Frequent High Expression of Kita-Kyushu Lung Cancer Antigen-1 (KK-LC-1) in Gastric Cancer

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Abstract. *Background: The tumor-associated antigen Kita-Kyushu lung cancer antigen-1 (KK-LC-1) has been reported as not being expressed in normal tissues, except for the testis, and in the setting of non-small cell lung cancer. The present study demonstrated that KK-LC-1 is expressed in gastric cancer. Materials and Methods: We analyzed the expression of KK-LC-1 and cancer/testis antigens (CTAs) in surgical specimens of 49 gastric carcinomas. The expression of KK-LC-1 and CTAs was assessed using reverse transcription-polymerase chain reaction. Results: KK-LC-1 expression was observed in gastric carcinomas. The number of lesions with expression of KK-LC-1, Melanoma antigen gene encoding-A1 (MAGE-A1), MAGE-A3 and New York Esophageal squamous cell carcinoma-1 (NY-ESO-1) was 40 (81.6%), 17 (34.7%), 22 (44.9%) and 8 (16.3%) out of the 49 specimens, respectively. Conclusion: KK-LC-1 should be categorized as a CTA. The frequency of KK-LC-1 expression was higher than that of the other CTAs. KK-LC-1 might be a useful target for immunotherapy and in diagnosis of gastric cancer.*

Gastric cancer is the second most common cause of cancer death in Japan. The majority of cases of gastric cancer are of advanced stage at diagnosis, and the survival rate thus remains poor, although medical and surgical treatments have improved. Therefore, there is a great need for novel therapeutic approaches for treating patients with advanced gastric cancer. Cancer immunotherapy is one of the best candidates for cancer therapy. However, few powerful targets for immunotherapy against gastric cancer are currently available.

A large number of tumor-associated antigens (TAAs) have been identified in various human cancer types (1-3). In particular, the cancer/testis antigens (CTAs) are attractive targets for immunotherapy against cancer because they are either expressed minimally or not at all in normal tissues, except for germline tissues, and aberrantly expressed in a range of human cancer types. CTAs are also attractive targets for cancer immunotherapy. Moreover, CTAs are potentially advantageous molecules for confirming systemic diagnosis of malignancy based on their specific expression patterns. However, knowledge regarding the frequency of expression of individual CTAs is not adequate for application in diagnosis of cancer.

Fukuyama *et al*. reported the identification of a new TAA designated Kita-Kyushu lung cancer antigen-1 (KK-LC-1), which has an epitope peptide recognized by a cytotoxic T-lymphocytes (CTL)(4). KK-LC-1 is not expressed in normal tissues, except for the testis, whereas it is frequently expressed in non-small cell lung cancer (NSCLC), at a rate of 33% (5). According to these findings, KK-LC-1 may be categorized as a CTA. However, this molecule does not meet the requirements for a CTA in that it is not expressed in malignancies affecting different organs.

The purpose of the present study was to assess the expression of KK-LC-1 in gastric cancer in order to confirm whether it is a CTA. Furthermore, we assessed the relationship between the expression of KK-LC-1 and that of other CTAs and examined whether KK-LC-1 is a good candidate as a target for immunotherapy for gastric cancer.

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Materials and Methods

The current study protocol was approved as No23-8 by the Human Ethics Review Committee of Kitasato University Medical Center, Japan, and signed informed consent was obtained from each subject prior to collection of the tissue samples used in this study.

Patients. A total of 85 patients underwent surgical resection of gastric cancer at the Department of Surgery, Kitasato University Medical Center, Kitamoto, Japan, during the period of June 2011-June 2013. We obtained 55 specimens from these patients. After excluding six patients with multiple gastric carcinomas, we examined 49 patients with gastric cancer. The patients' medical records, including findings for clinical data, preoperative examination results, details of the surgical procedure, histopathological findings and TNM stage, were also reviewed. All resected specimens, including the primary tumor and systemically dissected lymph nodes, were examined for the determination of tumor histology and the extent of lymph node metastasis. The clinicopathological findings were classified according to the Japanese Classification of Gastric Carcinoma [14th edition (6)]. The background factors and characteristics of the 49 patients are shown in Table I.

