Anti-KIAA0513 Antibody as a Broad-spectrum Biomarker of Mortal Atherosclerotic and Cancerous Diseases

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Research

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Abstract

Background: Numerous antibody biomarkers have been reported for cancer and atherosclerosis-related diseases. The major complications of atherosclerosis and diabetes mellitus (DM) are acute ischemic stroke (AIS), cardiovascular disease (CVD), and chronic kidney disease (CKD). Cancer development is accompanied by arterial disorders such as angiogenesis and atherosclerosis, and DM is a risk factor for certain cancers. Atherosclerosis-related diseases and cancers are therefore interrelated and could be detected by a common biomarker.

Methods: We employed the protein array method for the initial screening of antigens and employed the amplified luminescent proximity homogeneous assay-linked immunosorbent assay (AlphaLISA) to evaluate antibody levels in serum samples.

Results: The protein array identified KIAA0513 as an antigen recognized by serum IgG antibodies in the sera of patients with atherosclerosis. We then prepared recombinant glutathione S-transferase-fused KIAA0513 protein. AlphaLISA showed significantly higher serum antibody levels against KIAA0513 protein in patients with AIS, transient ischemic attack, DM, CVD, obstructive sleep apnea syndrome (OSAS), CKD, and solid cancers, such as esophageal, gastric, colon, lung, and breast cancer, than in healthy donors. A receiver operating characteristic (ROC) analysis revealed that the highest areas under the ROC curves of anti-KIAA0513 antibodies were for esophageal cancer, nephrosclerosis-type CKD, and DM. Spearman's correlation analysis revealed that serum anti-KIAA0513 antibody levels were associated with maximum intima-media thickness and plaque score, which are indices of atherosclerosis and stenosis.

Conclusion: Serum anti-KIAA0513 antibody markers appear to be useful for diagnosing AIS, TIA, DM, CVD, OSAS, CKD, and solid cancers and might reflect common arterial alterations leading to atherosclerotic and cancerous diseases.

Background

In recent years, various disease biomarkers have been discovered, and the development of simple blood tests is underway to determine the pathological condition, predict the onset of disease and its prognosis, and identify preventive/therapeutic targets. In terms of biomarker species, studies have reported enzyme, antigen, and, in recent years, nucleotide markers. However, there are still few reports on antibody markers, which include HSP60 [1], RPA2 [2], SOSTDC1 [3], PDCD11 [4], MMP1, CBX1, CBX5 [5], and DNAJC2 [6] for acute ischemic stroke (AIS); ATP2B4 [7], BMP-1 [2, 7], DHPS [8], SH3BP5 [9], prolyl carboxypeptidase [10], LRPAP1 [11], and ASXL2 [12] for atherosclerosis; nardilysin/NRD1 [13] for acute cardiac syndrome; and TUBB2C [14], insulin [15], glutamic acid decarboxylase [16], adiponectin [17], and GADD34 [18] for diabetes mellitus (DM).

The anti-p53 antibody is a typical antibody biomarker for cancer that has been put into practical use for diagnosing, monitoring, and predicting the prognosis of esophageal cancer (EC) and head and neck

cancer [19, 20]. Further application of the serological identification of antigens by cDNA expression cloning and the protein array method have identified autoantibodies against TACSTD2 [21], SLC2A1 [22], TRIM21 [23], myomegalin [24], makorin 1 [25], ECSA [26], cyclin L2 [27], and LRPAP1 [11] for EC; FIR/PUF60 for colorectal cancer (CRC) [28] and gastric cancer (GC) [29]; SH3GL1 [30] and filamin C [31] for glioma; EP300-interacting inhibitor of differentiation 3 for nonfunctional pancreatic neuroendocrine tumors [32]; and coatmer protein complex subunit epsilon/COPE [33], NBL1/DAN [34], and SNX16 [35] for obstructive sleep apnea syndrome (OSAS). Here, we report on serum antibodies against KIAA0513 (s-KIAA0513-Ab) as a broad-spectrum biomarker applicable to atherosclerosis-related diseases such as ischemic stroke, cardiovascular disease (CVD), chronic kidney disease (CKD), DM, and solid cancers.

Methods

Patient and control sera

This study was approved by the Local Ethical Review Board of the Chiba University Graduate School of Medicine (Chiba, Japan), as well as by the review boards of the participating hospitals. Sera were collected from patients who had provided written informed consent. Each serum sample was centrifuged at 3000 *g* for 10 min, and the supernatant was stored at – 80°C until use, avoiding the repeated freezing/thawing of samples.

Serum samples collected from the patients with AIS, transient ischemic attack (TIA), asymptomatic ischemic stroke (Asympt-CI), chronic-phase cerebral infarction (cCI), and deep and subcortical white matter hyperintensity (DSWMH) were obtained from Chiba Prefectural Sawara Hospital. The stroke subtypes were determined according to the criteria of the Trial of Org 10172 in the Acute Stroke Treatment classification system [36], and large-artery atherosclerosis and small-artery occlusion (lacune) were included as AIS and ischemic stroke. Samples from patients with DM, CVD, and OSAS were obtained from Chiba University Hospital. CVD included acute myocardial infarction (AMI) and unstable angina (UA). The serum samples of patients with AIS, TIA, and AMI were obtained within 2 weeks after disease onset. Samples collected from patients with CKD were obtained from the Kumamoto cohort [37, 38], whereas those collected from patients with EC, GC, CRC, lung cancer (LC), and breast cancer (BC) were obtained from the Department of Surgery, Toho University Hospital. Serum samples from healthy donors (HDs) were obtained from Chiba University, Port Square Kashiwado Clinic, the National Hospital Organization, Shimoshizu Hospital, and Chiba Prefectural Sawara Hospital. For comparisons with TIA and AIS, serum samples from HDs were selected from patients who exhibited no abnormalities in cranial magnetic resonance imaging.

ProtoArray® screening

The initial screening was performed using ProtoArray® Human Protein Microarrays v4.0 (Thermo Fisher Scientific, Waltham, MA), which were loaded with 9480 species of proteins, as described previously [9]. In total, we employed 20 serum samples (10 each from the patients and HDs) to detect antigens specifically recognized by IgG antibodies in the patient sera.

