The prognostic value of B7-H6 in esophageal squamous cell carcinoma

Huan Zhou
First Affiliated Hospital of Guangdong Pharmaceutical College
Jun Dong
Sun Yat-sen University Cancer Center
Liyi Guo
Huiyang Hospital Affiliated Southern Medical University
Xicheng Wang
First Affiliated Hospital of Guangdong Pharmaceutical College
Kailin Wang
First Affiliated Hospital of Guangdong Pharmaceutical College
Xiuyu Cai
Sun Yat-sen University Cancer Center
Shu Yang (y665925@126.com)
First Affiliated Hospital of Guangdong Pharmaceutical College https://orcid.org/0000-0002-6811-1917

Research article

Keywords: B7-H6, Esophageal squamous cell carcinoma, Immunohistochemistry, prognosis.

Posted Date: October 28th, 2019

DOI: https://doi.org/10.21203/rs.2.12817/v2

Version of Record: A version of this preprint was published at Scientific Reports on December 1st, 2019.
See the published version at https://doi.org/10.1038/s41598-019-54731-9.

Abstract

Background : B7-h6, a member of the B7 family molecules, participates in the clearance of tumor cells by binding to NKp30 on NK cells. The expression of B7-H6 in esophageal squamous cell carcinoma (ESCC) and the clinical significance is unknown. The goal of this study was to determine the expression of B7-H6 in ESCC and further explore its clinical significance. Patients and methods: We retrospectively collected clinical data from 145 patients diagnosed with ESCC between January 2007 and December 2008. These patients had all previously undergone surgical treatment for esophageal cancer, were clearly diagnosed, and had not received chemotherapy or radiotherapy. In addition, pathological tissue samples from the 145 patients were collected to detect the expression of B7-H6 by immunohistochemistry. The chi-square ($\chi 2$) test was used to analyse the relationships between B7-H6 and clinicopathological characteristics. The prognosis of the patients was analysed by Cox proportional hazards regression analysis and Kaplan-Meier analysis. Results: 133/145 (91.72%) of the ESCC tissue samples exhibited B7-H6 expression. The expression level of B7-H6 was correlated with T stage (P=0.036) and lymphatic metastasis status (P =0.044). According to the results of the ROC curve analysis, H-score =90 was selected as the cut-off value. The 145 patients were divided into two groups, the high B7-H6 expression (H-score≥90) group and the low B7-H6 expression (H-score≤90) group. Cox proportional hazards regression analysis indicated that tumour size (P =0.021), B7-H6 expression (P =0.025) and lymphatic metastasis status (P =0.049) were independent prognostic factors for ESCC. Kaplan-Meier analysis with the log-rank test demonstrated that the patients with high B7-H6 expression (P = 0.003), lymphatic metastasis (P <0.001) or a tumour size ≥ 3.0 cm (P = 0.001) had significantly worse survival than those with low B7-H6 expression, no lymphatic metastasis or a tumour size < 3.0 cm respectively. Conclusion: Our findings suggest that B7-H6 is widely expressed in ESCC samples. And B7-H6 may represent a predictor of poor prognosis for ESCC. Keywords: B7-H6, Esophageal squamous cell carcinoma, Immunohistochemistry, prognosis.

Background

Esophageal cancer is one of the most common malignant tumours in the world, ranking as the 6th most common cause of cancer-related death and the 8th most common cancer in the world, with an increasing incidence (1). Esophageal squamous cell carcinoma (ESCC) accounts for approximately 90% of esophageal cancer cases, which always in advanced stage when first diagnosed, with a low 5-year overall survival (OS) rate of about15%~25% (1). In recent years, although surgery, radiotherapy, chemotherapy, biological therapy and other comprehensive treatment methods have been widely used for the treatment of esophageal cancer, the prognosis and 5-year survival rate of esophageal cancer patients have remained very poor. Therefore, in-depth studies of the molecular mechanism underlying the occurrence and development of esophageal squamous cell carcinoma and the search for new molecular targets for diagnosis and prognostic monitoring have important clinical application value.

Tumour immune escape is an important molecular mechanism in the processes of tumourigenesis, invasion and metastasis. Tumour cells are usually unable to provide effective antigen signals, or the body has a defective immune response and is immunosuppressed, resulting in tumour cells being able to resist

attacks by immune effector cells and escape the body's immune monitoring (2). In this process, costimulatory molecules and their regulatory networks play an important role. Costimulatory molecules are mainly divided into two superfamilies, B7/CD28 and TNF/TNFR (3). Recently discovered new members of the B7 family of costimulatory molecules, such as B7-H1, B7-H3, B7-H4 and B7-H6, are widely expressed in many human tumour tissue types and can participate in the negative regulation of the T/natural killer (NK) cell-mediated antitumour immune response, thus attracting wide attention (3, 4). Studies have revealed that the expression of PD-L1 (B7-H1) and B7-H3 in esophageal cancer is associated with patient prognosis (5-7). Ling Wang et al reported B7-H3 and B7-H4 were widely expressed in esophageal squamous cell carcinoma tissues. High B7-H3 and B7-H4 expression was associated with advanced TNM stage and lymph node metastasis. Patients with both B7-H3 and B7-H4 high-expressed tumors had the poorest prognosis (8). Additionally, Lijie Chen et al demonstrated that B7-H4 expression level was associated with ESCC progression and survival, by reducing tumor immunosurveillance (9).

