KK-LC-1 May Be An Effective Prognostic Biomarker for Gastric Cancer

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Abstract

Background: To detect the protein expression of Kitakyushu lung cancer antigen 1 (KK-LC-1) in gastric cancer (GC) specimens, and to analyze the linear association of KK-LC-1 protein expression with clinical pathological data and prognosis.

Methods: A total of 94 patients in this study were all GC patients with surgical resection. KK-LC-1's protein expression in GC tissue was detected by immunohistochemistry. This report applies Histological score (H-score) to evaluate KK-LC-1's expression. Chi-square test, Kaplan-Meier method and Cox regression were used to analyze the linear association between KK-LC-1 expression and clinicopathological data and prognosis.

Results: KK-LC-1's protein expression in the cytoplasm of tumor tissue was found to be significantly higher than that in normal tissue (P <0.001). If we apply the median value of H-value as the cut-off point, it suggests that overall survival for GC patients with high KK-LC-1 expression levels in the cytoplasm was good (P = 0.016), and still had statistical significance after 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 deaths in the world. Most patients with gastric cancer usually cannot be diagnosed until late, so the prognosis is usually poor. The 5-year survival rate of all patients, including surgical patients, does not exceed 30% [1]. It is suggested that tumor tissue type, TNM stage as well as patient's physical and mental conditions are all important factors affecting the treatment of gastric cancer [2]. Metastatic GC can provide fewer treatment options, and its goal is palliative treatment rather than therapeutic goals. Although a number of chemotherapy drugs have been shown to be effective in treating gastric cancer, one fact we cannot ignore is that there are limitations using the targeted therapy for GC, which is mainly used for vascular endothelial growth factor (VEGF) pathway and HER2 targeted drugs. Some of the latest research results in the field, and information about the genetic background of the disease, may give us more opportunities for targeted therapy of GC [3].

In addition, various molecular biomarkers have pointed out that they are efficient being a diagnosis and prognostic tool for gastric cancer, but these biomarkers still need further validation before they can be used in daily clinical practice. At present, the only biomarkers used for GC are carcinoembryonic antigens CA 19 – 9, CA-50 [4]and CA-72 [5]. However, they are limited in lacking the high sensitivity and specificity required to evaluate GC diagnosis and prognosis, and the clinical treatment effect of these biomarkers is doubtful.

To date, no novel / reliable molecular markers have been introduced in the content of secondary prevention strategies [6]. Therefore, there is an urgent need to explore and find new prognostic biomarkers to improve the accuracy of prognosis prediction and seize the treatment opportunities of GC patients.

Cancer-testis antigen (CTA) are characterized by its spontaneous immunogenicity and unique expression pattern. These antigens are normally expressed only in germ cells of normal human testis and placenta, but they are usually activated in tumor cells [7, 8]. T cells and anti-CTA protein antibodies can be detected in cancer patients [9–13], suggesting that abnormal expression of CTA antigen can induce tumor tissues to produce an adaptive immune response. Since CTAs are tumor specific, they are believed to be potential effective targets for new therapeutic strategies, such as immunotherapy [14, 15]. At the same time, CTAs' expression in several types of cancer has potential significance for prognosis [16]. Although CTA's expressions are studied in various studies focusing on cancer, there are still few studies on gastric cancer [16–18]. CT83, also known as KK-LC-1, which is a CTA recognized by CTLs [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, in early stages of GC, high CT83 expression rates can be frequently detected [24].

Previous research by our team has shown that KK-LC-1's mRNA expression is related to the prognosis of gastric cancer (the article has been submitted). Therefore, KK-LC-1 protein's expression in GC specimens are studies in this project and we also analyzed the relationship 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) were obtained from Shanghai Outdo Biotech. The tissue chip samples were all gastric adenocarcinomas. The samples included 94 gastric cancer tissues and 84 adjacent tissues. The operation time was May 2007 to February 2008, and the date of last follow-up was July 2015. The diameter of each sample spot was 1.5 mm, and the thickness of the tissue section was 4 μm. The EnVision+ detection system (Dako) was used per the manufacturer's instructions. From these tissue arrays, 85 pairs of GC specimens and corresponding adjacent normal tissue specimens were obtained, as well as ten individual cancer tissue specimens. Surgical type was categorized as curative or noncurative resection. Radical resection (R0) refers to the complete removal of the tumor, no residue under the microscope, and complete removal of the naked eye, but the residual under the microscope (R1) or both the naked eye and pathology (R2) is considered non-curative. Collect clinicopathological data from the patient's medical record and follow up the patient since the surgery date. Applying the 7th edition of Cancer system of American Joint Committee, the GC patients were staged. The end point of follow-up is overall survival (OS), which refers to the interval between the date of surgery and the end of a cancer-related death. This study was approved by the Ethics Committee of Shanghai Outdo Biotech Company.

