# KK-LC-1 may be an effective prognostic biomarker for gastric cancer

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# Abstract

**Background:** To detect the expression of Kita-Kyushu lung cancer antigen-1 (KK-LC-1) in gastric cancer (GC) specimens and analyze the associations between KK-LC-1 expression and clinicopathological parameters and clinical prognosis.

**Methods:** A total of 94 patients with GC who underwent surgical resection were enrolled in this study. The expression of KK-LC-1 in GC tissues was detected by immunohistochemistry. The assessment of KK-LC-1 expression was conducted using the H-scoring system. H-score was calculated by the multiplication of the overall staining intensity with the percentage of positive cells. The expression of KK-LC-1 in the cytoplasm and was scored to achieve respective H-score values. The correlations between KK-LC-1 expression and clinicopathological parameters and clinical prognosis were analyzed using Chi-square test, Kaplan-Meier method and Cox regression.

**Results:** In the cytoplasm, the expression of KK-LC-1 in tumor tissues was significantly higher than that in normal tissues ($P < 0.001$, respectively). Using the median H-score as the cutoff value, it was discovered that, GC patients with higher levels of KK-LC-1expression in the cytoplasm, had favorable overall survival ($P = 0.016$), and it was still statistically meaningful in Cox regression analysis. At the same time, the study found that there was a negative correlation between KK-LC-1's protein expression and the pathological grade of the tumor ($P = 0.036$).

**Conclusions:** Our research data shows that KK-LC-1's expression in GC is higher than that of normal tissues, which is associated with a longer overall survival in GC. KK-LC-1 can be used as a biomarker for GC patients with good prognosis.


# Background

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide. The majority of cases of gastric cancer are usually not diagnosed until an advanced stage, and therefore the outcome is often poor, with a 5-year survival rate of no more than 30%, including patients who have undergone surgery [1]. The treatment of GC is dependent on the type of cancer tissue, the TNM staging and the general condition of the patient [2]. Metastatic GC gives us fewer options in dealing with the disease, aiming for palliative rather than curative goal. While the chemotherapeutic agents that have proven effectiveness in the treatment of GC are more than a few, we cannot ignore the fact that the targeted therapy for GC is still very limited, mainly to vascular endothelial growth factor (VEGF) pathway-and HER2-targeted agents. Recent achievements in the field of epigenetics and genetic background of the disease may enhance our chances of targeted therapeutic options in GC [3].

On the other hand, multiple molecular biomarkers had shown their potential efficacy as diagnostic and prognostic tools in GC but they still need further validation to be used in the day-to-day clinical practice. Up till the time being, the only used markers for GC in clinical practice are carcino-embryonic antigens; CA

19-9, CA-50[4] and CA-72 [5], which lack the high sensitivity and specificity that is needed in assessing diagnosis and prognosis of GC, making their efficacy questionable.

To date, no novel/reliable molecular marker has been introduced in the frame of secondary prevention strategies, so far [6] .Therefore, there is an urgent need to explore novel prognostic biomarkers to increase the accuracy of prognosis prediction and seize therapeutic opportunities for GC patients.

Cancer-testis antigens (CTAs) are characterized by their spontaneous immunogenicity and distinct expression patterns normally restricted to germ cells of the testis and placenta but frequently are activated in tumor cells [7,8]. T cells and antibodies against CTAs proteins are detectable in patients with cancer [9-13], suggesting that the abnormal expression of CTAs antigens in tumors could induce adaptive immune response. Because of their tumor specificity, CTAs are considered potential targets for new treatment strategies including immunotherapy [14,15], and the expression of CTAs has potential significance for the prognosis of several types of cancer [16]. While studies have reported the expression patterns of CTAs in various cancer types, fewer studies have focused on gastric cancer [16-18]. CT83, also known as Kita-Kyushu lung cancer antigen-1 (KK-LC-1) was a CTA recognized by cytotoxic T lymphocytes (CTL) [19]. It is a gene that encodes a member of the Cancer-testis antigenic protein family and is only expressed in malignant tumor tissue and testicular germ cells [20-23]. Futawatari N et al found that high CT83 expression rates can be frequently detected in early stages of GC [24] .

Previous research by our team has shown that mRAN expression of KK-LC-1 is related to the prognosis of gastric cancer (the article has been submitted). Therefore, in this study, we detected the expression of KK-LC-1 protein in GC specimens and analyzed the associations between KK-LC-1 protein expression and clinicopathological parameters and prognosis.

