Serum anti-LRPAP1 is a common biomarker for digestive organ cancers and atherosclerotic diseases

Makoto Sumazaki | Hideaki Shimada | Masaaki Ito | Fumiaki Shiratori | Eiichi Kobayashi | Yoichi Yoshida | Akihiko Adachi | Tomoo Matsutani | Yasuo Iwadate | Seiichiro Mine | Tosho Machida | Ikuo Kamitsukasa
Masahiro Mori | Kazuo Sugimoto | Akiyuki Uzawa | Satoshi Kuwabara | Yoshio Kobayashi | Mikiko Ohno | Eiichiro Nishi | Yoshiro Maezawa | Minoru Takemoto | Koutar Yokote | Hirotaka Takizawa | Koichi Kashiwado | Hideo Shin | Takashi Kishimoto | Kazuyuki Matsushita | Sohei Kobayashi | Rika Nakamura | Natsuko Shinmen | Hideyuki Kuroda | Xiao-Meng Zhang | Hao Wang | Ken-ichiro Goto | Takaki Hiwasa

1Department of Gastroenterological Surgery and Clinical Oncology, Toho University Graduate School of Medicine, Tokyo, Japan
2Department of Neurological Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan
3Department of Neurological Surgery, Chiba Prefectural Sawara Hospital, Chiba, Japan
4Department of Neurological Surgery, Chiba Cerebral and Cardiovascular Center, Chiba, Japan
5Department of Neurosurgery, Eastern Chiba Medical Center, Chiba, Japan
6Department of Neurology, Chiba Rosai Hospital, Chiba, Japan
7Department of Neurology, Chibaken Saiseikai Narashino Hospital, Chiba, Japan
8Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan
9Department of Biochemistry and Genetics, Graduate School of Medicine, Chiba University, Chiba, Japan
10Department of Cardiovascular Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan
11Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan
12Department of Pharmacology, Shiga University of Medical Science, Shiga, Japan
13Department of Endocrinology, Hematology and Gerontology, Graduate School of Medicine, Chiba University, Chiba, Japan
14Department of Diabetes, Metabolism and Endocrinology, School of Medicine, International University of Health and Welfare, Chiba, Japan
15Port Square Kashiwado Clinic, Kashiwado Memorial Foundation, Chiba, Japan
16Department of Neurology, Kashiwado Hospital, Chiba, Japan
17Department of Neurosurgery, Higashi Funabashi Hospital, Chiba, Japan
18Department of Molecular Pathology, Graduate School of Medicine, Chiba University, Chiba, Japan
19Division of Clinical Genetics and Proteomics, Department of Laboratory Medicine, Chiba University Hospital, Chiba, Japan
20Medical Project Division, Research Development Center, Fujikura Kasei Co., Saitama, Japan
21Department of Anesthesia, The First Affiliated Hospital, Jinan University, Guangzhou, China

Correspondence
Hideaki Shimada and Takaki Hiwasa,
Department of Gastroenterological Surgery and Clinical Oncology, Toho University Graduate School of Medicine, 5-21-16

Abstract
Some cancers are related to atherosclerotic diseases; therefore, these two types of disease may share some antibody biomarkers in common. To investigate this, a first
INTRODUCTION
Cancer biomarkers are indispensable for diagnosing cancer at an early stage, monitoring during treatment, and making prognoses. In addition to enzyme, antigen, nucleic acid, and antibody markers, there are various other markers, such as revenue, antigen, nucleic acid, and antibody markers. Our group has reported the discovery of antibodies against Trop2/intercellular adhesion molecule 1 (ICAM-1) as a target antigen of ESCC and colorectal carcinoma and some atherosclerosis-related diseases such as acute ischemic stroke (AIS) and diabetes mellitus (DM). 

It is known that autoantibodies develop in patients with atherosclerotic diseases such as acute ischemic stroke (AIS) and acute myocardial infarction (AMI). For example, there are antibodies against Hsp60, RPA2, SOSTDC1, PDCD11, MMP1, CX1, and CBX5 for AIS; ATP2B4, BMP-1, DHPS, SH3BP5, and prolylcarboxypeptidase for atherosclerosis; nardilysin (NRD1) for acute coronary syndrome; and TUBB2C for diabetes mellitus (DM).