Immunohistochemistry and H-scoring for KK-LC-1

Perform immunohistochemical staining manually, and carefully handle each slide in strict accordance with the instructions. Anti-KK-LC-1 antibody (CL4762, Abcam, United Kingdom, 100 μL), produced in mice, was applied in the expression analysis. Immunohistochemical slide staining results were independently evaluated by two experienced pathologists. H-scoring system is used to evaluate KK-LC-1 staining results [25-28], and we estimate the H-score by multiplying the total staining intensity of each section by the percentage of the number of positive cells. The staining intensity was divided into 4 levels varying from 0 to 3: 0 represents negative; 1 represents weak; 2 represents medium and 3 represents strong, and the percentage of positives was between 0 and 100. In general, the final H-score obtained was between 0 and 300. We stained KK-LC-1 in the cytoplasm and nucleus separately and obtained their respective values by scoring.

Statistical analysis

This study used SPSS 17.0 software for statistical analysis. To evaluate sample distribution, Kolmogorov-Smirnov nonparametric test was applied. Mann-Whitney U test was used to compare variables with abnormal distribution, chi-square test was used to compare qualitative variables. OS was compared with Kaplan-Meier method and log rank test. All potential factors related to prognosis from univariate analysis were input into the Cox regression model. As for identifying independent prognostic factors, this paper applies multi-factor Cox regression analysis. All P values were bidirectional, and *P*-values that are less than 0.05 were considered to be statistically significant.

Results

Clinicopathological characteristics and survival data

The average and median ages of patients undergoing gastric cancer surgery were 65 and 66 (range 45-83 years old). Patients that covered a ratio of 75.5% in this study were male, and the ratio of male patients to female patients was 3: 1. 95.7% of them are underwent radical resection, of which moderately to highly differentiated adenocarcinoma was found in 24.4% of cases. Patients with lymph node metastasis and patients with distant metastasis were 21.2% and 4%, respectively. Table 1 lists more information about this result.

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

KK-LC-1 staining was quantified and analyzed (Table 2A and Figure 1). In the tissue arrays, all stained specimens were located in the cytoplasm, this research did not find any strong positive (3+) staining in the specimens. H-score of tumor tissue and normal tissue separately were calculated separately and the empirical results are reported in Table 2B and Figure 2, and we found that the median H-score of KK-LC-1 in the cytoplasm of tumor tissue was 100 (range 0-250). In normal tissues, we calculated that the median H score of KK-LC-1 was 50 (range 0-150).

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

In both tumor and normal tissues, KK-LC-1 is found to be only expressed in the cytoplasm. As shown in the results of the Kolmogorov-Smirnov test, the H score values are abnormally distributed. Therefore, we chose the Mann-Whitney U test to analyze the data (Table 3A). In normal tissues and tumor tissues, KK-LC-1 is found to be only expressed in the cytoplasm. At the same time, we observed that KK-LC-1's expression was higher in tumor tissues than in normal tissues (P< 0.001).

Due to the difference in the median H score of tissue staining, the whole GC patients were divided into two subsamples. Univariate and multivariate analysis results indicate that, favorable OS (as is displayed in figure 3A), are generally those with high H-scores in the cytoplasm. Univariate survival analysis (Table 3B) showed that KK-LC-1 expression (P = 0.016), stage T (P = 0.002), stage N (P = 0.001) and clinical stage (P <0.001) were associated with OS in GC. In our study, age, gender, tumor size, M stage and pathological grade had no significant effect on GC patients. From the empirical results of Cos regression analysis, it showed that T stage, N stage and higher expression of KK-LC-1 protein were independent prognostic factors of OS (Table 3B).

Relationship between KK-LC-1 expression and clinicopathological data

In Table 4, we marked the association between KK-LC-1's expression and the clinicopathological data in detail. KK-LC-1 expression was not significantly associated with age, gender, tumor size, TNM stage and clinical stage. However, we found that higher expression of KK-LC-1 was observed in GC patients with low pathological grades.