B7-H6 is a newly identified ligand in the B7 family (10, 11). It is a type I transmembrane protein that shows considerable homology with the B7-H1 and B7-H3 proteins (12). The extracelluar region contains one IgV-like domain and one IgC-like domain. Gordon Joyce et al. have verified that the extracellular domain of NKp30 directly and selectively interacts with the extracellular domain of B7-H6, which used residue mutation strategy (13). Studies have shown that B7-H6 can be activated by binding to the activating receptor NKp30 on the surface of NK cells and that B7-H6 promotes TNF- and IFN-mediated killing of target cells by NK cells, which is one of the important mechanisms of NK cell-mediated antitumour immunity (12). B7-H6 mRNA expression was found in human primary lymphoma, leukemia, ovarian cancer, brain tumour, breast cancers, renal cell carcinoma , and various sarcomas potentially express large amounts of B7-H6 (11). The expression level of the B7-H6 protein was significantly upregulated in cancer tissue compared with normal tissue, which was closely related to the clinicopathological characteristics and prognosis of patients (14-17). However, until now, no reports have studied the clinical significance of B7-H6 expression in ESCC. In the present study, we used immunohistochemistry to investigate B7-H6 expression in esophageal squamous cell carcinoma tissue samples and to analyse the clinical implications of this expression.

Materials And Methods

Patient characteristics

We retrospectively collected clinical data from 145 patients with esophageal squamous cell carcinoma between January 2007 and December 2008. These patients had all undergone surgical treatment for esophageal cancer, were clearly diagnosed, and had not received chemotherapy or radiotherapy. In addition, pathological tissue samples from the 145 patients were collected to detect the expression of B7-H6 by immunohistochemistry. In addition, 7 non-malignant esophageal tissue samples from the non-malignant portion of the esophagus were collected and used as controls. The clinicopathological data of all patients were available and were included in the statistical analysis.

Antibodies and major reagents

A rabbit anti-human B7-H6 polyclonal antibody (ab121794) was purchased from Abcam (Cambridge, MA, USA; dilution 1/100), and a horseradish peroxidase (HRP)-conjugated anti-mouse/rabbit secondary antibody was purchased from Dako (Glostrup, Denmark). A DAB colour developing kit was purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China; cat. no. zli-9017).

Immunohistochemistry

Paraffin-embedded tissue samples were cut into 5 μm serial sections and baked for 30 minutes in a 60°C constant-temperature box. The sections were deparaffinized with xylene and rehydrated through a graded ethanol series. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution for 10 minutes, and antigen retrieval was performed at 100°C for 20 minutes in a sodium citrate buffer solution (0.01 mmol/L, pH 6.0). After being soaked in distilled water for 10 minutes, the sections were incubated with 10% foetal bovine serum to block nonspecific binding. Next, the sections were incubated with the rabbit anti-human B7-H6 polyclonal antibody at 4°C overnight and then incubated with the HRP-conjugated goat anti-mouse/rabbit secondary antibody at room temperature for 60 minutes. The excess secondary antibody was removed by washing with TBS and developed with DAB colourant; the sections were stained with haematoxylin, dehydrated with an alcohol gradient, dried and sealed with neutral resin.

Evaluation of immunohistochemical (IHC) staining

The esophageal squamous cell carcinoma tissue samples were examined by two independent senior pathologists who were not informed of the patients' clinicopathological characteristics. The B7-H6 immunohistochemical staining results were analyzed according to a previously described method: (9, 18) H-score = (% tumour cells unstained × 0) + (% tumour cells stained weakly × 1) + (% tumour cells stained moderately × 2) + (% tumour cells stained strongly × 3). The staining intensity was scored as "0" (no staining), "1" (weakly stained), "2" (moderately stained), or "3" (strongly stained). The H-scores ranged from 0 (100% negative tumour cells) to 300 (100% strongly stained tumour cells). The results from the two pathologists were averaged and used in the statistical analyses.

Statistical analyses

Overall survival (OS), which was defined as the time from surgery to patient death or last follow-up, was used as a measure of prognosis. The final follow-up date was December 24, 2018. SPSS software (version 13.0; IBM Corp., Armonk, NY, USA) was used for data analysis. The correlation between the B7-H6 expression level and different clinicopathological parameters was analysed by the chi-square ($\chi 2$) test, and survival data were analysed by univariate and multivariate Cox regression analyses, receiver operating characteristic (ROC) curve analysis, and Kaplan-Meier analysis with the log-rank test. Statistical significance was defined at $P<0.05$.