On the other hand, it was reported some time ago that atherosclerosis may be linked to cancer to varying degrees. For example, patients with esophageal, stomach, intestinal, or lung cancer have more severe coronary atherosclerotic blockage. The degree of atherosclerosis of the coronary arteries and aorta was positively and significantly related to the presence, size, multiplicity, and degree of atypia of adenomatous polyps. Diabetes was shown to be a risk factor linked with pancreatic, colorectal, endometrial, and prostate cancer. More recently, the prevalence of colorectal adenoma was found to be greater in patients with low-grade coronary atherosclerosis or significant coronary artery disease, suggesting a molecular connection between atherogenesis and tumorigenesis.

In the present study, we report the anti-lipoprotein receptor–related protein–associated protein 1 (LRPAP1) antibody (LRPAP1-Ab) as a common marker of digestive organ cancers and atherosclerotic diseases-related diseases.

MATERIALS AND METHODS
2.1 Patient and healthy donor (HD) sera
This study was approved by the Local Ethical Review Boards of the Toho University Graduate School of Medicine and of the Chiba University Graduate School of Medicine (Chiba, Japan) as well as the review boards of the cooperating hospitals. Serum was collected from patients who had provided written informed consent. Each serum sample was centrifuged at 2000 g for 10 minutes, and the supernatant was stored at −80°C until use. Repeated thawing and freezing of samples was avoided.

KEYWORDS
antibody biomarker, atherosclerosis, colorectal carcinoma, esophageal squamous cell carcinoma, gastric cancer
2.2 | SEREX screening

We performed immunoscreening by using a modified version of previously published methods. In order to screen for clones that were immunoreactive against sera of patients with ESCC or AIS, we used a human ESCC cell line T.Tn cDNA library in λZAP II phage\(^6\),\(^12\) and a human aortic endothelial cell cDNA library in Uni-ZAP XR Premade Library (Stratagene).\(^{20,21}\) *Escherichia coli* (E coli) XL1-Blue MR\(^F\) was infected with Uni-ZAP XR phage. The expression of resident cDNA clones was induced after blotting infected bacteria onto nitrocellulose membranes (NitroBind, Osmonics), which were pretreated with 10 mmol/L isopropyl-\(β\)-D-thiogalactoside (IPTG; Wako Pure Chemicals). To obtain monoclonality, positive clones were re-cloned two additional times, as previously described.\(^6\),\(^12\),\(^15,18,21\)

2.3 | Sequence analysis of identified antigens

We converted the monoclonalized phage cDNA clones to pBlue-script phagemids by in vitro excision using ExAssist helper phage (Stratagene). Plasmid DNA was obtained from the E coli SOLR strains transformed by the phagemids. Following sequencing of inserted cDNAs, homologous analysis was performed using a public database provided by the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4 | Expression and purification of LRPAP1 protein

Amino-terminals (amino acids 1-275) of coding sequences of LRPAP1 cDNA were recombined into the EcoRI/Xhol site of pGEX-4T-3 (GE Healthcare Life Sciences), followed by confirmation by DNA sequencing. Expression of the cDNA product was induced by treating pGEX-4T-3-LRPAP1-transformed E coli with 0.1 mmol/L IPTG for 4 hours at 25°C; the cells were subsequently lysed in BugBuster Master Mix (Merck Millipore). Glutathione S-transferase (GST)-tagged LRPAP1 protein was purified by glutathione-Sepharose (GE Healthcare Life Sciences) column chromatography according to the manufacturer’s instructions and dialyzed against phosphate-buffered saline, as previously described.\(^18,21,23\)

2.5 | Western blotting analysis

GST-tagged LRPAP1 as well as GST was purified as described above. GST and GST-LRPAP1 proteins (0.3 µg) were separated by SDS-polyacrylamide gel electrophoresis and electrically transferred onto nitrocellulose membranes (Advantec). The membranes were blocked using blocking solution (0.5% skim milk powder in a buffer comprising 20 mmol/L Tris–HCl [pH 7.6], 137 mmol/L NaCl, and 0.1% Tween 20), and the blotted proteins were probed with primary antibodies including anti-GST (goat; Rockland), anti-LRPAP1 (rabbit; Aviva Systems Biology), or sera from HDs or patients with ESCC (#4 and #5). After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-goat IgG, anti-rabbit IgG, and anti-human IgG (Santa Cruz Biotechnology), immunoreactivity was detected using Immobilon™ Western HRP Substrate (Merck KGa), as previously described.\(^6\),\(^12\),\(^18,20,23\)