Expression and purification of KIAA0513 protein

We cloned a sequence encoding cDNA of the isoform c of human KIAA0513 (accession number: NP_001284695.1) into pGEX-6P (Cytiva, Pittsburgh, PA). We induced expression of the cDNA product by treating *Escherichia coli* (*E. coli*) KRX (Promega, Madison, WI) cells harboring the pGEX-6P-KIAA0513 and pMINOR with 0.5 mM isopropyl- β -D-thiogalactoside at 37°C for 3 h [39]. The cells were lysed by sonication in phosphate-buffered saline containing 2 mM dithiothreitol. The proteins were purified using Glutathione-Sepharose 4 Fast Flow medium (Cytiva) and HiPrep 26/10 Desalting column (Cytiva). Purified glutathione S-transferase (GST)-KIAA0513 was concentrated to 3.5 mg/ml in phosphate-buffered saline containing 2 mM dithiothreitol.

Western blotting

Purified GST-KIAA0513 and the control GST proteins were electrophoresed through sodium dodecyl sulphate-polyacrylamide (11%) gels, followed by blotting onto nitrocellulose membranes. The membranes were blocked with 0.1% dry milk in 150 mM NaCl, 20 mM Tris-HCl (pH 7.6), and 0.1% Tween-20 (TBS-T) and then treated with anti-GST, anti-KIAA0513, or the patients' sera overnight. The membranes were washed 5 times with TBS-T and treated with HRP-conjugated secondary antibodies for 20 min. After 5 washes with TBS-T, the addition of Immobilon (Merck KGaA, Darmstadt, Germany) produced luminescence, which was detected by LuminoGraph II (Atto, Tokyo, Japan), as described previously [5, 6, 11].

Amplified luminescence proximity homogeneous assay-linked immunosorbent assay (AlphaLISA)

AlphaLISA was performed in 384-well microtiter plates (white opaque OptiPlate™, Perkin Elmer, Waltham, MA) containing either 2.5 μ L of 1:100-diluted serum with 2.5 μ L of GST or GST-KIAA0513 proteins (10 μ g/mL) in AlphaLISA buffer (25 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/mL dextran-500, and 0.05% ProClin-300). We incubated the reaction mixture at room temperature for 6–8 h, then added anti-human IgG-conjugated acceptor beads (2.5 μ L at 40 μ g/mL) and glutathione-conjugated donor beads (2.5 μ L at 40 μ g/mL), and incubated the mixture at room temperature in the dark for 1–14 days. We measured the chemical emissions using an EnSpire Alpha microplate reader (PerkinElmer), as described previously [5–12]. We calculated the specific reactions by subtracting the emission counts of the GST control from the counts of GST-fusion proteins.

Immunohistochemical staining

The formalin-fixed paraffin-embedded EC tissues were sectioned into 4- μ m-thick slices, which were deparaffinized, blocked with Hyper Peroxide Block and Protein Block (Rabbit Specific HRP/DAB Detection Kit, Abcam, Cambridge, UK), reacted with primary anti-KIAA0513 antibodies (rabbit polyclonal antibodies, Atlas Antibodies, Bromma, Sweden) at 0.75 μ g/mL for 18 h at 4°C, incubated with biotinylated anti-rabbit IgG, and reacted with streptavidin conjugated to horseradish peroxidase reagent (Streptavidin Peroxidase, Abcam). Lastly, we visualized the reaction with a chromogen (diaminobenzidine) in DAB Substrate

(Abcam). We then counterstained the sections with hematoxylin, dehydrated them, and mounted them as described [11].

Nested case-control study

We conducted a nested case-cohort study using the abovementioned AlphaLISA detection antibody levels. This study was nested within the Japan Public Health Center-based Prospective Study [40–42], which involved approximately 30,000 Japanese individuals aged 40–69 years at a baseline period from 1990–1994 who had provided informed consent. The serum samples employed were from 202 cases of incidental AIS in the cohort that occurred between baseline and 2008 and from 202 controls whose age (within 2 years), sex, date of blood sampling (within 3 months), time since last meal (within 4 h), and study location (Public Health Center area) were matched with those of the cases. We used a conditional logistic regression model to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs).

Statistical analysis

We employed the Mann–Whitney *U* test to determine the significant differences between 2 groups and the Kruskal–Wallis test (Mann–Whitney *U* test with Bonferroni correction applied) to evaluate the differences among ≥ 3 groups. We calculated the correlations using Spearman's correlation analysis and logistic regression analysis. All the statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc.). We assessed the predictive values of the putative disease markers via a receiver operating characteristic (ROC) curve analysis and set the cutoff values to maximize the sums of sensitivity and specificity. We evaluated patient survival using the Kaplan–Meier method and compared them using the log-rank test. All tests were 2-tailed, and *P*-values < 0.05 were considered to indicate statistically significant differences.

Results

Recognition of KIAA0513 by serum components from patients with atherosclerosis

We employed a ProtoArray loaded with 9480 protein species to identify the antigens recognized by antibodies in the sera of patients with atherosclerosis. We found that KIAA0513 isoform c (Accession Number: BC030280.1) reacted with 6 of the 10 serum samples from the patients with atherosclerosis and with only 1 of the 10 samples from the HDs. Subsequently, GST-fused full-length KIAA0513 protein was expressed in *E. coli* and purified by affinity-chromatography.

Presence of autoantibodies against KIAA0513 in the sera of patients with AIS, TIA, DM, EC, or CC

We examined the presence of autoantibodies against KIAA0513 in sera by Western blotting (Fig. 1). GST-KIAA0513 protein reacted with commercial anti-GST and anti-KIAA0513 antibodies, whereas the control GST reacted with anti-GST but not with anti-KIAA0513 antibodies. GST-KIAA0513 protein was also recognized by serum IgG antibodies in the patients with AIS (#07065 and #070684), TIA (#02337), DM (#22226), EC (#EC-6), and CRC (#Co-58) but not in the HDs (#09101). GST alone showed no apparent reaction with any serum from the patients or HDs.