Results

Study population

Patient characteristics are presented in Table 1. The median age of the study population was 60 years (range from 34 to 88 years). There were 82 cases (56.6%) of grade I-II disease and 63 cases (43.4%) of grade III- IV disease, according to TNM staging. Of the 145 patients examined, 79 (54.5%) died before the end of the observation period.

B7-H6 expression in esophageal tissue

In order to determine the expression levels of the B7-H6 protein in ESCC tissue, immunohistochemical analysis was performed (Figure 1). This approach revealed that B7-H6 was present in 133/145 (91.72%) of the samples. B7-H6 was always localized in the cytoplasm of the esophageal tumour cells, while weak B7-H6 staining was found in non-malignant esophageal tissue. In the 145 samples of esophageal squamous cell carcinoma, the median, maximum and minimum values of the B7-H6 staining H-score were 40, 180 and 0, respectively.

Relationships between B7-H6 and clinicopathological characteristics

The associations between the clinicopathological characteristics of the patients with esophageal squamous cell carcinoma and B7-H6 expression are presented in Table 2. To investigate the associations between the clinicopathological characteristics of esophageal cancer and B7-H6 protein expression, the 145 patients were divided into two major subgroups according to the intensity of the B7-H6 immunohistochemical staining as follows: $0 \leq$ H-score < 40 and H-score ≥ 40 (40 was the median value). This stratification revealed that B7-H6 expression was significantly correlated with T stage (P=0.036) and lymphatic metastasis status (P=0.044); however, B7-H6 expression was not associated with other clinicopathological parameters, including gender, age, tumour size, tumour location, differentiation degree, TNM stage, local recurrence status and metastasis status (P>0.05).

Diagnostic value of B7-H6 in ESCC

ROC curve of the expression B7-H6 was plotted in order to analyze its diagnostic value in ESCC (Figure 2). The result showed that the AUC and 95% (CI) were 0.599 and 0.507–0.692, which meant that it had diagnostic capability to some extent.

Survival outcomes

With H-score =90 as the cut-off value, the 145 patients with esophageal squamous cell carcinoma were divided into two groups: a low B7-H6 expression group (H-score $\leq$ 90) and a high B7-H6 expression group (H-score > 90). Univariate and multivariate Cox regression analyses of the 145 patients showed that tumour size (P=0.021), B7-H6 expression (P=0.025) and lymphatic metastasis status (P=0.049) were independent prognostic factors in esophageal squamous cell carcinoma, as shown in Table 3. To further study the prognostic value of tumour size, lymphatic metastasis status and B7-H6 expression in ESCC, Kaplan-Meier analysis with the log-rank test was performed. The survival analysis demonstrated that the

patients with tumour size ≥3.0 cm (P = 0.001, median survival time of 44.0 months, Figure 3A), lymphatic metastasis (P <0.001, median survival time of 23.8 months, Figure 3B) or high B7-H6 expression (P = 0.003, median survival time of 29.9 months, Figure 3C)had significantly worse survival than those with low B7-H6 expression (median survival time of 117.3 months), no lymphatic metastasis (median survival time was not achieved) or a tumour size less than 3.0 cm (median survival time was not achieved), respectively.

Discussion

The superfamily member B7/CD28 have previously been shown to serve potential roles in the immune response, and these molecules have been revealed to be effective diagnostic markers and therapeutic targets in cancer (3, 19). The newly discovered member of the B7 family B7-H6 interacted with its receptor on NK cells, namely, NKp30, and played an important role in NK cell-mediated immune responses (11).

NK cells were important immune cells in the body. It was a core cell of the natural immune system and can eliminate tumour cells. NK-cells activation was regulated by some activation receptors or inhibition receptors on the cell surface (10). The major activating receptors included NKG2D and the natural cytotoxicity receptors (NCRs) such as NKp46, NKp30, and NKp44 (20). NKp30 can promote NK cells to recognize and kill tumor cells, either alone or together with other stimulation receptors (21-23). The HLA–B-associated transcript 3 (BAT3) and the pp65 proteins have been shown to bind NKp30, but they don't correspond to tumour cell surface ligands because pp65 was a human cytomegalovirus tegument protein (24) and BAT3 was a nuclear protein released upon heat shock treatment (25). B7-H6 is a potent ligand for NKp30, and it doesn't bind any other CD28 family members nor other NCRs (12). B7-H6 expressed on tumour cells contacted NKp30 through the complementarity-determining region (CDR)-like loops of its V-like domain (26). NK cells eliminate B7-H6-expressing tumour cells either directly via cytotoxicity or indirectly by cytokine secretion[11]. Eva Schlecke et al. illustrated that tumour cells impeded NK-mediated recognition by metalloprotease-mediated shedding of B7-H6 (27). Soluble B7-H6 generated by ectodomain shedding is another form of B7-H6 (11). Soluble B7-H6 was capable to inhibit the binding of anti-NKp30 mAbs to NKp30 and to prevent NKp30-mediated NK cell triggering (27,28). Taken together, these data on B7-H6/ NKp30 interaction provided a theoretical basis for the development of novel cancer treatments.