2.6 | Amplified luminescence proximity homogeneous assay (AlphaLISA)

AlphaLISA was performed in 384-well microtiter plates (white opaque OptiPlate™, Perkin Elmer) containing either 2.5 µL 1:100-diluted serum with 2.5 µL GST or GST-LRPAP1 protein (10 µg/mL) in AlphaLISA buffer (25 mmol/L HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 µg/mL dextran-500, and 0.05% ProClin-300). The reaction mixture was incubated at room temperature for 6-8 hours, following which anti-human IgG–conjugated acceptor beads (2.5 µL at 40 µg/mL) and glutathione-conjugated donor beads (2.5 µL at 40 µg/mL) were added and incubated prior to another incubation at room temperature in the dark for 1-14 days. Chemical emissions were read on an EnSpire Alpha microplate reader (PerkinElmer), as previously
Specific reactions were calculated by subtracting the alpha counts of the GST control from the counts of GST-fusion proteins.

2.7 Immunohistochemical staining

Formalin-fixed paraffin-embedded ESCC tissues were sectioned at 4-µm thickness. The sections were deparaffinized, pretreated with Cell Conditioning 1 (CC1, Ventana Medical Systems), reacted with primary anti-LRPAP1 antibodies (rabbit polyclonal antibodies, Atlas Antibodies) at 2 µg/mL for 32 minutes at room temperature, visualized by Ventana's DAB detection kit (view DAB detection kit, Ventana Medical Systems), and counter stained with Hematoxylin II (Ventana Medical Systems) and Bluing Reagent (Ventana Medical Systems).

2.8 Statistical analyses

The Mann-Whitney U test was used to determine significant differences between two groups. The Kruskal-Wallis test was used to evaluate differences among more than three groups. Survival curves were calculated using the Kaplan-Meier method. Correlations were calculated using Spearman's correlation analysis. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software). The predictive values of putative disease markers were assessed via a receiver-operating characteristic (ROC) curve analysis, and the cut-off values were set to maximize the sums of sensitivity and specificity. All tests were two-tailed, and P values of <0.05 were considered statistically significant.

3 RESULTS

3.1 Recognition of LRPAP1 by serum components of patients with ESCC or AIS

SEREX screening identified an antigen recognized by antibodies in the sera of both patients with ESCC and those with AIS; this antigen was low-density LRPAP1 (Accession Number: NM_002337). Subsequently, GST-fused LRPAP1, which contained full-length LRPAP1 protein, was expressed in E.coli and purified by affinity chromatography.

3.2 Presence of serum antibodies against purified proteins in patients with ESCC

Using Western blotting, we confirmed the presence of antibodies against GST-fusion LRPAP1 protein in sera from patients with ESCC. GST and GST-LRPAP1 were recognized by anti-GST antibodies as reactions of 26- and 64-kDa proteins, respectively (Figure 1).

Conversely, GST-LRPAP1, but not GST, reacted with commercial anti-LRPAP1 antibody and the serum antibodies of ESCC patients #4 and #5.

3.3 Serum levels of LRPAP1-Abs in ESCC, GC, and CRC

We examined the levels of LRPAP1-Abs in the sera of patients with ESCC, GC, and CRC obtained from Toho University, Omori Medical Center. Sera of HDs were obtained from Port Square Kashiwado Clinic and Higashi Funabashi Hospital. The average ages (± SDs) of HDs and patients with ESCC, GC, or CRC were 57.05 ± 7.79, 67.01 ± 10.65, 68.14 ± 10.91, and 66.65 ± 11.64, respectively. Sample numbers of males/females of HDs, and patients with ESCC, GC, or CRC were 104/88, 155/37, 137/55, and 118/74, respectively. The AlphaLISA results demonstrated that serum antibody levels against the LRPAP1 protein were significantly higher in patients with ESCC, GC, or CRC than in HDs (Figure 2A). At a cutoff value of the average HD value +2 SD, the LRPAP1-Ab positivity rates in HDs and patients with ESCC, GC, or CRC were found to be 1.1%, 10.9%, 3.6%, and 6.8%, respectively (Table 1). The antibody levels were highest in patients with ECSS compared with other patients and HDs, which may be related to the fact that LRPAP1 was identified by SEREX screening using sera of patients with ESCC. The LRPAP1-Ab levels were also higher in patients with LC than those in HDs (Figure S1).