# Methods

### Tissue Microarrays

Tissue arrays containing multiple human gastric cancer tissues (HStm-Ade180Sur-17), was obtained from Shanghai Outdo Biotech. The tissue chip samples were all gastric adenocarcinomas. The gastric cancer tissues included 95 points, and the adjacent tissues included 85 points. The operation time was from 2007.05 to 2008.2, and the follow-up time was up to 2015.7. The follow-up time was calculated from the postoperative period, and the average survival time was 3.2 years. The diameter of each point is 1.5 mm, and the thickness of the tissue section is 4 μm. The EnVision+ detection system (Dako) was used per the manufacturer's instructions. Eighty-five pairs of GC specimens and adjacent normal tissue specimens were acquired from this tissue arrays, as well as ten individual cancer tissue specimens. Surgical types were categorized as curative and noncurative resections. Curative resection (R0) referred to *en bloc* resection with a negative surgical margin, while the presence of microscopic (R1) or macroscopic (R2) residual cancer was considered noncurative. The clinicopathological data were collected from the medical records and the patients were followed from the date of surgery. Patients with GC were staged according to the 8th edition of American Joint Committee on Cancer system. The endpoint was overall

survival (OS), defined as the time interval from the date of surgery to the cancer-related death. The study above was approved by the Ethics Committee of Shanghai Outdo Biotech Company.

### Immunohistochemistry and H-scoring for KK-LC-1

Immunohistochemical staining was conducted manually and each slide was strictly processed in accordance with the immunohistochemical protocol. Anti- KK-LC-1 antibody (CL4762, Abcam, the United Kingdom, 100uL), produced in mouse, was used for biomarker expression analysis. The immunohistochemical slides were evaluated independently by two experienced pathologists. The assessment of KK-LC-1 staining was conducted using the H-scoring system [25-28], and H-score was calculated by the multiplication of the overall staining intensity with the percentage of positive cells. The staining intensity was graded from 0 to 3 (0 = negative, 1 = weak, 2 = medium, 3 =strong) and the positive percentage increased from 0 to 100. Theoretically, the final H-score values were obtained with a range from 0 to 300. KK-LC-1 staining in the cytoplasm and nucleus was scored separately to achieve respective values.

### Statistical analysis

Statistical analyses were performed using SPSS 17.0 software (Chicago, IL, United States). Kolmogorov-Smirnov test was used to assess the distribution of the data and to decide the selection of statistical method. Chi-square test was used to compare qualitative variables and Mann-Whitney *U* test was used to compare the abnormally distributed variables. Kaplan-Meier method and log-rank test were used to compare OS. All potential prognostic factors on univariate analyses were entered into the Cox regression model. Cox regression multivariate analysis was performed further to identify the independent prognostic factors. All *P*-values were two sided and considered statistically significant when less than 0.05.

## Results

### Clinicopathological characteristics and survival data

The mean and median ages of patients at surgery were 65 and 66 years (range, 45-83 years), separately. The majority (75.5%) of patients were male and the female: male ratio was 1:3. Most (95.7%) of patients underwent curative resection and moderately to well-differentiated adenocarcinomas were found in 24.4% of cases. Nodal involvement and distant metastasis occurred in 21.2% and 4% of patients, respectively. More information about the cohort is listed in Table 1.

### Staining and H-scoring for KK-LC-1 expression

KK-LC-1 staining was counted and analyzed (Table 2A and Figure 1). In the tissue arrays, all stained specimens were located in the cytoplasm, and no strong positive (3+) staining was found in the specimens. We calculated the H-score in both tumor and normal tissues (Table 2B and Figure 2), and it was found that, in tumor tissues, KK-LC-1 exhibited a median H-score of 100 (range, 0-250) in the cytoplasm. While in normal tissues, the median H-scores observed for KK-LC-1 was 50 (range, 0-150).

### Various comparisons and cut-off values of KK-LC-1 expression results

Indicated by Kolmogorov-Smirnov test, the values of H-score were distributed abnormally. Therefore, Mann-Whitney *U* test was selected for analyses (Table 3A). In both tumor and normal tissues, KK-LC-1 is only expressed in cytoplasm. Moreover, we found that expression of KK-LC-1 in tumor tissues were higher normal tissues ($P < 0.001$).

Patients with GC were divided into two groups according to the median H-score values of the staining. We found that patients with higher H-score values in the cytoplasm had favorable OS using univariate and multivariate analyses (Figure 3A). Univariate survival analysis (Table 3B) revealed that KK-LC-1 expression ($P = 0.016$), T stage ($P = 0.002$), N stage ($P = 0.001$), Pathological Stages ($P < 0.001$) were associated with OS in patients with GC, while age, gender, tumor size, M stage and pathological grades had no significant influence on the survival in our study. Cox regression multivariate analysis revealed that T stage, N stage and KK-LC-1 protein expression were independent prognostic factors for OS (Table 3B).

### Correlations between KK-LC-1 expression and clinicopathological parameters

The correlations between KK-LC-1 expression and clinicopathological parameters are detailed in Table 4. There were no significant associations between KK-LC-1 expressions and age, gender, tumor size, TNM stages or Pathological Stages. However, higher KK-LC-1 expression were observed in patients with low pathological grades.