Elevation of s-KIAA0513-Abs levels in the patients with AIS and TIA

We then examined the s-KIAA0513-Abs levels in the patients with AIS or TIA. Sera from HDs and patients with AIS and TIA were obtained from Chiba Prefectural Sawara Hospital. The AlphaLISA results revealed that s-KIAA0513-Ab levels were significantly higher in the patients with AIS or TIA than in the HDs (Fig. 2a). Using the cutoff values of the average plus 2 standard deviations (SDs) of the HD values, the s-KIAA0513-Ab positivity rates for the HDs, patients with AIS, and those with TIA were 0.0%, 7.6%, and 15.6%, respectively (Table 1). The ROC analysis revealed that the areas under the ROC curves (AUCs) of s-KIAA0513-Abs were 0.6439 (95% CI 0.587–0.700) for AIS (Fig. 2b) and 0.6604 (95% CI 0.563–0.758) for TIA (Fig. 2c). Thus, TIA (which can be a prodromal stage of AIS) and AIS were equally associated with the s-KIAA0513-Ab marker.

Table 1

Comparison of the serum antibody levels of healthy donors (HDs) vs. those of patients with acute ischemic stroke (AIS) or transient ischemic attack (TIA).

Sample information		HD	AIS	TIA
	Total sample number	139	228	44
	Male/Female	87/52	129/99	24/20
	Age (Average $\pm$ SD)	51.6 $\pm$ 12.8	77.0 $\pm$ 11.1	68.5 $\pm$ 12.1
Subject group	Type of value	KIAA0513-Ab		
HD	Average	92,849		
	SD	36,115		
	Cutoff value	165,080		
	Positive number	0		
	Positive (%)	0.0%		
AIS	Average	110,913		
	SD	40,351		
	Positive number	11		
	Positive (%)	7.6%		
	<i>P</i> (AIS vs HD)	< 0.001		
TIA	Average	122,303		
	SD	42,845		
	Positive number	5		
	Positive (%)	15.6%		
	<i>P</i> (TIA vs HD)	< 0.01		
<p>Upper panel indicates the numbers of total samples and samples from male and female samples as well as ages [average $\pm$ standard deviation (SD)]. The lower panel summarizes the serum antibody levels (Alpha photon counts) examined by AlphaLISA using purified KIAA0513-GST protein as an antigen. Cutoff values were determined as the average HD values plus two SD, and positive samples higher than the cutoff value were scored. <i>P</i> values were calculated using the Kruskal-Wallis test. <i>P</i> values < 0.05 and positive rates > 10% are marked in bold text. A scatter dot plot of the same results is shown in Fig. 2a.</p>				

Elevation of serum antibody levels against KIAA0513 in patients with DM

We next examined the s-KIAA0513-Abs levels in the patients with DM. Serum samples from HDs and patients with DM were obtained from Chiba University and Chiba University Hospital. The s-KIAA0513-Abs levels were significantly higher in the samples from the patients with DM than in those from HDs (Fig. 3a). At a cutoff value equivalent to the average plus 2 SDs of the HD specimen values, the positive rates of s-KIAA0513-Ab in the HDs and patients with DM were 2.5% and 26.5%, respectively (Table 2). We performed an ROC analysis to evaluate the ability of these antibody markers to indicate the presence of DM. The AUC for s-KIAA0513-Abs was 0.736, yielding a sensitivity and specificity of 50.55% and 87.65%, respectively (Fig. 3b).

Table 2

Comparison of the serum antibody levels of HDs vs. those of patients with diabetes mellitus (DM).

Sample information		HD	DM
	Total sample number	81	275
	Male/Female	46/35	158/117
	Type 1 DM/Type 2 DM	-	26/216*
	Age (Average ± SD)	45.2 ± 11.0	63.1 ± 12.0
Subject group	Type of value	KIAA0513-Ab	
HD	Average	33,698	
	SD	18,121	
	Cutoff value	69,941	
	Positive number	2	
	Positive (%)	2.5%	
DM	Average	55,282	
	SD	29,897	
	Positive number	73	
	Positive (%)	26.5%	
	<i>P</i> (vs HD)	< 0.001	
The upper panel indicates the numbers of total samples, samples from male and female subjects, and samples from patients with type 1 and type 2 DM as well as ages (average ± SD). The lower panel summarizes the serum antibody levels examined by AlphaLISA using purified KIAA0513-GST protein as an antigen as described in the legend of Table 1. A scatter dot plot of the same results is shown in Fig. 2a. * 33 had undetermined DM.			

Association between s-KIAA0513-Abs levels and CVD and OSAS

Next, we examined the antibody levels in serum samples from patients with CVD obtained from Chiba University Hospital. Given that OSAS is related to atherosclerosis and is associated with a high risk of AIS and CVD [33 – 36], we also examined the sera of patients with OSAS obtained from Chiba University Hospital. Compared with those of the HDs, the s-KIAA0513-Abs levels were significantly higher in the patients with CVD or OSAS (Fig. 4a), although the positive rates in the patients with CVD and those with OSAS were not so high (10.3% and 11.6%, respectively) (Table 3). The ROC analysis revealed that the s-KIAA0513-Abs AUCs for CVD and OSAS were 0.649 (95% CI 0.582–0.716) (Fig. 4b) and 0.646 (95% CI 0.562–0.731) (Fig. 4b and 4c), respectively. Compared with the low *P*-value (< 0.0001) of s-KIAA0513-Ab for CVD, the *P*-value for OSAS was 0.001 (Table 3), suggesting a lesser association of the s-KIAA0513-Ab marker with OSAS than with CVD.

Table 3

Comparison of the serum antibody levels of HDs vs. those of patients with cardiovascular disease (CVD) or obstructive sleep apnea syndrome (OSAS).

Sample information		HD	CVD	OSAS
	Total sample number	76	97	86
	Male/Female	43/33	81/16	59/27
	Age (Average $\pm$ SD)	45.1 $\pm$ 11.5	66.3 $\pm$ 11.4	57.8 $\pm$ 12.5
Subject group	Type of value	KIAA0513-Ab		
HD	Average	43,398		
	SD	21,374		
	Cutoff value	86,146		
	Positive number	4		
	Positive (%)	5.3%		
CVD	Average	57,531		
	SD	22,460		
	Positive number	10		
	Positive (%)	10.3%		
	P (CVD vs HD)	< 0.001		
OSAS	Average	55,650		
	SD	27,493		
	Positive number	10		
	Positive (%)	11.6%		
	P (OSAS vs HD)	< 0.01		
<p>The upper panel indicates the numbers of total samples and samples from male and female samples as well as ages (average $\pm$ SD). The lower panel summarizes the serum antibody levels examined by AlphaLISA using purified KIAA0513-GST protein as an antigen as described in the legend of Table 1. A scatter dot plot of the same results is shown in Fig. 3.</p>				

