6348-6357.
40. Martins I, Wang Y, Michaud M, et al. Molecular mechanisms of ATP secretion during immunogenic cell death. *Cell Death Differ.* 2014;21(1):79-91.
41. Michaud M, Martins I, Sukkurwala AQ, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science.* 2011;334(6062):1573-1577.
42. Rao S, Tortola L, Perlot T, et al. A dual role for autophagy in a murine model of lung cancer. *Nat Commun.* 2014;5:3056.
43. Yuan X, Zhang J, Li D, et al. Prognostic significance of tumor-associated macrophages in ovarian cancer: a meta-analysis. *Gynecol Oncol.* 2017;147(1):181-187.
44. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell.* 2010;141(1):39-51.
45. Kubota K, Moriyama M, Furukawa S, et al. CD163(+)/CD204(+) tumor-associated macrophages contribute to T cell regulation via interleukin-10 and PD-L1 production in oral squamous cell carcinoma. *Sci Rep.* 2017;7(1):1755.
46. Zeng H, Zhang W, Gong Y, Xie C. Radiotherapy activates autophagy to increase CD8(+) T cell infiltration by modulating major histocompatibility complex class-I expression in non-small cell lung cancer. *J Int Med Res.* 2019;47(8):3818-3830.
47. Wang X, Wu W, Gao J, et al. Autophagy inhibition enhances PD-L1 expression in gastric cancer. *J Exp Clin Cancer Res.* 2019;38(1):140.
48. Ladoire S, Enot D, Senovilla L, et al. The presence of LC3B puncta and HMGB1 expression in malignant cells correlate with the immune infiltrate in breast cancer. *Autophagy.* 2016;12(5):864-875.
49. Buccarelli M, Marconi M, Pacioni S, et al. Inhibition of autophagy increases susceptibility of glioblastoma stem cells to temozolomide by igniting ferroptosis. *Cell Death Dis.* 2018;9(8):841.
50. Galluzzi L, Baehrecke EH, Ballabio A, et al. Molecular definitions of autophagy and related processes. *EMBO J.* 2017;36(13):1811-1836.