## Discussion

In the present study, we found that the overall expression of KK-LC-1 in tumor tissues was significantly higher than that in normal tissues, and GC patients with higher KK-LC-1 expression had a better prognosis. Fukuyama et al.[29] also found similar results that KK-LC-1 gene expression was higher in tumor regions than in non-tumor regions, and KK-LC-1 was occasionally expressed at non-tumour sites of stomachs carrying tumours. In our experimental results, the expression rate of KK-LC-1 in tumor tissues was 95.7%. By contrast, akiko et al. also found that the gene expression rate of KK-LC-1 was shown to be as high as 81.6%, which was higher than the rates of other CTAs [18]. In a study of triplenegative breast cancer, the expression rate of KK-LC-1 was reported to be 75% [20]. These findings are similar to ours, all suggesting that KK-LC-1 is highly expressed in tumors. However, there are no reports in the literature about the expression of tumor-associated antigens in gastric cancer as high as KK-LC-1, indicating that KK-LC-1 is an ideal therapeutic target..In terms of diagnostic applications, tumor-associated antigens that are highly expressed in early stage cancers are considered useful targets. At present, there are few reports about the expression of KK-LC-1 gene and protein and tumor prognosis. After that, more research results are needed to verify.

It was revealed in our current study that there were significant negative correlations between KK-LC-1 expression and pathological grade. The higher the pathological grade, the lower the expression of KK-LC-

1 in the tissue, and the prognosis will be poor. In contrast, the lower the pathological grade, the higher the expression of KK-LC-1 in the tissue, and the better the prognosis of the patient.. This result also indirectly shows the reliability of our experimental data. Therefore, we speculated that KK-LC-1 protein may be related to the early state of the tumor and thus related to a good prognosis. KK-LC-1 may serve as a positive biomarker indicative of prognosis and make a strategic choice for clinicians. For instance, patients with higher levels of KK-LC-1 expression perhaps could benefit better from the adjuvant chemo- or radio-therapy, in contrast to the ones with lower expression. However, studies have also shown that hepatocellular carcinoma (HCC) tissues exhibited increased levels of KK-LC-1. High KK-LC-1 level independently predicted poor survival outcome. KK-LC-1 promoted cell growth, migration, invasion and epithelial-mesenchymal transition in vitro and in vivo [30]. Taken together, aberrant KK-LC-1 expression, either increased or decreased expression, is clearly correlated with tumorigenesis. Therefore, KK-LC-1 may play different roles in disparate malignancies, and more thorough research studies are still encouraged to verify the exact relationships between KK-LC-1 and cancers, as well as the subtle mechanisms.

To date, some mechanisms have been revealed in several tumors, concerning KK-LC-1 and neoplasia. It was unveiled that, hypomethylated CpG islands have been reported to be associated with the activation of CT genes in several types of cancers. *CT45*, a 6-member family of X-linked CT genes, is upregulated in epithelial ovarian cancer. Elevated level of CT45 expression is demonstrated to be induced by promoter hypomethylation [30]. In lung adenocarcinoma, *PIWIL1* has been identified as an extremely highly expressed CT gene. Promoter DNA hypomethylation of PIWIL1 causes its overexpression [31]. However, functional and mechanistic investigation of KK-LC-1 in human malignancies has been few reported before.

Of note, our study may provide theoretical support for immunotherapy and targeted therapy in different tumors concerning KK-LC-1. The Human Protein Atlas (http://www.proteinatlas.org) data-sets also revealed that *CT83* transcripts were expressed in lung cancer, stomach cancer, colorectal cancer, urothelial cancer, cervix cancer, breast cancer, and various tumor cell lines (U-266/70, HeLa, U-266/84, K-562, U-2OS). We speculate that CT83 may be related to the body's anti-tumor response. In normal tissues, the expression level of CT83 is very low. However, when a tumor occurs, the expression level of CT83 may be related to the body's immune response to the tumor. The higher the expression level of CT83, the stronger the body's ability to resist tumors, and therefore the better the prognosis. In the study of early diagnosis of GC, researchers such as Futawatari found that a higher CT83 expression rate can often be detected in the early stages of GC [24]. Therefore, CT83 can be used as a potential marker for early diagnosis and treatment of GC.

Our study has several limitations. It was a single-institutional investigation and the number of cases was relatively small, restricting the power of statistical analysis. Finally, some patients received postoperative chemotherapy or radiotherapy, and the effects of these adjuvant therapies on prognosis were not considered, in spite of limited survival benefits brought by them. For these reasons above, multiinstitutional investigations and prospective studies are required to explore the expression of KK-LC-1 in GC, and further evaluate its clinical significance in a larger cohort of patients.

# Conclusions

In summary, our project indicates that KK-LC-1's protein expression in GC is higher than that in neighboring tissues. High levels of KK-LC-1 protein expression are associated with longer overall survival in GC. KK-LC-1 is a good biomarker for patients with GC.

# Abbreviations

KK-LC-1: Kitakyushu lung cancer antigen 1 (CT83); GC: gastric cancer; VEGF: vascular endothelial growth factor; CTA: Cancer-testis antigen; OS: overall survival; PDT: photodynamic therapy

# Declarations


### Acknowledgments

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### Authors' contributions

JJ and YL designed the study and wrote the manuscript. AQ W and JH C helped with data management and carried out statistical analysis. Teachers WZ and JH G classify the tissues after immunohistochemical staining. LP L and YL performed project administration. All authors have read and approved the manuscript.