In recent years, immune checkpoint inhibition with antibodies that block cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) have led to meaningful improvements in survival and have brought tumour immunotherapy into a new era (29,30) .Several clinical studies in esophageal cancer using PD-1 inhibitors, such as nivolumab or pembrolizumab, are in progress with recent promising results (31-33). However, the relationship between PD-1/PD-L1 expression in esophageal cancer tissue and prognosis remains controversial. To date, no good biomarker has been found to guide treatment and prognosis. Abnormal B7-H6 expression was found in many human cancer types, suggesting that B7-H6 expressed important clinical significance. The present study provided the first investigation into the relationship between the prognostic and clinical value of B7-H6 expression in ESCC tissue. IHC staining

demonstrated that B7-H6 expression was present in most ESCC tissue samples, which was consistent with the findings of other studies (14,17,34). We also found that the expression level of B7-H6 correlated with T stage and lymphatic metastasis status, which demonstrated that B7-H6 expression might be a marker to identify the T stage and lymphatic metastasis status of esophageal squamous cell carcinoma. This result is similar to the findings of studies in gastric carcinoma, ovarian cancer, non-small cell lung cancer, astrocytoma, breast cancer and other cancers (14,15,17,34-36). In addition, Cox regression analysis and the log-rank test revealed that B7-H6 expression level was independent prognostic factor for ESCC. Patients with high B7-H6 expression had significantly worse survival than those with low B7-H6 expression, suggesting that high B7-H6 expression is a predictor of poor prognosis. This result is similar to data from other researchers (15,35,36). Thus, B7-H6 may be regarded as a possible biomarker for predicting the OS of ESCC patients, and also serve as an independent prognostic index .In the past three years, some studies of the knockdown of B7-H6 expression in tumours have been carried out (16,37,38) and indicated that B7-H6 may be a potential therapeutic target in several human cancers. Therefore, it is believed that further studies on the expression of B7-H6 at the gene level and the knockdown of B7-H6 expression may also have certain clinical value in determining the prognosis of ESCC patients.

Still, our study has some limitations. Firstly, its retrospective nature, potential selection bias, and confounding bias, were unavoidable. Secondly, all tumour samples were all from patients of China, which may differ from other ethnics and region. Thirdly, it may be more meaningful to detect the B7-H6 expression in the protein and gene level using western bolt, enzyme-linked immunosorbent assay (ELISA) or gene chip. And further validate the phenotype change via altering the expression of B7-H6 in ESCC is meaningful.

Conclusions

Our present study indicated that B7-H6 was widely expressed in ESCC tissues and can serve as an independent prognostic marker for ESCC.

Abbreviations

AUC area under the curve

CI confidence interval

ESCC esophageal squamous cell carcinoma

HR hazard ratio

IHC immunohistochemistry

NCRs natural cytotoxicity receptors

OS overall survival

ROC receiver operating characteristic

TNM tumour-node-metastasis

Declarations

Acknowledgments

The authors would like to thank the patients who participated in this study, and two independent senior pathologists for technical support.

Funding

This study was supported by the Science and Technology Planning Project of the Guangdong Esophageal Cancer Research Institute (No Q201607) and 2016 youth teacher training program of sun yat-sen university (No 051703).

Availability of data and materials

The dataset supporting the conclusions of this article is available in the repository [the Research Data Deposit public platform], [RDDA2019001075 in http://www.researchdata.org.cn].