3.4 Survival analysis

The LRPAP1-Ab levels were divided into four groups (lowest first, second, third, and highest fourth quartiles) and analyzed for prognoses. Kaplan-Meier plotting showed that the highest quartile had poorer prognoses than other groups (Figure 2B). The half
survival period of the highest quartile was approximately 22 months, whereas that of other groups was 40-42 months, although the differences were not significant (P values of the survival of the highest quartile vs the lowest, second, and third quartiles were 0.451, 0.290, and 0.127, respectively).

### 3.5 Immunohistochemical analysis of antigenic LRPAP1 protein

We examined the expression levels of LRPAP1 antigenic protein in ESCC tissues using immunohistochemical staining. Representative examples of staining are shown in Figure 2C. ESCC tissues were heavily stained by anti-LRPAP1 antibody, whereas surrounding healthy esophageal tissues were not. The cytoplasmic localization of LRPAP1 protein is consistent with previous reports. Thus, the expression levels of LRPAP1 may account for some, if not all, of the development of serum LRPAP1-Abs.

### 3.6 Elevation of serum antibody levels against LRPAP1 in patients with AIS or DM

We examined LRPAP1-Abs in HDs and patients with AIS, CVD, and DM. Sera of patients with AIS or DM were obtained from Chiba Rosai Hospital and Chiba University Hospital, respectively. Sera
of patients with CVDs such as AMI, unstable angina pectoris, effort angina pectoris, and old myocardial infarction were obtained from Kyoto University Hospital. HD sera were obtained from Chiba University, Chiba Prefectural Sawara Hospital, and Shimoshizu Hospital. The average ages (± SDs) of HDs and patients with AIS, CVD, or DM were 44.75 ± 12.38, 67.04 ± 10.80, 65.16 ± 11.19, and 61.25 ± 11.38 years, respectively. The ratios of males to females of HDs, AIS, CVD, and DM patients were 50/46, 73/23, 66/30, and 56/40, respectively. A total of 96 specimens of each of HDs, AIS, CVD, and type II DM patients were simultaneously assayed by AlphaLISA on a 384-well plate. The levels of LRPAP1-Abs were significantly higher in patients with AIS or DM, but not CVD, compared with those in HDs (Figure 3A). At a cutoff value of the average HD value +2 SD, the positive rates were 4.2% in HDs, 12.5% in AIS, 11.5% in CVD, and 18.8% in DM patients (Table 2).

### 3.7 Elevation of LRPAP1-Abs levels in patients with stroke

We examined further the levels of LRPAP1-Abs in 696 sera from controls and stroke patients, including 226 specimens from HDs, 228 from patients with AIS, 44 from patients with TIA, 17 from patients with asymptomatic cerebral infarction (asympt-Cl), 122 from patients with DSWMH, and 59 from patients with chronic-phase...
The serum LRPAP1-Ab levels were most closely correlated with cerebral infarction (cCI), all of which were obtained from Chiba Prefectural Sawara Hospital. The AlphaLISA results demonstrated that the serum antibody levels against LRPAP1 protein were significantly higher in patients with AIS or TIA, but not in other groups, compared with HDs (Figure 4A). Using cutoff values determined as described in the previous section, the LRPAP1-Ab positivity rates in HDs and patients with AIS, TIA, asympt-Cl, DSWMH, or cCI were found to be 3.5%, 6.1%, 13.6%, 0.0%, 1.6%, and 10.2%, respectively (Table 2). Patients with TIA showed the highest levels of LRPAP1-Ab in serum among the groups examined (Table 3). Therefore, elevated LRPAP1-Ab levels may reflect the cause but not the result of stroke.