Discussion

In this study, GC patients with higher level of KK-LC-1 expression are found to have a better prognosis, and the overall expression of KK-LC-1 protein in gastric cancer tissue was obviously higher than that in normal tissues. Fukuyama et al.[29] also found similar results: KK-LC-1 gene expression was found to be higher in tumor regions than in nontumor regions, and sometimes KK-LC-1 was found to be expressed in non-tumor sites that carrying tumors stomach tissue. In our experimental findings, KK-LC-1's protein expression rate in tumor tissues was 95.7%. By contrast, Akiko et al. also found KK-LC-1's gene expression rate can reach a percentage of 81.6%, which is significantly higher than others. [18] One study found that KK-LC-1's expression rate in triple negative breast cancer was 75%.[20] These findings are similar to ours, which suggests that KK-LC-1 is likely to be highly expressed in tumors. However, no existing studies focus on the expression of tumor-associated antigens in gastric cancer as high as KK-LC-1, suggesting that KK-LC-1 can be treated as an ideal therapeutic target. For clinical diagnostic applications, high expression of tumor-associated antigens in the early stages of cancer is often considered a useful target. At present, there are few reports about the expression of the KK-LC-1 gene and protein and tumor prognosis. Thus, more research results are needed for verification.

Generally, this study's findings suggest that there is a significant negative correlation between KK-LC-1's protein expression and pathological grade. The higher the pathological grade is, the lower the KK-LC-1's protein expression in the tissue and the poorer the prognosis. In contrast, the lower the pathological grade is, the higher the KK-LC-1's protein expression in the tissue and the better the prognosis of the patient. This result also indirectly shows the reliability of our experimental data. We hypothesized that KK-LC-1 protein can be associated with the early state of the tumor and thus related to a good prognosis. Therefore, KK-LC-1 can be used as a positive biomarker directly related to prognosis, which is able to provide clinicians with more choices. For example, patients with higher KK-LC-1's protein expression levels may obtain better results from adjuvant chemotherapy or radiotherapy than patients with lower expression levels. However, studies have also shown that the expression level of KK-LC-1 in hepatocellular carcinoma (HCC) is increased. High KK-LC-1 expression levels are associated with poor survival outcomes. At the same time, the study found that KK-LC-1 promotes cell growth, invasion, migration and epithelial-mesenchymal transition in vivo and in vitro. [30]. In summary, the abnormal KK-LC-1's protein expression is obviously related to the occurrence and development of tumors. Therefore, KK-LC-1 may play different roles in different malignant tumors, and researchers still need to conduct more in-depth research in order to verify the true relationship between KK-LC-1 and cancer and their involvement Specific mechanism.

To date, some mechanisms concerning KK-LC-1 and neoplasia have been revealed in several tumors. According to reports, the activation of CT genes in some types of cancer is related to hypomethylation of CpG islands. *CT45* is one of the 6 member families of the X-linked CT gene, and the expression of *CT45* associated with hypomethylation of promoter DNA is increased in epithelial ovarian cancer. The researchers believe that *CT45* expression may be a prognostic biomarker. [31]. In lung adenocarcinoma, *PIWIL1* is considered to be a highly expressed CT gene. Hypomethylation of the promoter DNA of *PIWIL1* can cause overexpression of CT genes [32]. However, So far, there are few reports on the function and mechanism of KK-LC-1 in human malignant tumors.

It is worth noting that our research findings can be regarded as a theoretical basis for immunotherapy and targeted therapy of different tumors involving KK-LC-1. It is suggested, based on the Human Protein Atlas database (http://www.proteinatlas.org), *CT83* transcripts are expressed in various tumor cell lines, including gastric cancer, colorectal cancer, breast cancer, urothelial cancer, lung cancer, and cervical cancer. Therefore, *CT83* may be a potential target for antibody-photosensitizer conjugate-based photodynamic therapy (PDT) in various malignant tumors. Ziyu Ye et al [33] developed a novel mouse anti-human CT83 monoclonal antibody (CT83 mAb 7G4), which can form an antibody-photosensitizer complex of 7g4-1-ga and can specifically recognize human CT83. In vitro experiments show that 7G4-1-Ga is more cytotoxic to CT83-expressing human cancer cells than 1-Ga. Therefore, anti-CT83 mAb may become an effective treatment for CT83-expressing tumor targeting PDT in the future.