Authors' contributions

S.Y and X.C conceived the original idea and designed the study. H.Z and K.W collected the date for the study, which were analysed by H.Z, J.D, and L.G. The date interpretation and manuscript drafting were performed by H.Z and J.D. The manuscript was revised by X.W, L.G, X.C, and S.Y. All authors reviewed the manuscript and gave the final approval for submission.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Sun Yat-sen University Cancer Center and written informed consent was provided by all patients based on the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Pennathur A, Gibson MK, Jobe BA, Luketich JD. Oesophageal carcinoma. Lancet. 2013;381(9864):400-12.
2. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565-70.
3. Lu B, Chen L, Liu L, Zhu Y, Wu C, Jiang J, et al. T-cell-mediated tumor immune surveillance and expression of B7 co-inhibitory molecules in cancers of the upper gastrointestinal tract. Immunologic research. 2011;50(2-3):269-75.
4. Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nature reviews Immunology. 2008;8(6):467-77.
5. Leng C, Li Y, Qin J, Ma J, Liu X, Cui Y, et al. Relationship between expression of PD-L1 and PD-L2 on esophageal squamous cell carcinoma and the antitumor effects of CD8(+) T cells. Oncology reports. 2016;35(2):699-708.
6. Lim SH, Hong M, Ahn S, Choi YL, Kim KM, Oh D, et al. Changes in tumour expression of programmed death-ligand 1 after neoadjuvant concurrent chemoradiotherapy in patients with squamous oesophageal cancer. European journal of cancer. 2016;52:1-9.
7. Song J, Shi W, Zhang Y, Sun M, Liang X, Zheng S. Epidermal growth factor receptor and B7-H3 expression in esophageal squamous tissues correlate to patient prognosis. OncoTargets and therapy. 2016;9:6257-63.
8. Wang L, Cao NN, Wang S, Man HW, Li PF, Shan BE. Roles of coinhibitory molecules B7-H3 and B7-H4 in esophageal squamous cell carcinoma. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2016;37(3):2961-71.
9. Chen LJ, Sun J, Wu HY, Zhou SM, Tan Y, Tan M, et al. B7-H4 expression associates with cancer progression and predicts patient's survival in human esophageal squamous cell carcinoma. Cancer immunology, immunotherapy : CII. 2011;60(7):1047-55.
10. Chen Y, Mo J, Jia X, He Y. The B7 Family Member B7-H6: a New Bane of Tumor. Pathology oncology research : POR. 2018;24(4):717-21.
11. Ni L, Dong C. New B7 Family Checkpoints in Human Cancers. Molecular cancer therapeutics. 2017;16(7):1203-11.
12. Brandt CS, Baratin M, Yi EC, Kennedy J, Gao Z, Fox B, et al. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. The Journal of experimental medicine. 2009;206(7):1495-503.
13. Joyce MG, Tran P, Zhuravleva MA, Jaw J, Colonna M, Sun PD. Crystal structure of human natural cytotoxicity receptor NKp30 and identification of its ligand binding site. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(15):6223-8.
14. Chen XJ, Shen J, Zhang GB, Chen WC. B7-H6 protein expression has no prognostic significance in human gastric carcinoma. Pathology oncology research : POR. 2014;20(1):203-7.
15. Zhou Y, Xu Y, Chen L, Xu B, Wu C, Jiang J. B7-H6 expression correlates with cancer progression and patient's survival in human ovarian cancer. International journal of clinical and experimental

pathology. 2015;8(8):9428-33.
16. Wu F, Wang J, Ke X. Knockdown of B7-H6 inhibits tumor progression and enhances chemosensitivity in B-cell non-Hodgkin lymphoma. International journal of oncology. 2016;48(4):1561-70.
17. Zhang X, Zhang G, Qin Y, Bai R, Huang J. B7-H6 expression in non-small cell lung cancers. International journal of clinical and experimental pathology. 2014;7(10):6936-42.
18. Chen L, Di D, Luo G, Zheng L, Tan Y, Zhang X, et al. Immunochemical staining of MT2-MMP correlates positively to angiogenesis of human esophageal cancer. Anticancer research. 2010;30(10):4363-8.
19. Seliger B, Quandt D. The expression, function, and clinical relevance of B7 family members in cancer. Cancer immunology, immunotherapy : CII. 2012;61(8):1327-41.
20. Kaifu T, Escaliere B, Gastinel LN, Vivier E, Baratin M. B7-H6/NKp30 interaction: a mechanism of alerting NK cells against tumors. Cellular and molecular life sciences : CMLS. 2011;68(21):3531-9.
21. Pende D, Rivera P, Marcenaro S, Chang CC, Biassoni R, Conte R, et al. Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. Cancer research. 2002;62(21):6178-86.
22. Pende D, Parolini S, Pessino A, Sivori S, Augugliaro R, Morelli L, et al. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. The Journal of experimental medicine. 1999;190(10):1505-16.
23. Byrd A, Hoffmann SC, Jarahian M, Momburg F, Watzl C. Expression analysis of the ligands for the Natural Killer cell receptors NKp30 and NKp44. PloS one. 2007;2(12):e1339.
24. Arnon TI, Achdout H, Levi O, Markel G, Saleh N, Katz G, et al. Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. Nature immunology. 2005;6(5):515-23.
25. Pogge von Strandmann E, Simhadri VR, von Tresckow B, Sasse S, Reiners KS, Hansen HP, et al. Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. Immunity. 2007;27(6):965-74.
26. Wang L, Rubinstein R, Lines JL, Wasiuk A, Ahonen C, Guo Y, et al. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. The Journal of experimental medicine. 2011;208(3):577-92.
27. Schlecker E, Fiegler N, Arnold A, Altevogt P, Rose-John S, Moldenhauer G, et al. Metalloprotease-mediated tumor cell shedding of B7-H6, the ligand of the natural killer cell-activating receptor NKp30. Cancer research. 2014;74(13):3429-40.
28. Matta J, Baratin M, Chiche L, Forel JM, Cognet C, Thomas G, et al. Induction of B7-H6, a ligand for the natural killer cell-activating receptor NKp30, in inflammatory conditions. Blood. 2013;122(3):394-404.
29. Redman JM, Gibney GT, Atkins MB. Advances in immunotherapy for melanoma. BMC medicine. 2016;14:20.