### Table 2 Comparison of serum anti-LRPAP1 antibody (LRPAP1-Ab) levels between healthy donors (HDs) and patients with acute ischemic stroke (AIS), cardiovascular disease (CVD), and diabetes mellitus (DM) examined by amplified luminescence proximity homogeneous assay (AlphaLISA)

|        | LRPAP1-Ab |
|--------|-----------|
| **HD** | 3869      |
| Average|           |
| SD     | 1920      |
| Cutoff value | 7709 |
| Total no. | 96    |
| Positive no. | 4    |
| Positive rate | 4.2% |
| **AIS** |           |
| Average | 4873      |
| SD     | 2743      |
| Total no. | 96    |
| Positive no. | 12   |
| Positive rate | 12.5% |
| P value (AIS vs HD) | <0.05 |
| **CVD** |           |
| Average | 4493      |
| SD     | 2626      |
| Total no. | 96    |
| Positive no. | 11   |
| Positive rate | 11.5% |
| P value (CVD vs HD) | ns     |
| **DM**  |           |
| Average | 5312      |
| SD     | 2809      |
| Total no. | 96    |
| Positive no. | 18   |
| Positive rate | 18.8% |
| P value (DM vs HD) | <0.001 |

Note: Shown numbers are as described in Table 1; P values lower than 0.05 and positive rates higher than 10% were marked in bold. All data of the same results are shown in Figure 3.

Abbreviation: ns, not significant.

### 3.8 ROC analysis

The results of the ROC curve analysis are shown in Figures 2D-F, 3B,C, and 4B,C, which show areas under the curve (AUC), 95% confidence intervals (CI), cutoff values, sensitivity, specificity, and P values. Serum LRPAP1-Ab levels showed high AUC values against ESCC, CRC, DM, and TIA, of 0.6884, 0.6831, 0.6624, and 0.6988, respectively. The AUC against LC was 0.6359 (Figure S1B), which was somewhat higher than those against GC and AIS.

### 3.9 Correlation analysis

A comparative analysis of serum LRPAP1-Ab levels and participant data was performed using the same samples as shown in Figure 4A, from Chiba Prefectural Sawara Hospital. The antibody levels were then compared between male and female participants; with or without obesity; with or without diseases including DM, hypertension (HT), CVD, and dyslipidemia; and with or without smoking and alcohol intake habits. Comparisons using the Mann-Whitney U test revealed that LRPAP1-Ab levels were significantly higher in smokers compared with nonsmokers (Table 4). None of the other categories showed any significant differences in LRPAP1-Ab levels.

Spearman's rank-order correlation analysis was performed to determine if there were any correlations between serum antibody levels against the LRPAP1 protein and participant parameters, including general information such as age, height, weight, body mass index (BMI), and the degree of artery stenosis—the maximum intima-media thickness (max IMT). The following previously described blood test data were also included: albumin/globulin ratio (A/G), aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), total bilirubin (tBil), direct bilirubin (dBil), cholinesterase (CHE), γ-glutamyl transpeptidase (γ-GTP), total protein (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CRE), estimated glomerular-filtrating ratio (eGFR), uric acid (UA), thymol turbidity test (TTT), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), creatine kinase (CK), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate (IP), iron (Fe), C-reactive protein (CRP), low-density lipoprotein cholesterol (LDL-C), white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red-cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), procalcitonin (PCT), platelet distribution width (PDW), glutated hemoglobin (HbA1c), blood sugar (BS), smoking period, and alcohol intake frequency.

The serum LRPAP1-Ab levels were most closely correlated with smoking period (P < 0.0001) and partly related to WBC number (P = 0.0074) and PCT (P = 0.0386; Table 5). Although LRPAP1-Ab levels were elevated in patients with DM (Figure 3A), there was...
no apparent correlation between HbA1c and antibody levels ($P = 0.3101$), suggesting that LRPAP1-Ab levels do not directly reflect DM but may indirectly reflect lesions caused by DM.