Elevation of s-KIAA0513-Abs levels in patients with CKD

We next examined the antibody levels in the sera of patients with CKD, which is also closely related to atherosclerosis. CKD was divided into 3 groups as follows: type 1, diabetic kidney disease; type 2, nephrosclerosis; and type 3, glomerulonephritis. Samples from patients with CKD were obtained from the

Kumamoto cohort, and samples from HDs were obtained from Chiba University. Patients from all 3 CKD groups had significantly higher serum s-KIAA0513-Abs levels than the HDs (Fig. 5a). The s-KIAA0513-Ab positivity rates in the HDs and patients with types 1, 2, and 3 CKD were 6.1%, 29.0%, 37.5%, and 20.3%, respectively (Table 4), indicating that the highest positive rate was observed in the patients with type 2 CKD. The ROC analysis revealed s-KIAA0513-Abs AUCs as high as 0.7434 (95% CI 0.678–0.809) for type 1 CKD (Fig. 5b), 0.808 (95% CI 0.726–0.890) for type 2 CKD (Fig. 5c), and 0.691 (95% CI 0.618–0.764) for type 3 CKD (Fig. 5d).

Table 4

Comparison of s-KIAA0513-Ab levels of HDs versus those of patients with chronic kidney disease (CKD).

Sample information		HD	Type-1 CKD	Type-2 CKD	Type-3 CKD
Total sample number		82	145	32	123
Male/Female		44/38	106/39	21/11	70/53
Age (Average $\pm$ SD)		44.1 $\pm$ 11.2	66.0 $\pm$ 10.4	76.0 $\pm$ 9.8	62.0 $\pm$ 11.7
Alpha analysis (antibody level)		KIAA0513-Ab			
HD	Average	36,007			
	SD	28,697			
	Cutoff value	93,400			
	Positive No.	5			
	Positive rate (%)	6.1%			
Typ-1 CKD	Average	71,773			
	SD	44,434			
	Positive No.	42			
	Positive rate (%)	29.0%			
	<i>P</i> (vs HD)	< 0.001			
Type-2 CKD	Average	84,954			
	SD	47,414			
	Positive No.	12			
	Positive rate (%)	37.5%			
	<i>P</i> (vs HD)	< 0.001			
Type-3 CKD	Average	61,443			
	SD	41,110			
	Positive No.	25			
	Positive rate (%)	20.3%			
	<i>P</i> (vs HD)	< 0.001			

Types-1, -2, and - 3 CKDs correspond to diabetic kidney disease, nephrosclerosis, and glomerulonephritis, respectively. The upper panel indicates the numbers of all samples and samples from males and females as well as age (average $\pm$ SD). The lower panel summarizes the serum antibody levels examined by AlphaLISA using purified KIAA0513-GST protein as an antigen as described in the legend of Table 1. A scatter dot plot of the same results is shown in Fig. 4a.

s-KIAA0513-Ab levels in solid cancer

Given that atherosclerotic diseases are frequently related to cancer with certain common biomarkers being reported [17], we examined the serum samples from patients with EC, GC, CRC, LC, and BC obtained from Toho University Hospital. The s-KIAA0513-Abs levels were significantly higher in the samples from all patients with cancer than in those from the HDs (Fig. 6a, Table 5). The highest average value and positive rate of s-KIAA0513-Ab levels were observed for EC (Table 5). Likewise, the AUC values were highest for EC (0.830) and lowest for BC among the cancers examined (Fig. 6b–6f, Table 6).

Table 5
Comparison of s-KIAA0513-Ab levels between HDs and patients with cancer.

Sample information		HD	EC	GC	CC	LC	MC
	Total sample number	96	192	96	192	96	96
	Male/Female	51/45	155/37	68/28	119/74	0/96	58/38
	Age (Average $\pm$ SD)	57.9 $\pm$ 6.0	67.4 $\pm$ 9.8	68.7 $\pm$ 10.6	66.7 $\pm$ 11.7	60.9 $\pm$ 13.3	68.1 $\pm$ 9.6
Subject group	Type of value	KIAA0513-Ab					
HD	Average	36,199					
	SD	23,001					
	Cutoff value	82,201					
	Positive number	8					
	Positive (%)	8.3%					
EC	Average	87,535					
	SD	57,134					
	Positive number	86					
	Positive (%)	44.8%					
	P (EC vs HD)	< 0.001					
GC	Average	62,714					
	SD	54,540					
	Positive number	21					
	Positive (%)	21.9%					
	P (GC vs HD)	< 0.001					
CRC	Average	69,308					
	SD	58,449					
	Positive number	53					

Types of cancer diagnoses included esophageal cancer (EC), gastric cancer (GC), colon cancer (CC), lung cancer (LC), and breast cancer (BC). Purified KIAA0513 (2-302)-GST protein was used as antigen. Cutoff values were determined as the average HD values plus two SDs. *P* values were calculated by comparing the results of HDs and patients using the Kruskal-Wallis test. *P* values < 0.05 and positive rates > 10% are marked in bold text. A scatter dot plot of the same results is shown in Fig. 5a.

Sample information		HD	EC	GC	CC	LC	MC
	Positive (%)	27.6%					
	P (CRC vs HD)	< 0.001					
LC	Average	62,148					
	SD	57,101					
	Positive number	24					
	Positive (%)	25.0%					
	P (LC vs HD)	< 0.001					
BC	Average	50,270					
	SD	36,802					
	Positive number	18					
	Positive (%)	18.8%					
	P (MC vs HD)	0.002					
Types of cancer diagnoses included esophageal cancer (EC), gastric cancer (GC), colon cancer (CC), lung cancer (LC), and breast cancer (BC). Purified KIAA0513 (2-302)-GST protein was used as antigen. Cutoff values were determined as the average HD values plus two SDs. <i>P</i> values were calculated by comparing the results of HDs and patients using the Kruskal-Wallis test. <i>P</i> values < 0.05 and positive rates > 10% are marked in bold text. A scatter dot plot of the same results is shown in Fig. 5a.							

Table 6
Receiver operating characteristic (ROC) analysis.