Table I. *Characteristics of 49 patients with gastric cancer.*

Mean age, years (range)	70.0 (30-85)
Gender	
Male	33 (67.3%)
Female	16 (32.7%)
Depth of invasion	
T1	17 (34.7%)
T2	9 (18.4%)
T3	8 (16.3%)
T4	15 (30.6%)
Lymph node metastasis	
N0	23 (46.9%)
N1	9 (18.4%)
N2	8 (16.3%)
N3	9 (18.4%)
Histological type	
Papillary adenocarcinoma	2 (4.1%)
Tubular adenocarcinoma	23 (46.9%)
Mucinous adenocarcinoma	1 (2.0%)
Poorly-differentiated adenocarcinoma	19 (38.8%)
Signet ring cell carcinoma	1 (2.0%)
Carcinoma with lymphoid stroma	3 (6.1%)
Stage of disease	
I	20 (40.8%)
II	15 (30.6%)
III	9 (18.4%)
IV	5 (10.2%)

Tissue specimens. The collected tumor tissues obtained from the 49 patients with gastric cancer were immediately preserved in RNAlater (Life technologies, Carsbad, CA), and the specimens were allowed to incubate for penetration of RNAlater at 4˚C overnight and then stored at −80˚C until use.

RNA isolation, cDNA synthesis and PCR analysis of CTAs. Total RNA was isolated from the tumor specimens using BIO ROBOT EZ1 and EZ1 RNA Tissue Mini Kits (48) (both Qiagen, Hilden, Germany) according to the manufacturer's instructions and converted to cDNA using an oligo-p(dN) 6 random primer and Superscript II reverse transcriptase (both Life Technologies), respectively. β-Actin was used as an internal standard to assess the quality of RNA isolation. The expressed levels of β-actin, Melanoma antigen gene encoding-A1 (MAGE-A1) and -A3, and New York Esophageal squamous cell carcinoma -1(NY-ESO-1) were measured with TaqMan Gene Expression Assays ID Hs99999903_m1, Hs00607097_m1, H200366532_m1 and Hs00265824_m1, respectively, using the 7900HT Fast Real-Time PCR System (all Life Technologies). The cDNAs converted from the RNAs were measured according to the threshold cycle number (Ct) of β-actin and then the expression levels of the CTAs and KK-LC-1 were assessed, when Ct of β-actin was under 28. The expression of KK-LC-1 was examined using conventional reverse transcription polymerase chain reaction (RT-PCR), because an appropriate probe for detecting mRNA of KK-LC-1 has not been established. For RT-PCR of KK-LC-1, ATGAACTTCT ATTTACTCCTAGCGAGC and TTAGGTGGATTTCCGGTGAGG were used as specific primers, and annealing was performed at 67˚C for 40 cycles, yielding a 342-bp product.

Results

Expression of KK-LC-1 in seven specimens of gastric cancer is indicated in Figure 1. The number of specimens expressing KK-LC-1 in the patients with gastric cancer was 40 out of 49 (81.6%) (Table II). Expression of MAGE-A1, MAGE-A3 and NY-ESO-1 was detected in 17 (34.7%), 22 (44.9%) and 8 (16.3%) out of the 49 patients, respectively (Table II). Figure 2 shows the expression pattern of CTAs in each gastric cancer specimen, and Table III shows the correlations between KK-LC-1 expression and the expression of the other CTAs in the 49 cases. The expression of any CTA, including KK-LC-1, was observed in 47 out of the 49 cases (95.9%). Among these 47 cases, 17 tumors expressed KK-LC-1 synchronously with another CTA, whereas the expression of KK-LC-1 was found in 23 lesions without expression of other CTAs. The expressions of CTAs excluding KK-LC-1 were found in 24 out of the 49 cases (49.0%). The specimens that expressed NY-ESO-1 also expressed both MAGE-A1 and MAGE-A3 (Figure 2).

Discussion

The characteristics of KK-LC-1 tend to place it within the CTA category because it is not expressed in normal tissues, with the exception of the testis (4). While the expression of KK-LC-1 has been reported only in tissues of NSCLC (4, 5), the present study showed that KK-LC-1 is also expressed in gastric cancer and confirmed that KK-LC-1 may be categorized as a CTA because it is expressed in tumor cells in multiple organs.