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### Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shanghai Outdo Biotech Company. The patients enrolled all presented written informed consent.

### Consent for publication

Not applicable.

### Competing Interests

No conflicts of interest declared.

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# Tables

Due to technical limitations, the tables are provided in the Supplementary Files section.

# Figures

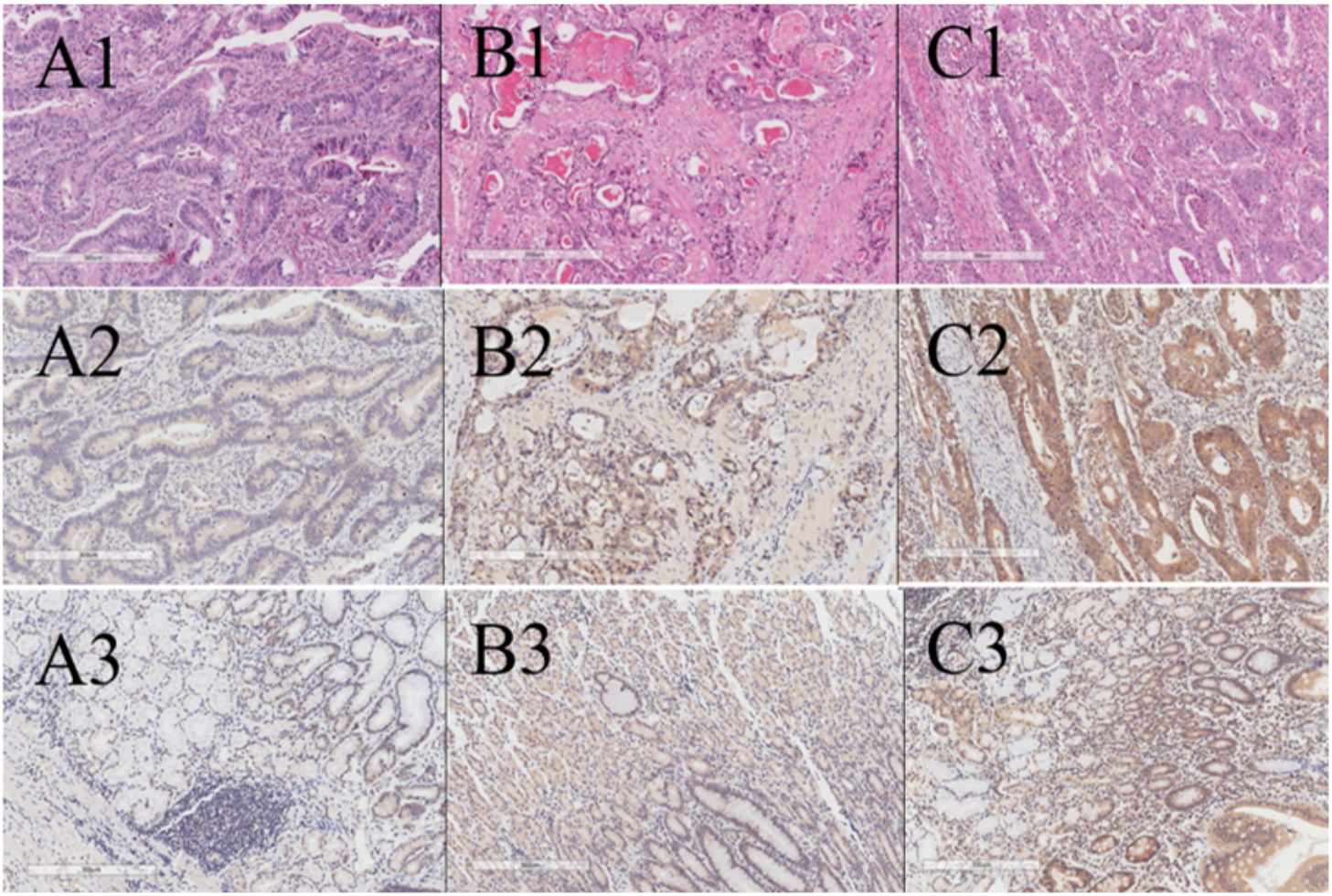
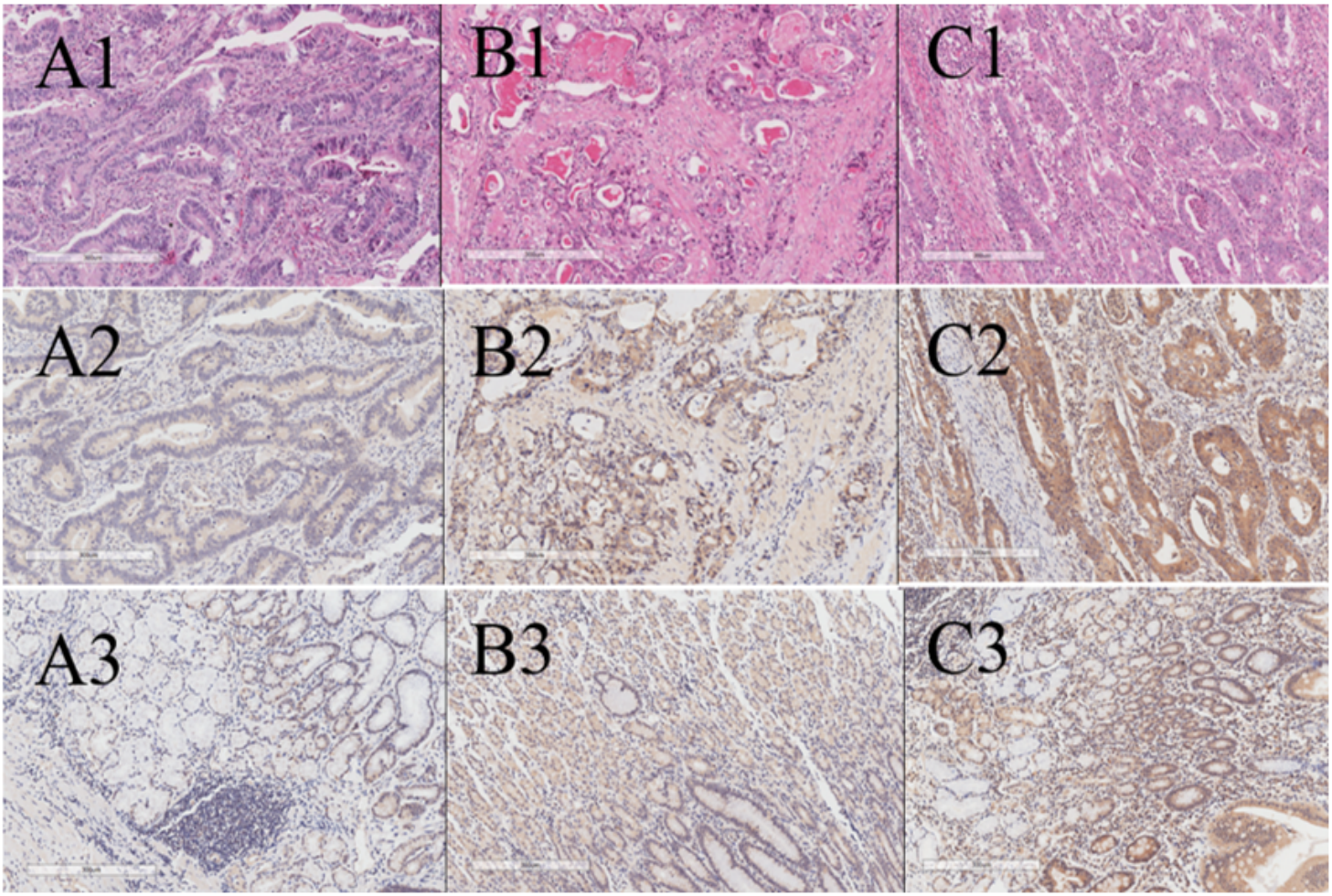