4 | DISCUSSION

SEREX screening identified LRPAP1 as an antigen recognized by serum IgG in patients with ESCC or atherosclerosis. Subsequent analyses demonstrated higher levels of serum antibodies against the LRPAP1 protein in patients with ESCC, GC, CRC, LC, AIS, TIA, and DM, but not CVD, compared with HDs (Tables 1-3; Figures 2-4 and S1). Further comparisons using the Mann-Whitney U test between LRPAP1-Ab–positive and –negative groups revealed that smoking was closely associated with LRPAP1-Ab levels (Table 4). Spearman’s correlation analysis of LRPAP1-Ab levels and participant parameters also confirmed that the smoking period was correlated with LRPAP1-Abs (Table 5). The sensitivity of ESCC was higher than that of GC and CRC (Figure 2B-D), possibly because the esophagus is more susceptible to the effects of smoking than the stomach and colon.40,41

We then examined LRPAP1-Ab levels in patients with atherosclerosis-related diseases, because LDL and LDL receptors are closely associated with atherosclerosis.42,43 LRPAP1-Ab levels were elevated in patients with DM (Figure 3A), whereas they showed no apparent correlation with HbA1c ($P = 0.3101$) or BS ($P = 0.1537$; Table 5). Complications arising from DM were not associated with LRPAP1-Ab levels (Table 4). These findings suggest that LRPAP1-Ab levels do not directly reflect DM but may indirectly reflect the atherosclerotic lesions caused by DM.44 DM, like smoking, is a risk factor for both cancer and atherosclerosis. It is possible that DM and smoking can cause a similar effect which results in the elevated expression of LRPAP1-Abs.

Thus far, some common biomarkers for both atherosclerosis-related diseases and cancer have been reported. The transcription factor 7-like 2 (TFG7L2) gene, which influences diabetes risk, is associated with incidence of colon cancer.45 Low circulating adiponectin concentrations are associated with type 2 DM, HT, dyslipidemia, coronary artery disease, stroke, colon cancer, and GC.46 Serum $\beta$-2
### TABLE 3  Comparison of serum anti-LRPAP1 antibody (LRPAP1-Ab) levels between healthy donors (HDs) and patients with AIS, transient ischemic attack (TIA), asymptomatic cerebral infarction (asympt-CI), deep and subcortical white matter hyperintensity (DSWMH), and chronic-phase CI (cCI) examined by amplified luminescence proximity homogeneous assay (AlphaLISA)

|              | HD            | AIS          | TIA          | Asympt-CI     | DSWMH        | cCI          |
|--------------|---------------|--------------|--------------|---------------|--------------|--------------|
| LRPAP1-Ab    | Average       | 4564         | 5299         | 6308          | 5747         | 4621         |
|              | SD            | 2659         | 2625         | 2985          | 2423         | 2207         |
|              | Cutoff value  | 9882         | 9882         | 9882          | 9882         | 9882         |
|              | Total no.     | 226          | 228          | 44            | 17           | 122          |
|              | Positive no.  | 8            | 14           | 6             | 0            | 2            |
|              | Positive rate | 3.5%         | 6.1%         | 13.6%         | 0.0%         | 1.6%         |
|              | P value (AIS vs HD) | <0.01 | <0.001 | | | ns |

### TABLE 4  Correlation analysis of antibody levels against LRPAP1-GST protein with data of subjects of the Sawara Hospital cohort

| Sex          | Male | Female |
|--------------|------|--------|
| Sample number| 395  | 270    |
| LRPAP1-Ab level | 5140 | 5051  |
| SD           | 2712 | 2693   |
| P value (vs male) | 0.669 |        |

| Obesity      | BMI < 25 | BMI ≥ 25 |
|--------------|----------|----------|
| Sample number| 498      | 158      |
| LRPAP1-Ab level | 5138 | 5069  |
| SD           | 2661    | 2856    |
| P value (vs male) | 0.670 |        |

| Other disease | DM− | DM+ |
|---------------|-----|-----|
| Sample number | 525 | 135 |
| LRPAP1-Ab level | 5052 | 5338 |
| SD            | 2702  | 2688  |
| P value (vs DM−) | 0.143 |       |

| Other disease | HT− | HT+ |
|---------------|-----|-----|
| Sample number | 239 | 421 |
| LRPAP1-Ab level | 4914 | 5221 |
| SD            | 2615  | 2743  |
| P value (vs HT−) | 0.153 |       |