30. Carlo MI, Voss MH, Motzer RJ. Checkpoint inhibitors and other novel immunotherapies for advanced renal cell carcinoma. Nature reviews Urology. 2016;13(7):420-31.
31. Kojima T, Doi T. Immunotherapy for Esophageal Squamous Cell Carcinoma. Current oncology reports. 2017;19(5):33.
32. Kudo T, Hamamoto Y, Kato K, Ura T, Kojima T, Tsushima T, et al. Nivolumab treatment for oesophageal squamous-cell carcinoma: an open-label, multicentre, phase 2 trial. The Lancet Oncology. 2017;18(5):631-9.
33. Doi T, Piha-Paul SA, Jalal SI, Saraf S, Lunceford J, Koshiji M, et al. Safety and Antitumor Activity of the Anti-Programmed Death-1 Antibody Pembrolizumab in Patients With Advanced Esophageal Carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2018;36(1):61-7.
34. Guo JG, Guo CC, He ZQ, Liu ZG, Wang Y, Mou YG. Clinical significance of B7-H6 protein expression in astrocytoma. OncoTargets and therapy. 2016;9:3291-7.
35. Chen L, Feng J, Xu B, Zhou Y, Zheng X, Wu C, et al. Expression of B7-H6 expression in human hepatocellular carcinoma and its clinical significance. Cancer cell international. 2018;18:126.
36. Sun J, Tao H, Li X, Wang L, Yang J, Wu P, et al. Clinical significance of novel costimulatory molecule B7-H6 in human breast cancer. Oncology letters. 2017;14(2):2405-9.
37. Che F, Xie X, Wang L, Su Q, Jia F, Ye Y, et al. B7-H6 expression is induced by lipopolysaccharide and facilitates cancer invasion and metastasis in human gliomas. International immunopharmacology. 2018;59:318-27.
38. Jiang T, Wu W, Zhang H, Zhang X, Zhang D, Wang Q, et al. High expression of B7-H6 in human glioma tissues promotes tumor progression. Oncotarget. 2017;8(23):37435-47.

Tables

Table 1. Clinical characteristics of 145 patients

Characteristic	Number (%)
Age(Years)	
Median	60
Range	34-88
<60	71(49.0)
≥60	74(51.0)
Gender	
Male	107(73.8)
Female	38(26.2)
Tumor size (cm)	
3.0	69(47.6)
≥3.0	75(51.7)
Tumour location	
Upper third	14(9.7)
Middle third	90(62.1)
Lower htird	41(28.3)
Differentiated degree	
Low	29(20.0)
Middle	60(41.4)
Hign	32(22.1)
Low-middle	13(9.0)
Middle-high	11(7.6)
T stage	
T1	13(9.0)
T2	23(15.9)
T3	96(66.2)
T4	13(9.0)
Lymphatic metastasis	
Yes	64(44.1)
No	81(55.9)
TNM stages	
I+II	82(56.6)
III+IV	63(43.4)
Death	
Yes	79(54.5)
No	66(45.5)

Table 2. Correlation between the B7-H6 expression level and the patients' clinical parameters

Clinical parameters	Cases	B7-H6 expression level			P-value
		H-score<40	H-score≥40	χ2	
Gender				0.096	0.756
Male	107	51	56		
Female	38	17	21		
Age(Years)				0.055	0.815
<60	71	34	37		
≥60	74	34	40		
Tumour size ,cm				0.652	0.419
< 3.0	69	35	34		
≥3.0	75	33	42		
Tumour location				0.66	0.719
Upper third	14	8	6		
Middle third	90	41	49		
Lower third	41	19	22		
Differentiated degree				1.389	0.846
Low	29	14	15		
Middle	60	30	30		
High	32	13	19		
Low-middle	13	5	8		
Middle-high	11	6	5		
T stage				8.526	0.036[a]
T1	13	11	2		
T2	23	9	14		
T3	96	43	53		
T4	13	5	8		
Lymphatic metastasis				4.062	0.044[a]
Yes	64	24	40		
No	81	44	37		
TNM stages				0.73	0.393
I+II	82	41	41		
III+IV	63	27	36		
Local recurrence or metastasis				2.794	0.095
Yes	30	10	20		
No	115	58	57		

Note: [a]P<0.05.

Table 3. Univariate and multivariate analyses of prognosis in esophageal squamous cell carcinoma

	Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value
Age, years (<60 vs≥60)	1.043 (0.670-1.621)	0.853		
Gender (male vs female)	0.814 (0.503-1.315)	0.400		
Tumour location				
Upper third vs Lower third	1.192(0.528-2.693)	0.673		
	0.891(0.535-1.484)			
Middle third vs Lower third		0.658		
Tumor size, cm (<3.0 vs ≥3.0)	2.095(1.323-3.316)	0.002[a]	1.749(1.089-2.807)	0.021[a]
Pathological stage				
T1 vs T4	0.161 (0.044-0.587)	0.006[a]	0.400(0.092-1.745)	0.223
T2 vs T4	0.400 (0.169-0.948)	0.037[a]	0.507(0.140-1.842)	0.302
T3 vs T4	0.567(0.288-1.118)	0.101	0.580(0.258-1.305)	0.188
Lymphatic metastasis (yes vs no)	2.914(1.856-4.577)	<0.001[a]	2.157(1.002-4.644)	0.049[a]
TNM stages (I-II vs III-IV)	2.912 (1.849-4.586)	<0.001[a]	1.186 (0.474-2.969)	0.715
B7-H6 expression level (low vs high)	2.053(1.266-3.329)	0.004[a]	1.751(1.071-2.861)	0.025[a]

Note: [a]P<0.05.