However, the research process of our study has some potential limitations. This is a relatively single study with relatively few sample cases and few statistical analysis tools. Finally, some patients received postoperative chemotherapy or radiotherapy. Although the survival period was limited, the results did not

consider these adjuvant therapies' impact on prognosis. Therefore, multi-angle studies and further researches are needed to explore KK-LC-1's expression in GC and we need to evaluate its clinical efficiency in a wider range 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

Ethic approval

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.

Availability of data and material

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

Author 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. LP L and YL performed project administration. All authors have read and approved the manuscript.

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Competing Interest

No conflicts of interest declared.

Conflicts of Interest Statement:

There are not competing interests to disclose.

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Tables

Table 1
Clinicopathological characteristics of the tissue microarrays

Characteristic	n (%)	Characteristic	n (%)
Age(yr)		N0	20(21.3)
≤ 65	44(46.5)	N1	17(18.1)
65	50(53.2)	N2	22(23.4)
Gender		N3a	29(30.9)
Female	23(24.5)	N3b	6(6.4)
Male	71(75.5)	Pathological grades	
Tumor Size(cm)			23(24.5)
≤ 5	38(40.4)	-	13(13.8)
5	56(59.6)		50(53.2)
T Stage		-	8(8.5)
T1a	1(1.1)	Clinical Stage	
T1b	2(2.2)	A	2(2.1)
T2	9(9.6)	B	4(4.3)
T3	62(66.0)	A	13(13.8)
T4a	18(19.1)	B	15(16.0)
T4b	2(2.1)	A	22(23.4)
M Stage		B	29(30.9)
M0	90(95.7)	C	5(5.3)
M1	4(4.3)		4(4.3)

Table 2A. Kita−Kyushu lung cancer antigen−1 staining results in tumor and normal tissues

Cytoplasmic staining	Positive cell rate (%) Median (range)		*n* (%)	
	Tumor tissues	Normal tissues	Tumor tissues	Normal tissues
Negative	-	-	4(4.3)	8(9.5)
Positiv	100 (40-100)	90 (10-100)	90(95.7)	76(90.5)
< 1	90 (80-100)	60 (10-100)	19(20.2)	36(42.9)
1+	100 (40-100)	90 (20-100)	57(60.7)	40(47.6)
2+	100 (90-100)	-	14(14.8)	0(0)

Table 2B. H-score values in tumor and normal tissues

Group	*n*	Localization	Minimum	Maximum	Median
Tumor	94		0	250	100
Normal	84	Cytoplasm	0	150	50

Table3A. Comparison in tumor and normal tissues for Kita−Kyushu lung cancer antigen−1 expression

Group	*n*	Minimum	Maximum	Median	Mean rank	*P*
Tumor	94	0	250	100	111.00	< 0.001*
Normal	84	0	150	50	65.44	

* Statistically significant

Table3B. Univariate and multivariate analyses for Gastric cancer

Univariate	χ^2	*P* value	*OR* (95%CI)
Age	0.577	0.565	
Gender	0.871	0.351	
Tumor Size	2.632	0.105	
T Stage	9.868	0.002*	
N Stage	11.973		
M Stage	1.873	0.001*	
Pathological grades	3.753	0.053	
Clinical Stage	10.464	0.171	
Protein Expression	5.848		
Multivariate		0.001*	
T Stage			3.466(1.394-8.622)
N Stage		0.016*	2.986(1.308-6.814)
Protein Expression		0.007*	0.591(0.364-0.961)
		0.009*	
* Statistically significant		0.034*	

Table 4 Correlation between KK-LC-1 expression and clinicopathological parameters

Parameter	Low expression	High expression	χ^2	*P* value
Age				
≤65	29	15	0.963	0.326
> 65	28	22		
Gender				
Female	15	8	0.268	0.605
Male	42	29		
Tumor Size (cm)				
≤5	21	17	0.772	0.380
> 5	36	20		
T Stage				
T1+2	6	6	0.652	0.419
T3+4	51	31		
M Stage				
M0	54	36	0.361	0.548
M1	3	1		
N Stage				
Negative	12	8	0.004	0.947
Positive	45	29		
Pathological grades				
< Ⅲ	17	19	4.400	0.036*
≥Ⅲ	40	18		
Clinical Stage				
Ⅰ + Ⅱ	19	15	0.505	0.477
Ⅲ+Ⅳ	38	22		