	AIS	TIA	DM	CVD	OSAS
AUC value	0.6439	0.6604	0.7361	0.6492	0.6463
Cutoff value	> 108861	> 106592	> 50364	> 63974	> 40530
Sensitivity (%)	57.02	63.64	50.55	34.18	73.26
Specificity (%)	67.63	64.75	87.65	89.47	52.63
95% CI	0.5873 to 0.7004	0.5633 to 0.7575	0.6789 to 0.7933	0.5821 to 0.7163	0.5616 to 0.7309
<i>P</i> value	< 0.0001	0.0014	< 0.0001	< 0.0001	0.0013
	Type-1 CKD	Type-2 CKD	Type-3 CKD		
AUC value	0.7434	0.8080	0.6906		
Cutoff value	> 47004	> 29559	> 43344		
Sensitivity (%)	65.52	100.00	63.41		
Specificity (%)	73.81	50.00	69.05		
95% CI	0.6783 to 0.8085	0.7264 to 0.8897	0.6177 to 0.7635		
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001		
	EC	GC	CRC	LC	MC
AUC value	0.8300	0.6772	0.7279	0.6513	0.6076
Cutoff value	> 48378	> 47940	> 43924	> 48049	> 46726
Sensitivity (%)	72.92	55.21	61.98	50.00	41.67
Specificity (%)	80.21	79.17	75.00	80.21	78.13
95% CI	0.7818 to 0.8782	0.6009 to 0.7535	0.6681 to 0.7876	0.5736 to 0.7289	0.5281 to 0.6872
Area under the curve (AUC), 95% confidence interval (95% CI), cutoff value, sensitivity (%), specificity (%), and <i>P</i> value of the ROC analysis are shown. Purified GST-KIAA0513 protein was used as an antigen. <i>P</i> values lower than 0.05 and AUCs higher than 0.7 are marked in bold.					

	AIS	TIA	DM	CVD	OSAS
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0100
Area under the curve (AUC), 95% confidence interval (95% CI), cutoff value, sensitivity (%), specificity (%), and <i>P</i> value of the ROC analysis are shown. Purified GST-KIAA0513 protein was used as an antigen. <i>P</i> values lower than 0.05 and AUCs higher than 0.7 are marked in bold.					

We then examined whether s-KIAA0513-Ab levels are related to the postoperative survival of patients with EC or GC. We divided the s-KIAA0513-Ab levels into positive and negative groups using the cutoff values obtained by the ROC analysis (Fig. 6b, 6c). Although there were no significant differences in overall survival between the s-KIAA0513-Ab-positive and negative EC groups ($P = 0.351$), the antibody-positive group presented a poor prognosis in the late postoperative stage (40–60 weeks) (Fig. 7a). In contrast, there was no apparent difference in survival between the s-KIAA0513-Ab-positive and negative GC groups (Fig. 7b).

We examined the expression levels of KIAA0513 antigenic protein in EC tissues using immunohistochemical staining. Figure 7c shows representative examples of the staining. EC tissues were heavily stained by anti-KIAA0513 antibody, whereas surrounding healthy esophageal tissues were not. KIAA0513 protein was localized in the cytoplasm, which is consistent with a previous report [43]. Thus, the high KIAA0513 expression levels might account for some, if not all, of the development of serum KIAA0513-Abs.

Correlation analysis

We performed a correlation analysis of s-KIAA0513-Ab levels and participant data using 665 specimens from Chiba Prefectural Sawara Hospital, including 139 specimens from HDs, 225 from patients with AIS, 44 from patients with TIA, 17 from patients with Asympt-Cl, 122 from patients with DSWMH, 59 from patients with cCl, and 41 from disease controls. In this analysis, we employed the Mann–Whitney U test to compare s-KIAA0513-Ab levels between the male and female participants, with or without DM, hypertension, CVD, dyslipidemia, and obesity [body mass index (BMI) ≥ 25] and with or without smoking and alcohol intake habits. We observed a significant difference in s-KIAA0513-Ab levels only between the patients with hypertension and those without hypertension (Table 7).

Table 7

Correlation analysis of antibody levels against KIAA0513 protein with data of subjects in the Sawara Hospital cohort

Sex		Male	Female
Sample number		396	268
KIAA0513-Ab level	Average	10,136	104,138
	SD	39,889	40,969
<i>P</i> value (vs Male)			0.1466
Other disease		DM–	DM+
Sample number		525	135
KIAA0513-Ab level	Average	101,818	101,727
	SD	40,022	41,930
<i>P</i> value (vs DM-)			0.5031
Other disease		HT–	HT+
Sample number		239	421
KIAA0513-Ab level	Average	96,476	104,821
	SD	38,988	40,905
<i>P</i> value (vs HT-)			0.0091
Other disease		CVD–	CVD+
Sample number		623	37
KIAA0513-Ab level	Average	101,376	108,939
	SD	39,757	49,697
<i>P</i> value (vs CVD-)			0.1177
Other disease		Lipidemia–	Lipidemia+
Sample number		475	185
KIAA0513-Ab level	Average	103,028	98,647
	SD	40,591	39,808

The subjects were divided as follows: sex (male and female); presence (+) or absence (–) of complication of DM, hypertension (HT), CVD, or dyslipidemia, and lifestyle factors (smoking and alcohol intake habits). Antibody levels (alpha counts) were compared using the Kruskal-Wallis test (upper panel) and the Mann–Whitney *U* test (lower panels). Sample numbers, averages and SDs of counts as well as *P* values are shown. Significant correlations ($P < 0.05$) are marked in bold.

Sex		Male	Female
<i>P</i> value (vs Lipidemia-)			0.2716
Obesity		BMI < 25	BMI > 25
Sample number		498	158
KIAA0513-Ab level	Average	102,071	101,390
	SD	40,475	40,488
<i>P</i> value (vs Alcohol-)			0.7490
Life style		Non-smoker	Smoker
Sample number		344	319
KIAA0513-Ab level	Average	98,991	104,832
	SD	38,563	42,055
<i>P</i> value (vs non-smoker)			0.0673
Life style		Alcohol–	Alcohol+
Sample number		238	419
KIAA0513-Ab level	Average	102,663	101,364
	SD	39,883	40,759
<i>P</i> value (vs Alcohol-)			0.4595
The subjects were divided as follows: sex (male and female); presence (+) or absence (–) of complication of DM, hypertension (HT), CVD, or dyslipidemia, and lifestyle factors (smoking and alcohol intake habits). Antibody levels (alpha counts) were compared using the Kruskal-Wallis test (upper panel) and the Mann–Whitney <i>U</i> test (lower panels). Sample numbers, averages and SDs of counts as well as <i>P</i> values are shown. Significant correlations ($P < 0.05$) are marked in bold.			