| Other disease | CVD− | CVD+ |
|---------------|------|------|
| Sample number | 623  | 37   |
| LRPAP1-Ab level | 5114 | 5051 |
| SD            | 2686  | 2963  |
| P value (vs CVD−) | 0.754 |       |

| Other disease | Lipidemia− | Lipidemia+ |
|---------------|------------|------------|
| Sample number | 475        | 185        |
| LRPAP1-Ab level | 5102 | 5311 |
| SD            | 2629    | 2882    |
| P value (vs Lipidemia−) | 0.855 |       |

| Lifestyle | Nonsmoker | Smoker |
|----------|-----------|--------|
| Sample number | 344 | 319   |
| LRPAP1-Ab level | 4702 | 5542  |
| SD            | 2331   | 2999   |
| P value (vs nonsmoker) | 0.0006 |       |

| Lifestyle | Alcohol− | Alcohol+ |
|----------|----------|----------|
| Sample number | 238 | 419    |
| P value (vs nonsmoker) | 0.0006 |      |

Note: Shown numbers are as described in Table 1. All data of the same results are shown in Figure 4.
Abbreviation: ns, not significant.
are shown. Significant correlations (P < 0.05) are marked in bold text.

Note: The subjects were divided as follows: sex (male and female); obesity, presence (+) or absence (−) of complication of diabetes mellitus (DM), hypertension (HT), cardiovascular disease (CVD), dyslipidemia, and lifestyle factors (smoking and alcohol intake habits). Antibody levels (Alpha counts) were compared using the Mann-Whitney U test (lower panels). Sample numbers, averages and SDs of counts as well as 4462 TABLE 4 (Continued)

| Lifestyle | Alcohol− | Alcohol+ |
|-----------|----------|----------|
| LRPAP1-Ab level | | |
| Average | 4748 | 5307 |
| SD | 2386 | 2859 |
| P value (vs Alcohol−) | 0.051 |

Other molecules and genes reported to be involved in both cancer and atherosclerotic diseases include adenosine monophosphate-activated protein kinase (AMPK), peroxisome proliferator-activated receptor-γ (PPAR-γ), plasminogen activator inhibitor-1 (PAI-1), dual-specificity tyrosine phosphorylation–regulated kinase 1B (DYRK1B), and methylenetetrahydrofolate reductase (MTHFR). In addition to these genomic, transcriptomic, and proteomic analyses, our immunomic screening has identified a novel marker that is a biomarker of kidney filtration, is associated with increased colorectal cancer risk. Proteomics analysis has revealed proteins related to atherosclerosis formation, including mimecan and cathepsin D, which have been identified as biomarkers of cancerous tumors. Other molecules and genes reported to be involved in both cancer and atherosclerotic diseases include adenosine monophosphate-activated protein kinase (AMPK), peroxisome proliferator-activated receptor-γ (PPAR-γ), plasminogen activator inhibitor-1 (PAI-1), dual-specificity tyrosine phosphorylation–regulated kinase 1B (DYRK1B), and methylenetetrahydrofolate reductase (MTHFR). In addition to these genomic, transcriptomic, and proteomic analyses, our immunomic screening has identified a novel marker that is a biomarker of kidney filtration, is associated with increased colorectal cancer risk. Proteomics analysis has revealed proteins related to atherosclerosis formation, including mimecan and cathepsin D, which have been identified as biomarkers of cancerous tumors. It is well known that low-density lipoprotein plays an important role in the development of atherosclerosis, which is a major receptor for oxidized low-density lipoprotein (ox-LDL), is important for maintaining the transformed state of cancer cells and for tumor growth. Kounnas et al. have reported that LRPAP1 binds to both low-density lipoprotein receptor-related protein 1 (LRP1) and low-density lipoprotein receptor-related protein 2 (LRP2) and can specifically inhibit ligand binding to these receptors. Variants of not only LRP1 but also LRP6 were associated with an increased risk of ischemic stroke. LRP1, LRP6, and TCF7L2 are involved in the Wnt signaling pathway, which plays an important role in the development of both atherosclerosis and cancer. Cytoplasmic LRPAP1 has been suggested to act as a chaperone, preventing ligand binding during receptor trafficking. Willnow et al. found that the export of LRP2 and very low-density lipoprotein receptor (VLDL) receptors from the ER is impaired in LRPAP1-deficient mice. Thus, LRPAP1 is indispensable for absorbing LDL and VLDL into cells by promoting the trafficking of the surface receptors. For cancer cells, the uptake of much lipid involved in LDL and VLDL is beneficial for producing large amounts of energy. On the other hand, the incorporation of large amounts of lipids into vascular endothelial cells and/or smooth muscle cells may facilitate the development of atheromatous plaques.