Figures

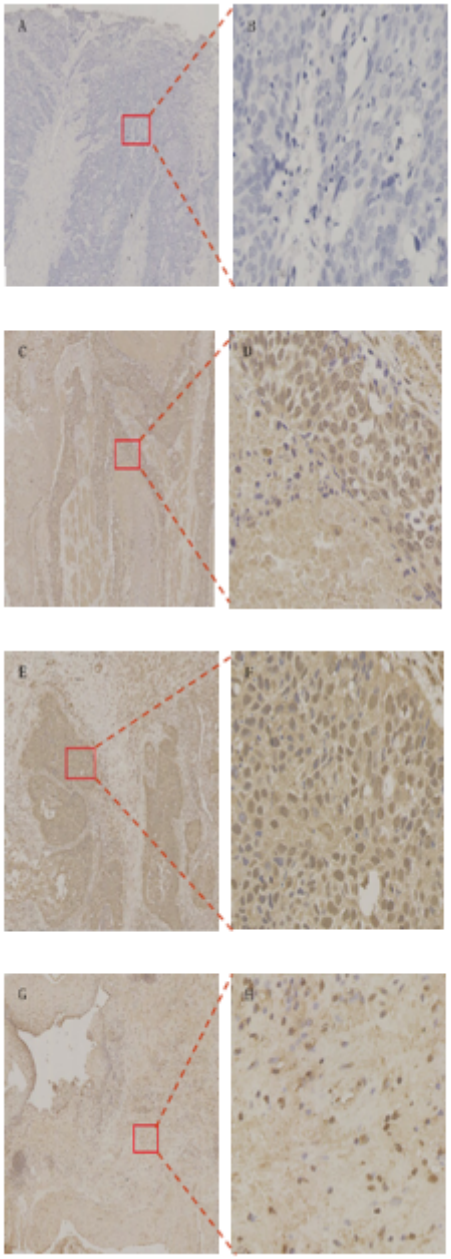

Figure 1

Immunohistochemical staining (magnification: left, 5×; right, 100×). Notes: Immunohistochemical staining was used to detect B7-H6 expression (brown) in ESCC tissue samples and adjacent normal tissue samples. (A–B) Negative B7-H6 expression in ESCC tissue samples. (C–D) Low B7-H6 expression in ESCC tissue samples. (E–F) High B7-H6 expression in ESCC tissue samples. (G–H) Low B7-H6 expression in adjacent normal tissue samples.