We performed a Spearman's correlation analysis to determine the correlation between the s-KIAA0513-Ab levels and the continuous variables of participant parameters, including general information such as age, height, weight, and BMI; degree of artery stenosis, including the maximum intima-media thickness (max-IMT); lifestyle factors such as smoking duration (years) and alcohol intake frequency (times/week); and blood test data. Supplementary Table S1 lists the average values of these parameters. There was a significant correlation between s-KIAA013-Abs and age, max-IMT, alkaline phosphatase, potassium, C-reactive protein (CRP), body sugar (BS), and smoking duration (Table 8) and an inverse relationship with height and weight. The correlation with max-IMT suggests that s-KIAA0513-Ab levels are associated with stenosis and atherosclerosis, which was further confirmed by using other cohorts. A Spearman's correlation analysis of the CKD cohort (300 participants) showed a significant correlation with plaque

score, max-IMT [44, 45], and cardio-ankle vascular index (CAVI) (right) [46] (Supplementary Table S2), which are indices of atherosclerosis. CRP was also associated with s-KIAA0513-Abs in the CKD cohort, suggesting the involvement of inflammation. In contrast, age, height, weight, BMI, and potassium levels showed no significant correlation with s-KIAA0513-Abs in the CKD cohort. AIS is closely related to age, which might be indirectly associated with s-KIAA0513-Abs.

Table 8
Correlation analysis of serum antibody levels against KIAA0513 with data on subjects in the Sawara Hospital cohort.

Parameter	Number of XY pairs	KIAA0513-Ab	
		<i>r</i> value	<i>P</i> value
Age	663	0.1959	< 0.0001**
Height	657	-0.1317	0.0007
Weight	661	-0.1093	0.0049
BMI*	656	-0.0414	0.2899
max-IMT	457	0.1763	0.0002
A/G	628	-0.0187	0.6402
AST (GOT)	655	0.0395	0.3134
ALT (GPT)	655	-0.0035	0.9278
ALP	600	0.0889	0.0295
LDH	631	0.0431	0.2798
tBil	637	0.0317	0.4250
CHE	506	-0.0387	0.3851
γ-GTP	609	-0.0099	0.8072
TP	633	-0.0184	0.6435
ALB	638	-0.0207	0.6014
BUN	654	-0.0454	0.2469
CRE	649	-0.0310	0.4303
eGFR	552	0.0432	0.3113
UA	492	-0.0138	0.7596
AMY	415	-0.0901	0.0667
T-CHO	563	-0.0446	0.2913
HDL-C	435	-0.0280	0.5600
TG	460	-0.0198	0.6719
Na	641	-0.0347	0.3808
K	641	-0.0899	0.0228

Parameter	Number of XY pairs	KIAA0513-Ab	
		<i>r</i> value	<i>P</i> value
Cl	641	-0.0392	0.3215
Ca	380	-0.0607	0.2376
IP	302	-0.0085	0.8824
Fe	311	-0.0083	0.8838
CRP	477	0.0917	0.0453
LDL-C	344	-0.0559	0.3011
WBC	650	0.0712	0.0697
RBC	650	-0.0220	0.5748
HGB	650	0.0117	0.7651
HCT	650	-0.0041	0.9174
MCV	650	0.0525	0.1814
MCH	650	0.0611	0.1199
MCHC	650	0.0401	0.3080
RDW	650	0.0437	0.2661
PLT	650	-0.0269	0.4942
MPV	650	-0.0354	0.3683
PCT	650	-0.0271	0.4900
PDW	650	-0.0342	0.3838
BS	596	0.0932	0.0229
HbA1c	505	-0.0212	0.6351
Smoking duration	656	0.1297	0.0009
Alcohol frequency	662	-0.0328	0.4000
Sample numbers, correlation coefficients (<i>r</i> values), and <i>P</i> values obtained by Spearman's correlation analysis are shown.			

Parameter	Number of XY pairs	KIAA0513-Ab	
		<i>r</i> value	<i>P</i> value
*Subjects' data used were age, height, weight, body mass index (BMI), maximum intima–media thickness (max-IMT), albumin/globulin ratio (A/G), aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (tBil), cholinesterase (CHE), γ-glutamyl transpeptidase (γ-GTP), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), estimated glomerular filtration rate (eGFR), uric acid (UA), amylase (AMY), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphate (IP), iron (Fe), C-reactive protein (CRP), low-density lipoprotein cholesterol (LDL-C), white blood cell number (WBC), red blood cell number (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), red cell distribution width (RDW), platelet number (PLT), mean platelet volume (MPV), procalcitonin (PCT), platelet distribution width (PDW), blood sugar (BS), glycated hemoglobin (HbA1c), smoking duration (year), and alcohol intake frequency (times/week).			
**Significant correlations (<i>P</i> < 0.05) are marked in bold.			

Japan Public Health Center (JPHC) cohort analysis

We conducted a case-control study nested within the Japan Public Health Center-based Prospective Study, which involved approximately 30,000 plasma samples [40–42]. The level of antibodies against the KIAA0513 protein was positively associated with the risk of AIS. The ORs (95% CIs) were 2.11 (1.17–3.81) and 2.23 (1.18–4.21) for those in the third and highest quartiles of antibody levels, respectively, compared with those in the lowest quartile (Table 9). These results indicate that the antibody markers against the KIAA0513 protein are useful for predicting the onset of AIS.

Table 9
Odds ratios of incident KIAA0513-Abs versus AIS.

KIAA0513-Ab vs AIS		
2nd	Matched OR	1.69
	95% CI	0.91–3.15
3rd	Matched OR	2.11
	95% CI	1.17–3.81
Max	Matched OR	2.23
	95% CI	1.18–4.21
Results of a case–control study nested within the Japan Public Health Center-based Prospective Study are shown. The odds ratios (ORs) and 95% confidence intervals (95% CIs) of the 2nd, 3rd, and the highest (max) quartiles versus the lowest quartile are shown for AIS with respect to the antibody levels of s-KIAA0513-Abs.		