* Statistically significant

Figures

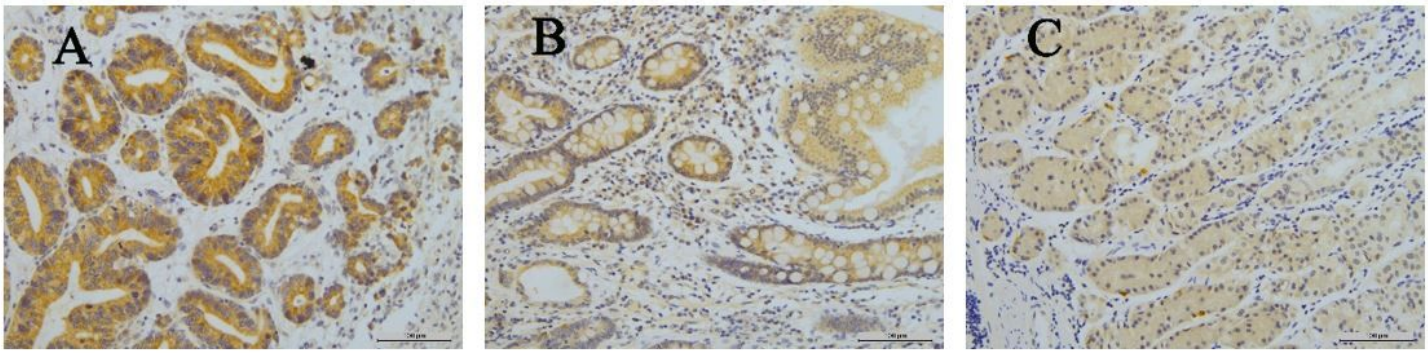

Figure 1

Immunohistochemical staining of tumor tissues (magnification, × 200). A-C: Cytoplasm staining with 2+, 1+ and <1 intensity, respectively.