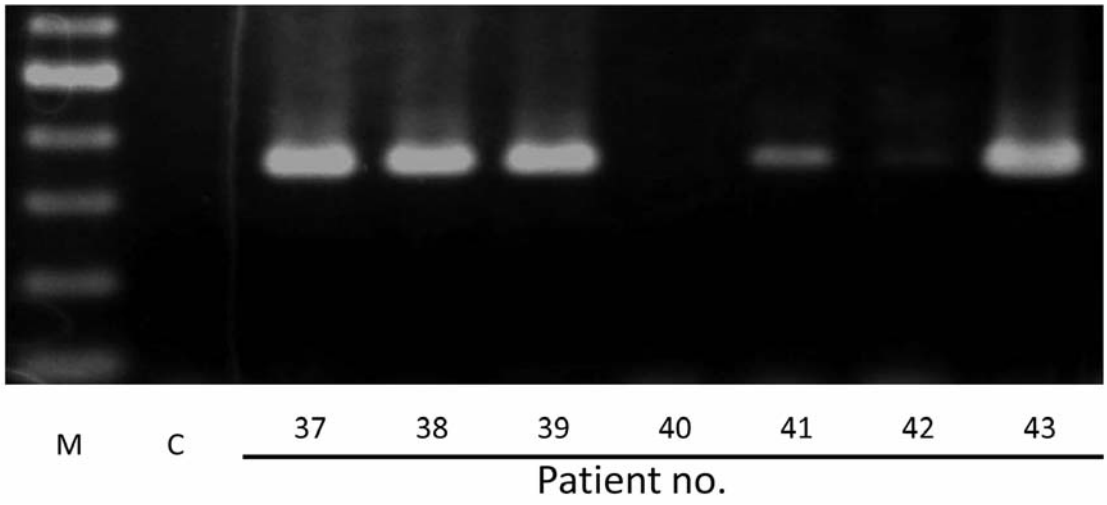

Figure 1. *Expression of Kita-Kyushu lung cancer antigen-1 (KK-LC-1) in the gastric tumors of seven patients with gastric cancer. The expression of KK-LC-1 was detected as a 342-bp band of the amplicon. KK-LC-1 was expressed in five out of these seven gastric specimens. The brightest band in lane M indicates 500 bp. M: 1000 bp ladder; C: negative control.*

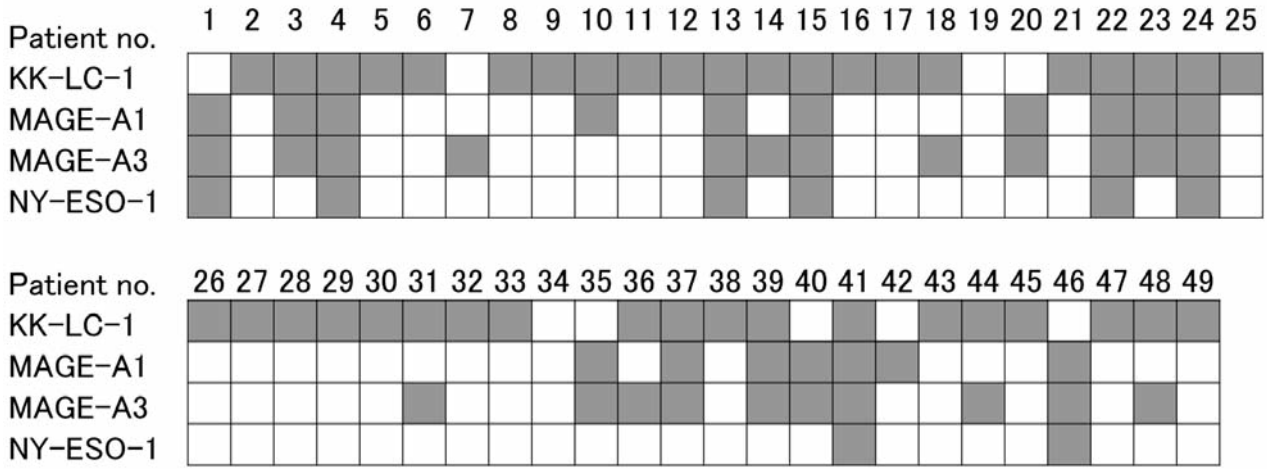

Figure 2. *Expression patterns of cancer/testis antigens (CTAs), including Kita-Kyushu lung cancer antigen-1 (KK-LC-1), in specimens from the 49 patients with gastric cancer. Solid squares indicate expression of that CTA in the specimen.*