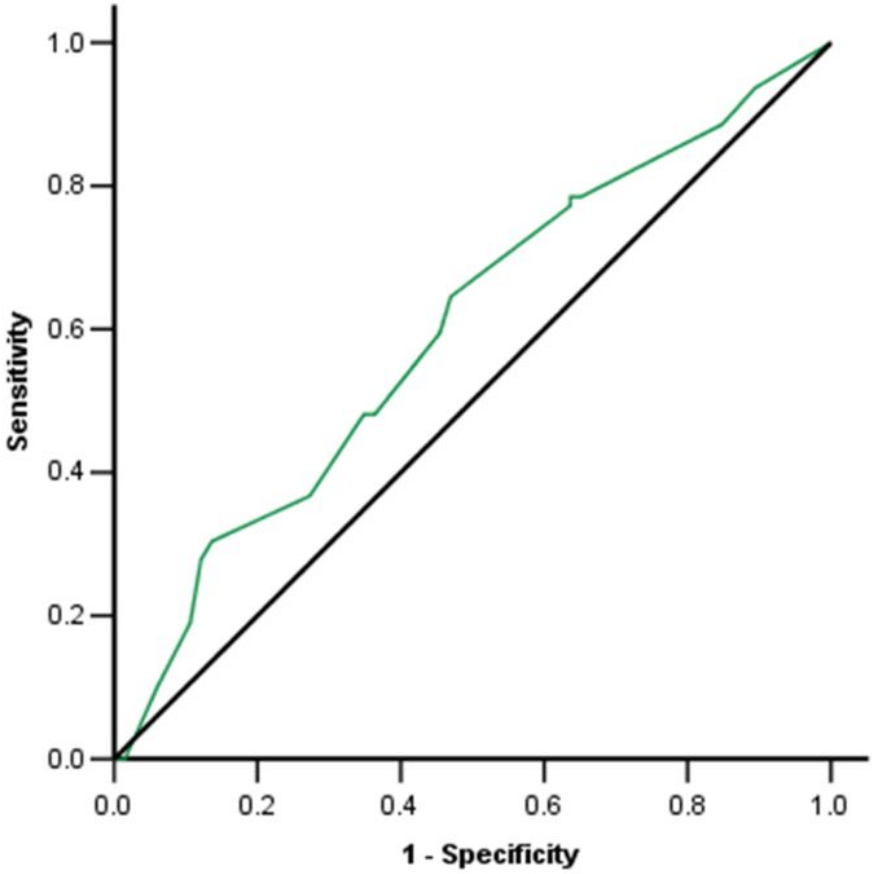

Figure 2

The ROC curve of B7-H6 in ESCC. AUC was 0.599, 95% CI, 0.507–0.692. Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic; CI, confidence interval.

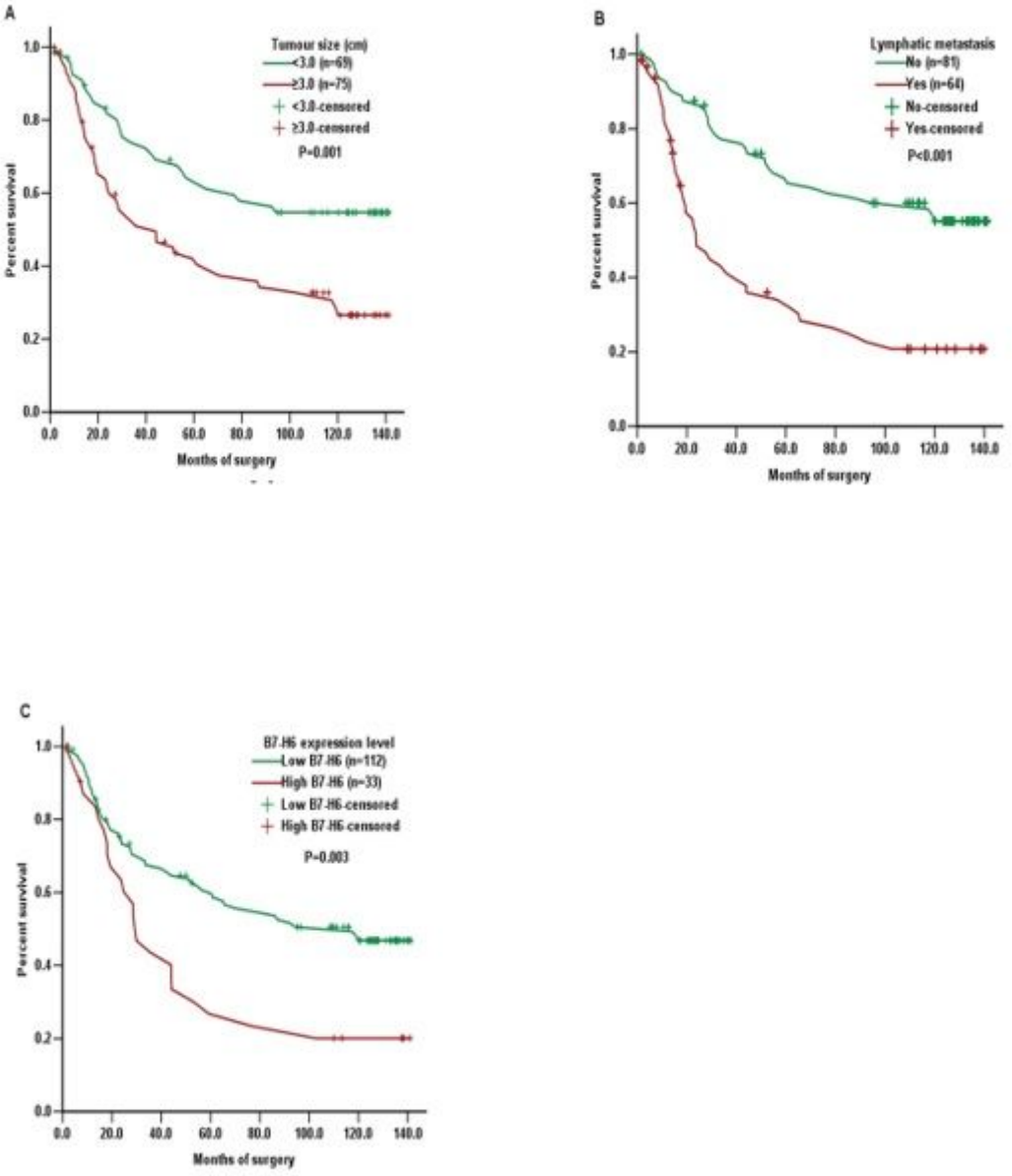

Figure 3

Kaplan-Meier survival analysis of the associations between overall survival and tumour size (Figure 3A, P=0.001), lymphatic metastasis status (Figure 3B, P□0.001) or B7-H6 expression (Figure 3C, P=0.003) in ESCC patients.