**Figure 1**

HE and Immunohistochemical staining of tissues (magnification, × 200). A1、B1 and C1: HE staining; A2、B2 and C2 : Immunohistochemical staining of cancer tissue, with<1, 1+ and 2+ intensity, respectively; A3、B3 and C3 : Immunohistochemical staining of adjacent tissue, with<1, 1+ and 1.5+ intensity, respectively. A1、A2 and A3 are the same sample, B1、B2 and B3 are the same sample, C1、C2 and C3 are the same sample.

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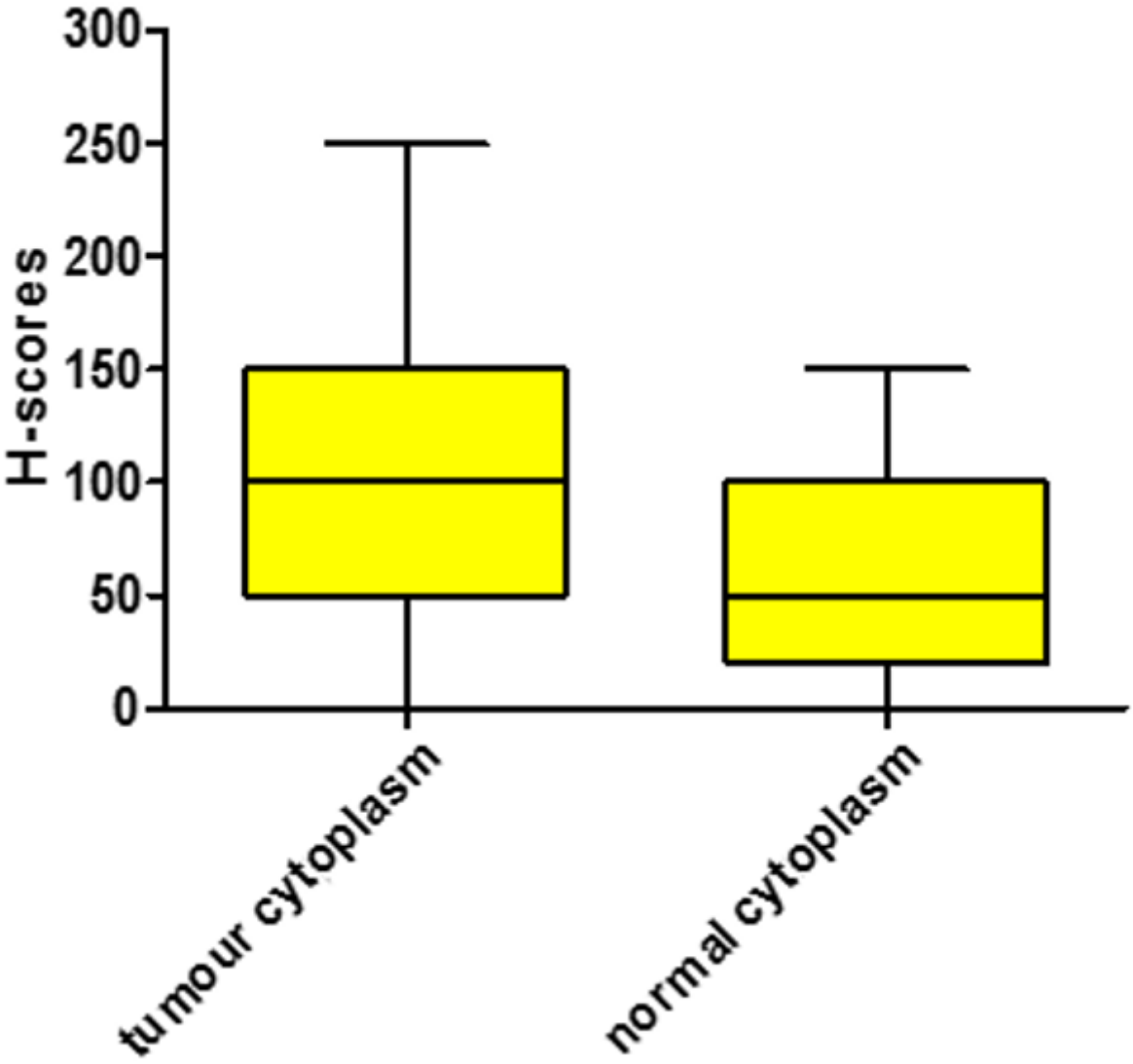
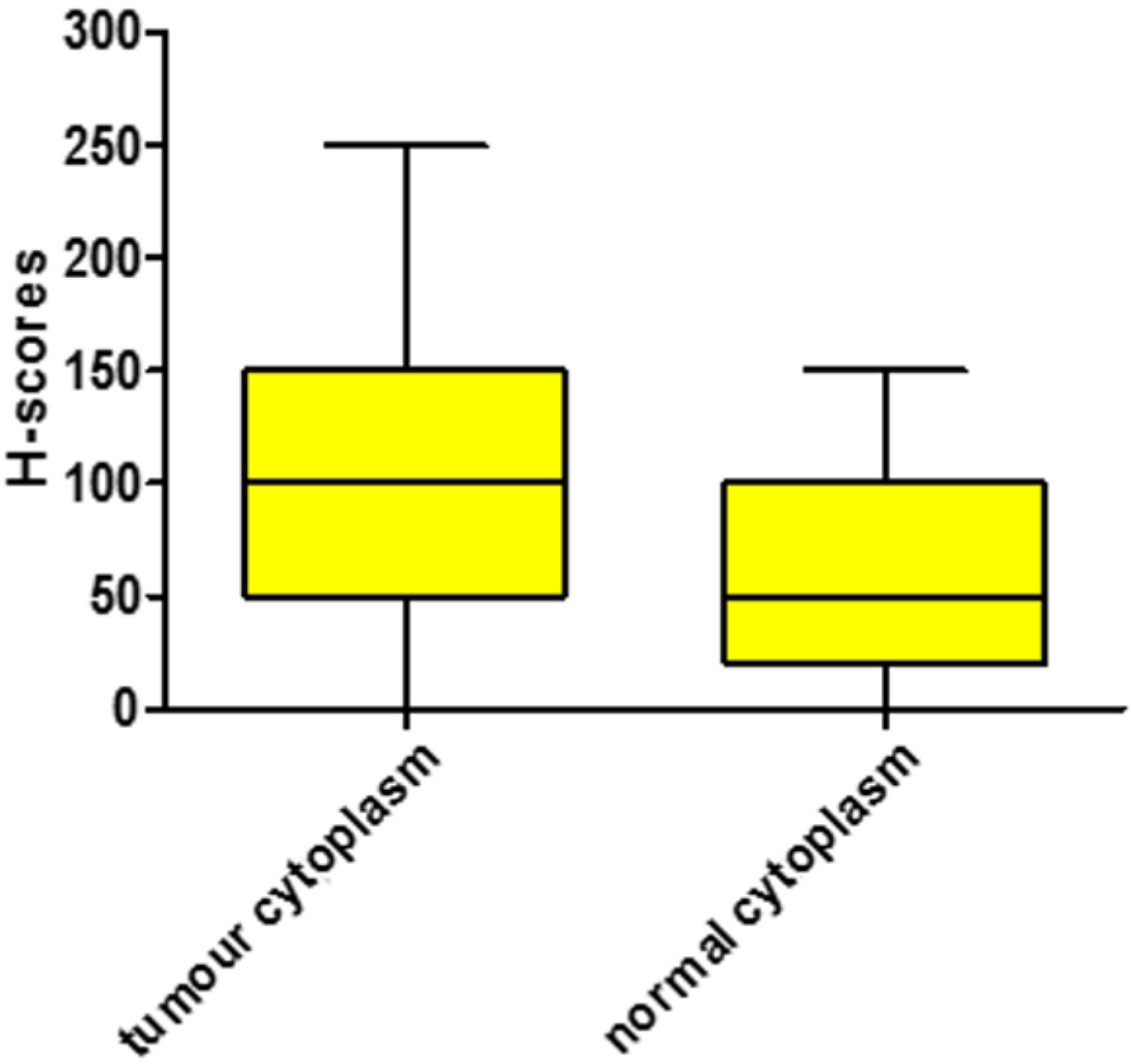