In comparison with that seen for the other CTAs tested in this study, the 81.6% frequency of KK-LC-1 expression in gastric tumors is markedly higher than the 34.7% observed for MAGE-A1, 44.9% observed for MAGE-A3 and 16.3% observed for NY-ESO-1. Similarly to the current results, the frequency of MAGE-A1, MAGE-A3 and NY-ESO-1 expression has been reported to be 24-41% (7-10), 38-43% (7, 8, 10) and 8-13% (7, 9, 11), respectively. To date, there exist several reports showing that specific carcinogens induce the *de novo* expression of specific CTAs in cell lines (12, 13). In particular, Fukuyama *et al*. showed that *Helicobacter pylori* induces the specific expression of CTAs (12), and more than 80% of cases of gastric cancer are caused by infection with *H. pylori* (14). *H. pylori* has the potential to induce the expression of specific CTAs in conjunction with the induction of malignant transformation of host cells (12). These observations indicate that specific CTAs, such as KK-LC-1, are frequently highly expressed in gastric cancer.

The expression and immunogenicity for T-cell recognition *via* TAAs are prerequisites for potential targeting by polyvalent cancer vaccines, and the identification of tumor antigens recognized by CTLs is required for the application

Table II. *Cancer/testis antigen (CTA) expression in 49 patients with gastric cancer.*

CTA	Positive	Negative	Positivity (%)
KK-LC-1	40	9	81.6
MAGE-A1	17	32	34.7
MAGE-A3	22	27	44.9
NY-ESO-1	8	41	16.3

Table III. *Detection of cancer-testis antigens (CTAs) in 49 patients with gastric cancer.*

Combination of CTAs	Number (N=49)	Detection rate (%)
Two CTAs including KK-LC-1		
KK-LC-1 and MAGE-A1	46	93.9
KK-LC-1 and MAGE-A3	46	93.9
KK-LC-1 and NY-ESO-1	42	85.7
Two CTAs excluding KK-LC-1		
MAGE-A1 and MAGE-A3	24	49
MAGE-A1 and NY-ESO-1	17	34.7
MAGE-A3 and NY-ESO-1	22	44.9
Three CTAs including KK-LC-1		
KK-LC-1, MAGE-A1 and MAGE-A3	47	95.9
KK-LC-1. MAGE-A1 and NY-ESO-1	46	93.9
KK-LC-1, MAGE-A3 and NY-ESO-1	46	93.9
Three CTAs excluding KK-LC-1		
MAGE-A1, MAGE-A3, and NY-ESO-1	24	49
Four CTAs including KK-LC-1		
KK-LC-1, MAGE-A1, MAGE-A3 and NY-ESO-1	47	95.9

of vaccines. The peptide KK-LC-$1_{76\text{-}84}$ is an epitope peptide that binds to human leukocyte antigen-B62 (HLA-B62) and is recognized by CTLs (4). Therefore, KK-LC-1 is an attractive antigen for use as a candidate vaccine in patients with HLA-B62-positive and KK-LC-1-expressing tumors. The HLA-B62-positive population is not large, accounting for fewer than 20% of Japanese and Caucasians (15, 16). To date, various reports have shown that CTAs have multiple epitope peptides binding to HLA-class I molecules (17-19). Hence, KK-LC-1 may have other epitope peptides that are recognized by CTLs in an HLA class I-dependent manner.

On the other hand, the detection of KK-LC-1 is useful for the detection of cancer cells. Detecting KK-LC-1, as mRNA or protein, in biopsy specimens, for the diagnosis of gastric or other types of cancer is not limited to HLA restriction, compared to vaccination with cancer immunotherapy, which requires HLA restriction. This method is attractive for detecting gastric cancer cells for diagnosis as a result of the 81.6% expression frequency of KK-LC-1, which is higher than the rate of detection of multiple CTAs. Other researchers have reported that the rates of detection of at least one CTA using three to 11 CTAs are below 74% (7, 9, 10, 20). Moreover, in this study, the rate of detection of at least one CTA using KK-LC-1, MAGE-A1 and MAGE-A3 was 95.6%.

In summary, KK-LC-1 is a CTA and potential target for cancer immunotherapy in addition to previously identified CTAs. In cases of gastric cancer, in particular, cancer immunotherapy targeting CTAs, including KK-LC-1, is expected to be applicable to most patients with gastric cancer, and diagnosis targeting this molecule has the potential to detect the majority of cancer cells in the stomach.

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