Discussion

Our initial ProtoArray screening identified KIAA0513 as an antigen, as recognized by serum IgG in patients with atherosclerosis, and subsequently recombinant GST-tagged KIAA0513 protein of 301 amino acids was purified. Western blotting confirmed the presence of autoantibodies against KIAA0513 (Fig. 1). Using the KIAA0513 protein as an antigen, we examined serum antibody levels by AlphaLISA. The results showed significantly higher s-KIAA0513-Abs levels in the patients with AIS, TIA, DM, CVD, OSAS, CKD, EC, GC, CC, LC, and MC than in the HDs (Figs. 2 – 6, Table 1 – 5). Among these diseases, the highest AUC values were observed for EC, type 2 CKD, and DM (Figs. 3, 5, and 6, Table 6). The close correlation between s-KIAA0513-Ab levels and HT (Table 7) might account for the association with OSAS, which is frequently accompanied by HT [47]. The Spearman's correlation analysis showed a significant correlation between s-KIAA0513-Ab and max-IMT, plaque score, and CAVI, all of which are indices of atherosclerosis-related lesions (Table 8, Supplementary Table S2) [44–46]. In contrast, s-KIAA0513-Ab levels were weakly correlated with BS ($P=0.023$) (Table 8), but completely unrelated to HbA1c, a typical DM marker. Thus, although the patients with DM showed high s-KIAA0513-Ab levels compared with the HDs, this antibody marker might not primarily reflect DM lesions but rather atherosclerotic lesions caused by DM. Given that angiogenesis is essential for the development of cancer, vascular malformation might be accompanied by the typical alterations in atherosclerosis. In fact, DM and arteriosclerotic diseases are cancer risk factors [48–50].

There are 3 known splicing variants of KIAA0513: isoform a (411 amino acids, NP_055547.1), isoform b (301 amino acids, NP_001273495.1), and isoform c (301 amino acids, NP_001284695.1). The full-length 301 amino acids of KIAA0513 isoforms c and b are exactly the same as the first 301 amino acids of KIAA0513 isoform a. We also purified GST-fused KIAA0513 isoform a and examined the serum antibodies using sera from HDs and patients with AIS and CVD. Both isoforms a and c of KIAA0513 showed higher antibody levels in the sera from patients with AIS or CVD than in the sera from HDs (Supplementary Fig. S1a and 1b). The reactivity of KIAA0513 isoform c against serum antibodies was closely correlated with that of KIAA0513 isoform a, although the former was higher than the latter (Supplementary Fig. S1c), implying that the major epitope sites of serum autoantibodies are located in the 301 amino acids of isoform c.

KIAA0513 mRNA expression has been observed predominantly in the neurons and glial cells of the brain, with low-level expression in most human tissues, whereas the KIAA0513 protein was exclusively found in the brain [43]. Among brain regions, the highest expression was in the cerebellum, cortex, hippocampus, pons, putamen, and amygdala. Using a yeast 2-hybrid analysis of a fetal brain cDNA library, Lauriat et al. [43] found that the N-terminal portion of KIAA0513 interacted with KIBRA, HAX1, and INTS4. A coimmunoprecipitation analysis revealed a physical association between KIAA0513 and KIBRA. Given that KIBRA, HAX1, and INTS4 are involved in synaptic and apoptotic signaling, KIAA0513 can also participate in these signaling pathways.

In addition to the KIAA0513-Abs employed in this study, autoantibodies against ATP2B4, BMP-1, DHPS, LRPAP1, and ASXL2, which are markers of atherosclerosis, were also elevated in the sera of patients with EC [7, 8, 11, 12], indicating that arterial abnormalities can also affect the carcinogenic process. In fact, angiogenesis is essential for the development of solid tumors [51], and diabetes and obesity, which induce arteriosclerosis, are risk factors for CRC and EC [52–54]. Given that all tissues and organs require oxygen and nutrition provided by arteries, the alteration of arterial structure and/or function can affect numerous tissues and organs. All tissues and organs present in a body can affect each other to some degree [55]. In other words, the AIS, CVD, and CKD caused by arteriosclerosis, the arteriosclerosis induced by DM, and the solid cancer caused by arterial lesions can be accompanied by arterial abnormalities. Markers associated with such abnormalities could therefore detect all of the above disorders.

Cancer, heart disease, cerebrovascular disease, and renal failure are the first, second, fourth, and eighth leading causes of death in Japan, respectively (Ministry of Health, Labor and Welfare 2018 vital statistics). Most of the other causes of death are unavoidable, such as senility and accidents. In other words, the onset and progression of cancer, heart disease, cerebrovascular disease, and renal failure (as well as their risk factor DM) can be suppressed by proper health management, such as early diagnosis and intervention. Surprisingly, cancer, heart disease, cerebrovascular disease, renal failure, and DM can be detected by the s-KIAA0513-Ab marker, making it applicable for diagnostic purposes and providing appropriate treatment, lifestyle guidance, etc., leading to improved quality of life.

As of 2020, numerous reports have shown that the presence of underlying diseases such as DM, heart disease, cerebrovascular disease, cancer, OSA, and kidney disease aggravate the coronavirus disease (COVID-19) [56–59]. The s-KIAA0513-Ab marker is therefore a highly useful tool for detecting patients with a higher mortality risk in COVID-19.

Antibody markers are generally more sensitive than antigen markers. Given that the KIAA0513 protein has particularly high antigenicity, this KIAA0513-Ab marker is extremely sensitive. Given the major life-threatening diseases this marker can detect, the KIAA0513-Ab marker could be referred to as a “supermarker”.

Conclusion

The serum anti-KIAA0513 antibody marker appears to be useful for diagnosing the progress of atherosclerosis, which can lead to the onset of life-threatening AIS, CVD, and cancer.