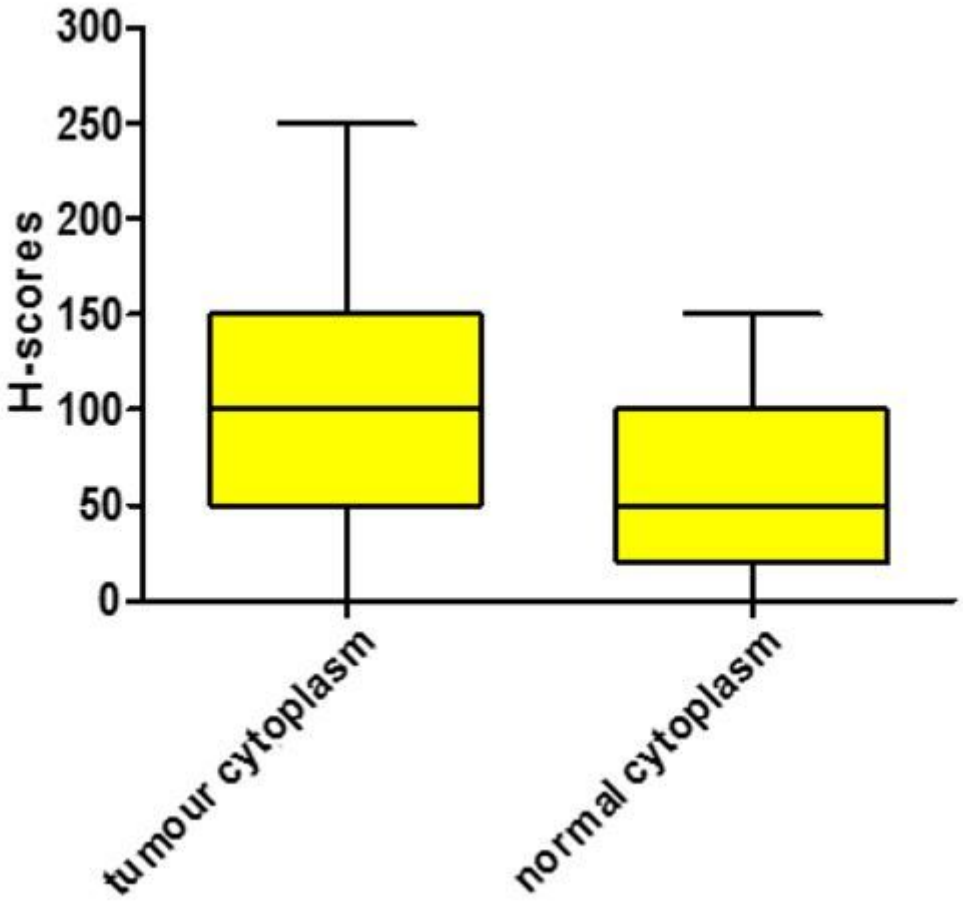

Figure 2

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

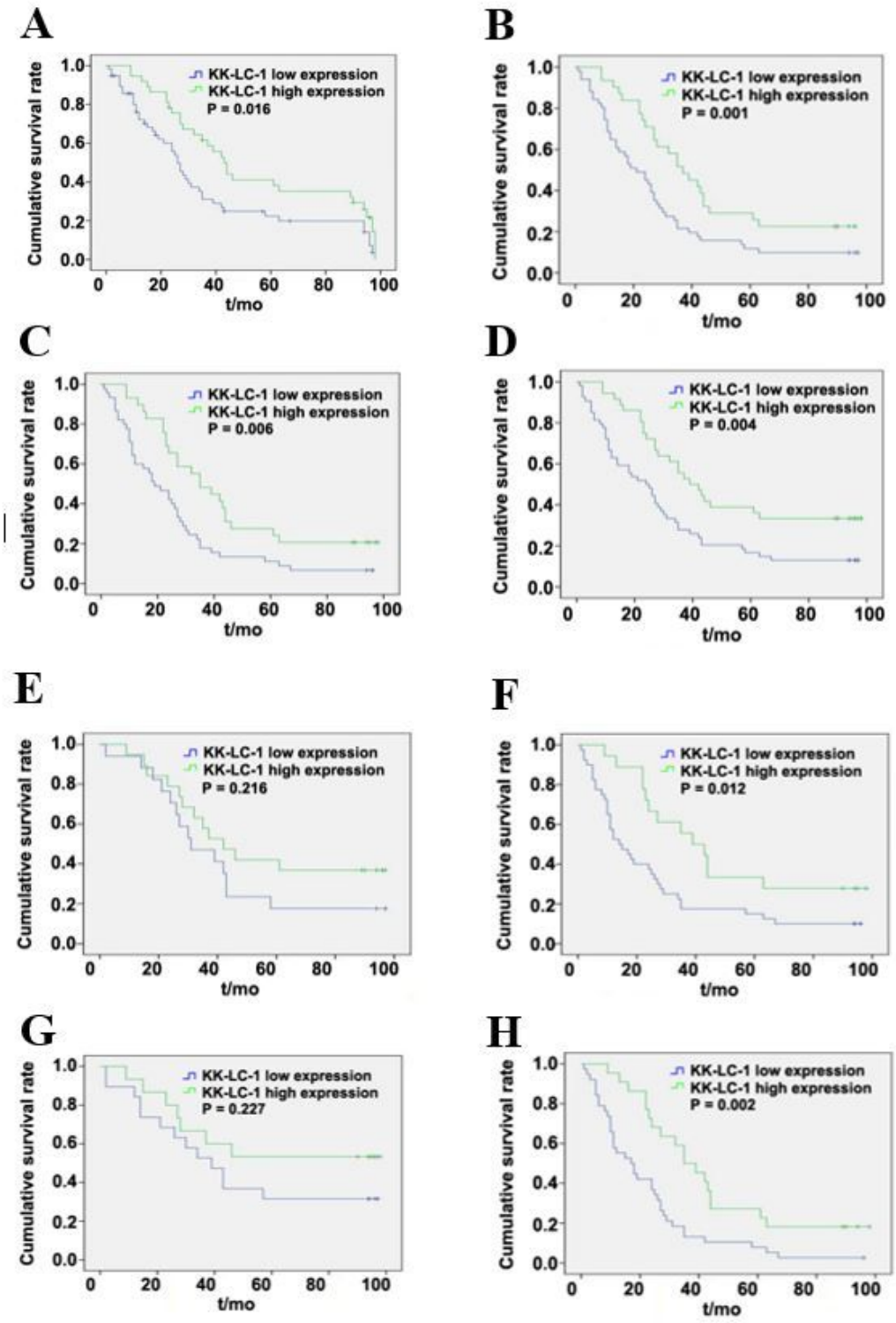

Figure 3

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: Clinical Stage ⅠⅡⅢ group; H: Clinical Stage Ⅰ+Ⅱ group. KK-LC-1: Kita-Kyushu lung cancer antigen-1