In both cancer and atherosclerosis, the disease develops gradually over several years. The early stages of the disease are sometimes accompanied by low-level tissue destruction and subsequent leakage.

**TABLE 5** Correlation analysis of serum antibody levels against purified LRPAP1-GST protein with data of subjects of the Sawara Hospital cohort

| | r value | P value |
|---|---------|---------|
| Age | 0.0361 | 0.3543 |
| Height (cm) | −0.0322 | 0.4101 |
| Weight (kg) | −0.0342 | 0.3797 |
| BMI | −0.0133 | 0.7334 |
| max IMT | 0.0744 | 0.1121 |
| A/G | 0.0593 | 0.1379 |
| AST | 0.0247 | 0.5289 |
| ALT | 0.0272 | 0.4870 |
| ALP | 0.0453 | 0.2675 |
| LDH | −0.0084 | 0.8340 |
| LAP | 0.0183 | 0.7380 |
| tBil | 0.0510 | 0.1987 |
| dBil | 0.0192 | 0.7487 |
| γ-GTP | 0.0352 | 0.3854 |
| TP | −0.0189 | 0.6359 |
| ALB | 0.0272 | 0.4929 |
| BUN | −0.0453 | 0.2477 |
| CRE | −0.0287 | 0.4660 |
| eGFR | 0.0653 | 0.1253 |
| UA | −0.0062 | 0.8903 |
| TTT | 0.1106 | 0.0586 |
| T-CHO | −0.0408 | 0.3344 |
| HDL-C | −0.0175 | 0.7153 |
| TG | −0.0318 | 0.4960 |
| CK | 0.0061 | 0.9328 |
| K | −0.0541 | 0.1710 |
| Cl | −0.0356 | 0.3688 |
| Ca | −0.0705 | 0.1701 |
| IP | −0.0214 | 0.7105 |
| Fe | 0.0358 | 0.5296 |
| CRP | 0.0439 | 0.3393 |
| LDL-C | −0.0418 | 0.4392 |
| WBC | 0.1049 | 0.0074 |
| RBC | 0.0456 | 0.2458 |
| HGB | 0.0674 | 0.0860 |
| HCT | 0.0617 | 0.1164 |
| MCV | 0.0432 | 0.2714 |
| MCH | 0.0613 | 0.1183 |
| MCHC | 0.0433 | 0.2709 |
| RDW | −0.0240 | 0.5409 |
| PLT | 0.0655 | 0.0950 |
| MPV | −0.0005 | 0.9894 |
| PCT | 0.0812 | 0.0386 |

(Continues)
of intracellular proteins. Repeated leakage of such antigenic proteins leads to amplified antibody expression with low antigen levels. Therefore, antibody markers are much more sensitive than antigen markers and could be useful for the early diagnosis of precancerous lesions and preonset detection of AIS. TIA, a prodromal symptom of AIS, consistently showed significantly higher LRPAP1-Ab levels than HD controls (Figure 4A). Thus, the early treatment of LRPAP1-Ab-positive patients may avoid the onset of disease. Serum LRPAP1-Ab levels appear to be a useful marker for the diagnosis of ESCC, GC, CRC, AIS, TIA, and DM, which are presumably caused by smoking.

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ORCID
Makoto Sumazaki  https://orcid.org/0000-0002-1466-8277
Hideaki Shimada  https://orcid.org/0000-0002-1990-8217
Kazuyuki Matsuhashita  https://orcid.org/0000-0001-6742-0761
Sohei Kobayashi  https://orcid.org/0000-0002-6152-177X
Takaki Hiwasa  https://orcid.org/0000-0002-0475-3881

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**Supporting information**
Additional supporting information may be found online in the Supporting Information section.

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