Abbreviations

95% CIs: 95% Confidence intervals; AIS: Acute ischemic stroke; AMI: Acute myocardial infarction; AlphaLISA: Amplified luminescence proximity homogeneous assay-linked immunosorbent assay; Asympt-CI: Asymptomatic ischemic stroke; AUC: Areas under the ROC curve; BC: Breast cancer; BMI: Body mass index; BS: Body sugar; CAVI: Cardio-ankle vascular index; cCI: Chronic-phase cerebral infarction;

CKD: Chronic kidney disease; CRC: Colorectal cancer; CRP: C-reactive protein; CVD: Cardiovascular disease; DM: Diabetes mellitus; DSWMH: Deep and subcortical white matter hyperintensity; EC: Esophageal cancer; GC: Gastric cancer; GST: Glutathione S-transferase; HD: Healthy donor; HT: Hypertension; LC: Lung cancer; max-IMT: Maximum intima-media thickness; OR: Odds ratio; OSAS: Obstructive sleep apnea syndrome; PBS: Phosphate-buffered saline; ROC: Receiver operating characteristic; SD: Standard deviations; SEREX: Serological identification of antigens by cDNA expression cloning; TIA: Transient ischemic attack; UA: Unstable angina

Declarations

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Authors' contributions

TH, TMac, SYo, HK, HS, and YI conceived and designed the study. TH, SYL, MK, BSZ, NSh, and RN performed the experiments and acquired the data. SM, MSu, FS, MSh, SYo, MT, KY, YK, KM, SK, JT, KT, and HT contributed reagents, materials, analysis tools or patient data. YY, EK, TMat, and ST analyzed and interpreted the data. YY, MI, SYa, MSa, KY, HI, and NSa performed the statistical analyses. TH, YY, TMac, MSh, KY, and YI drafted the manuscript.

All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

All authors confirm that tables and figures were not published previously.

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Availability of data and materials

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

Ethics approval and consent to participate

The present study was approved by the Local Ethical Review Board of Chiba University Graduate School of Medicine (Chiba, Japan) as well as the review boards of co-operating hospitals. Serum was collected from patients who had provided informed consent.

Consent for publication

Not applicable.

Competing interests

The present study was performed in collaboration with Fujikura Kasei Co., Ltd. RN, NS, and HK are employees of Fujikura Kasei Co., Ltd.

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Figures

Figure 1

Figure 1

Presence of antibodies against KIAA0513 in sera from patient with acute ischemic stroke (AIS), transient ischemic attack (TIA), diabetes mellitus (DM), esophageal cancer (EC), or colorectal cancer (CRC). Purified proteins of GST-KIA0513 (lane 1) and GST (lane 2) were electrophoresed through sodium dodecyl sulfate-polyacrylamide gel, followed by staining with Coomassie Brilliant Blue (CBB) or western blotting using anti-GST (αGST), anti-KIAA0513 (αKIAA0513), serum IgG from patients with AIS (AIS-07065, AIS-07684), TIA (TIA-02337), DM (DM-22226), EC (EC-6), or CRC (Co-58), and from a healthy donor (HD) (HD-09101).

Figure 2

Figure 2

Comparison of serum anti-KIAA0513 antibody (s-KIAA0513-Ab) levels between HDs and patients with AIS or TIA. (a) The s-KIAA0513-Ab levels of healthy donors (HDs) and patients with AIS or TIA were examined by AlphaLISA using GST-KIAA05132-302 protein as the antigen, followed by subtraction of the levels against control GST. A scatter dot plot of the s-KIAA0513-Ab levels is shown. The bars represent the average and $\pm$ SD. P values were calculated using the Kruskal-Wallis test. **, $P < 0.01$; ***, $P < 0.001$. The total (male/female) numbers, average ages $\pm$ standard deviations (SDs), average antibody levels $\pm$ SDs,

cutoff values, positive numbers, positive rates (%), and P values versus HDs are summarized and shown in Table 1. Receiver operating characteristic curve (ROC) analysis was performed to assess the abilities of s-KIAA0513-Abs to detect AIS (b) and TIA (c). Numbers in the figures indicate the areas under the curve (AUC), cutoff values for antibody levels, sensitivity, specificity, and 95% confidence interval (95% CI).

Figure 3

Figure 3

Comparison of s-KIAA0513-Ab levels between HDs and patients with DM. (a) GST-KIAA0513 protein was used as an antigen. Serum antibody levels in HDs and patients with DM were determined by AlphaLISA and are shown as a scatter dot plot, as described in the legend of Fig. 2. ***, $P < 0.001$. The same results

are summarized in Table 2. (b) Responses to s-KIAA0513-Abs was also evaluated using ROC analysis, and the numbers in figures are as described in the legend of Fig. 2.

Figure 4

Figure 4

Comparison of s-KIAA0513-Ab levels between HDs and patients with cardiovascular disease (CVD) or obstructive sleep apnea syndrome (OSAS). (a) Serum antibody levels against KIAA0513 protein were examined by AlphaLISA and are shown as a scatter dot plot, as described in the legend of Fig. 2. **, $P <$

0.01; ***, $P < 0.001$. The same results are summarized in Table 3. The ability of s-KIAA0513-Abs to detect CVD (b) and OSAS (c) were also evaluated using ROC analysis as described in the legend of Fig. 2.

Figure 5

Figure 5

Comparison of s-KIAA0513-Ab levels between HDs and patients with chronic kidney disease (CKD). (a) Serum antibody levels against KIAA0513 protein were compared between HDs and patients with type-1, -2, or -3 CKD and are shown as a scatter dot plot. Results are presented as described in the legend of Fig.

2. ***, $P < 0.001$ vs. HD specimens. Averages, SDs, cutoff values, total numbers, positive numbers, positive rates (%), and P values are shown in Table 4. (b–d) The abilities of s-KIAA0513-Abs to detect each type of CKD were also evaluated using ROC analysis.

Figure 6

Figure 6

Comparison of s-KIAA0513-Abs levels between HDs and patients with EC, gastric cancer (GC), CRC, lung cancer (LC), or breast cancer (BC). (a) Serum antibody levels against KIAA0513 protein were compared

between HDs and patients with cancer and are shown as a scatter dot plot. Results are presented as described in the legend of Fig. 2. **, $P < 0.01$; ***, $P < 0.001$ vs. HD specimens. Averages, SDs, cutoff values, total numbers, positive numbers, positive rates (%), and P values are summarized in Table 5. (b–f) The abilities of s-KIAA0513-Abs to detect each cancer were also evaluated using ROC analysis.

Figure 7

Figure 7

Survival analysis and immunohistochemical staining of cancer specimens. Comparison of overall survivals of the patients with EC (a) or GC (b) according to s-KIAA0513-Ab-positive (s-KIAA0513-Ab+) and negative (s-KIAA0513-Ab-) groups. Cutoff values of EC and GC were determined by ROC analysis (Fig. 6b and 6c, respectively). Statistical analyses were performed by the Log-Rank test. (c) Surgically resected EC tissue was stained using anti-KIAA0513 antibody (rabbit polyclonal antibodies, Atlas Antibodies).

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