## Figure 2

Box plot of Kita-Kyushu lung cancer antigen-1 H scores in tumors and normal tissues.

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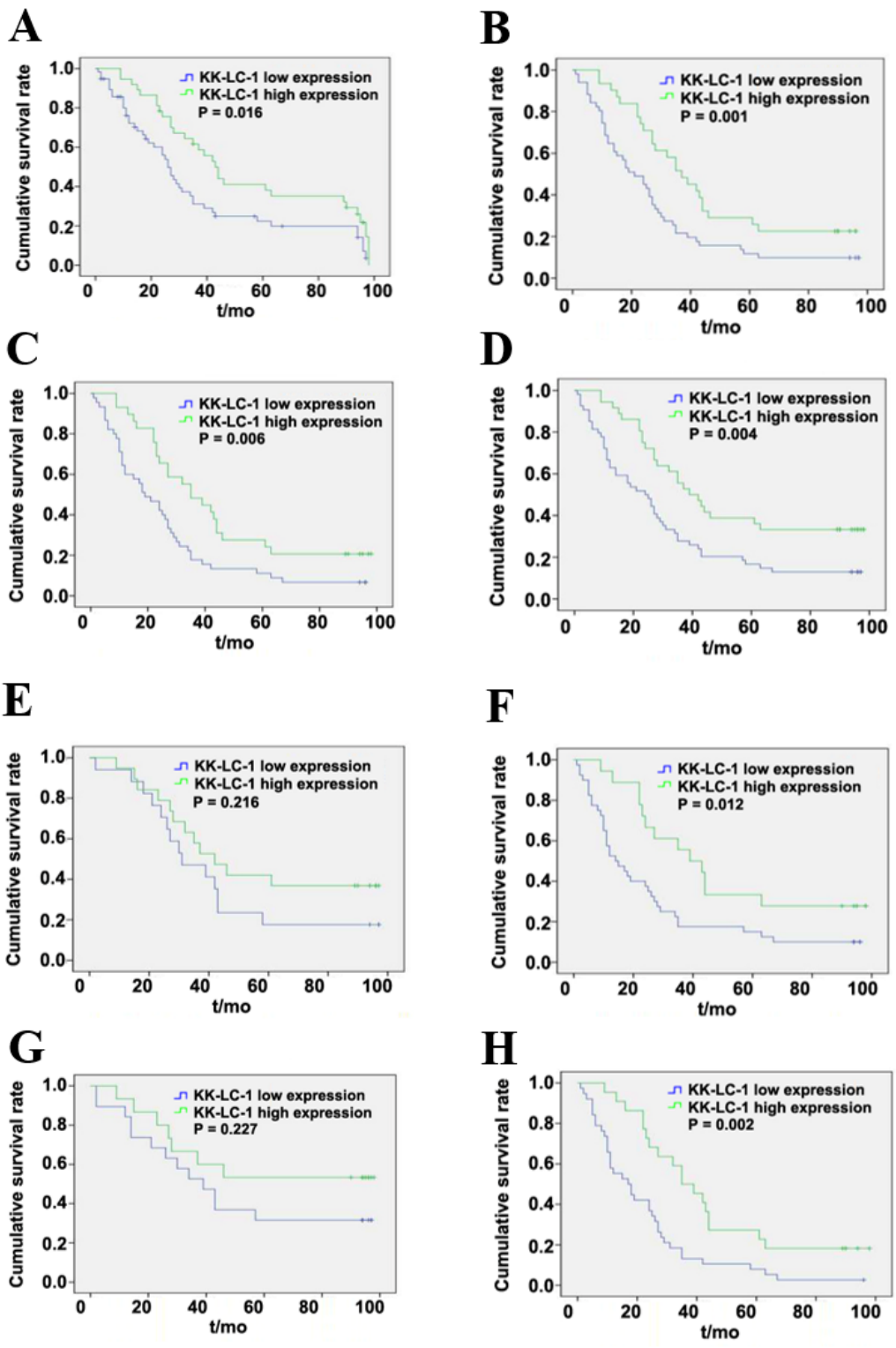
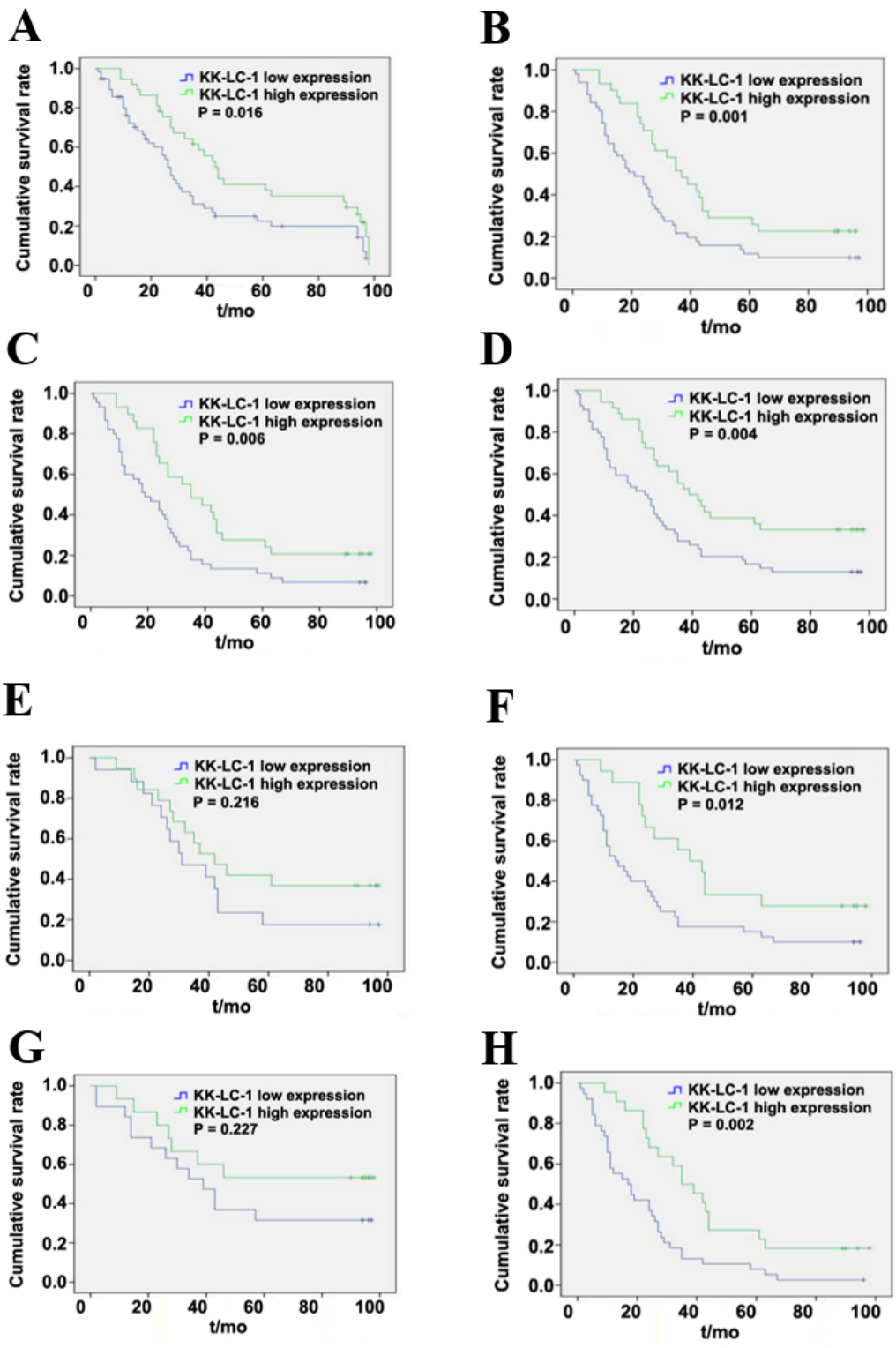



## Figure 3

Kaplan-Meier survival analyses in different subgroups, according to Kita-Kyushu lung cancer antigen-1 expression. A: The whole cohort; B: T3 + 4 group; C: Positive Nodal involvement group; D: M0 group; E: Pathological grades ⅠⅡ group; F: Pathological grades ≥Ⅱ; G: Pathological Stage ⅠⅡⅢ group; H: Pathological Stage Ⅱ+Ⅲ group. KK-LC-1: Kita-Kyushu lung cancer antigen-1

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