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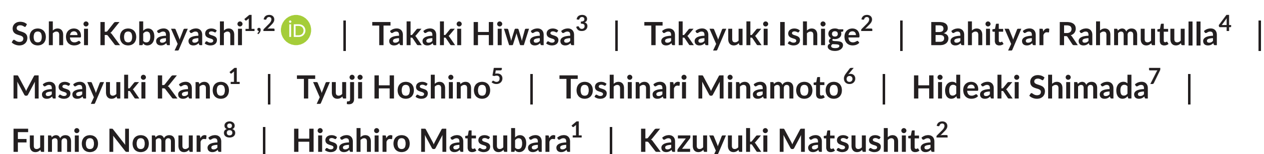
Anti-FIRΔexon2, a splicing variant form of PUF60, autoantibody is detected in the sera of esophageal squamous cell carcinoma



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Abstract

Anti-PUF60 autoantibodies are reportedly detected in the sera of patients with dermatomyositis and Sjögren's syndrome; however, little is known regarding its existence in the sera of cancer patients. FIR, a splicing variant of the *PUF60* gene, is a transcriptional repressor of *c-myc*. In colorectal cancer, there is an overexpression of the dominant negative form of FIR, in which exon 2 is lacking (FIRΔexon2). Previously, large-scale SEREX (serological identification of antigens by recombinant cDNA expression cloning) screenings have identified anti-FIR autoantibodies in the sera of cancer patients. In the present study, we revealed the presence and significance of anti-FIR (FIR/FIRΔexon2) Abs in the sera of patients with esophageal squamous cell carcinoma (ESCC). Our results were validated by an amplified luminescence proximity homogeneous assay using sera of patients with various cancer types. We revealed that anti-FIRΔexon2 Ab had higher sensitivity than anti-FIR Ab. Receiver operating characteristic (ROC) analysis was applied for evaluating the use of anti-FIRΔexon2 Ab as candidate markers such as anti-p53 Ab and carcinoembryonic antigen, and the highest area under the ROC curve was observed in the combination of anti-FIRΔexon2 Ab and anti-p53 Ab. In summary, our results suggest the use of anti-FIRΔexon2 Ab in combination with the anti-p53 Ab as a predictive marker for ESCC. The area under the ROC curve was further increased in the advanced stage of ESCC. The value of anti-FIRΔexon2 autoantibody as novel clinical indicator against ESCC and as a companion diagnostic tool is discussed.

KEYWORDS
AlphaLISA, anti-FIRΔexon2 autoantibody, esophageal squamous cell carcinoma, gastrointestinal cancer, SEREX

Abbreviations: AlphaLISA, amplified luminescence proximity homogeneous assay; AUC, area under the ROC curve; CEA, carcinoembryonic antigen; CFAP70, cilia and flagella-associated protein 70; FIR, FUBP1-interacting repressor; FIRΔexon2, splicing variant of FIR that lacks exon 2; FUBP1, FUSE binding protein 1; FUSE, far upstream element; GST, glutathione S-transferase; HD, healthy donor; IPTG, isopryl-β-D-thiogalactoside; KARS, lysyl-tRNA synthetase; ROC, receiver operating characteristic; SEREX, serological identification of antigens by recombinant cDNA expression cloning; SNX15, sorting nexin 15; SOHLH1, spermatogenesis and oogenesis specific basic helix-loop-helix 1.

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1 | INTRODUCTION

Esophageal squamous cell carcinoma is one of the most common and lethal gastrointestinal malignancies worldwide.[1,2] Despite multimodal treatments, such as radical surgery, chemotherapy, and radiotherapy, the 5-year survival rate of patients with ESCC remains extremely low due to its highly invasive and metastatic nature.[3,4] The pathophysiology of ESCC is not entirely understood; thus, further research into the discovery and development of effective biomarkers for ESCC diagnosis, prognosis, and treatment is warranted.

The SEREX is an effective screening method for identifying serum Ab-type tumor markers.[5] We have previously undertaken large-scale SEREX screenings where numerous candidates for ESCC SEREX antigens were identified and potential novel diagnostic markers for digestive organ cancers were discovered.[6-12]

In the majority of colorectal cancers, *c-myc* is overexpressed and required for tumor maintenance.[13,14] The FUSE is a sequence required for the proper expression of the human *c-myc* gene. It is located 1.5 kb upstream of the *c-myc* promoter P1 and binds FUBP1, a transcription factor that stimulates *c-myc* expression in a FUSE-dependent manner.[15,16] The FUBP1 regulates the proliferation and migration of cells and is overexpressed in hepatocellular carcinoma.[17-19] Yeast 2-hybrid analysis revealed that FUBP1 binds to a protein that has transcriptional inhibitory activity, termed FIR, and FIR was found to engage the TFIIH/p89/XPB helicase and repress *c-myc* transcription.[20]

Apoptosis is induced by FIR through *c-myc* suppression; thus, it is a suitable target for anticancer therapy.[21,22] FIRΔexon2, a splicing variant of FIR that lacks exon 2, failed to repress *c-myc* and inhibited FIR-induced apoptosis, suggesting that FIRΔexon2 is a dominant negative form of FIR in human cancers.[23] Alternatively, FIR is a splicing variant form of the poly(U)-binding-splicing factor, PUF60.[24,25] Anti-PUF60 autoantibodies are reportedly detected in the sera of patients with autoimmune diseases such as dermatomyositis, Sjögren's syndrome, and idiopathic inflammatory myopathy.[26,27] This suggests that the combination of anti-FIR Abs with other clinically available tumor markers, such as anti-p53 Abs, CEA, and CA19-9, could increase the specificity and accuracy of diagnosis.[28-32] The present study aimed to explore the presence and significance of anti-FIRΔexon2 Abs in the sera of patients with ESCC and to determine its use as a potential candidate marker.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

The present study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Sera of patients with ESCC (n = 95) were obtained from the Department of Frontier Surgery (Chiba University Hospital, Chiba, Japan). Sera of healthy donors (HDs) (n = 94) were obtained from the Higashi Funabashi Hospital, Funabashi City, Chiba, Japan. Written informed consent was obtained from all participants prior to the study. Each serum sample was centrifuged at 2000 *g* for 10 minutes and the supernatant was stored at −80°C until further use. Repeated thawing and freezing of the samples was avoided. This study was approved by the Local Ethical Review Board of the Chiba University, Graduate School of Medicine and the Higashi Funabashi Hospital.

2.2 | Screening by expression cloning

Recombinant DNA studies were undertaken with permission from the Chiba University Graduate School of Medicine and were carried out in accordance with the rules of the Japanese government. We used a λZAP II phage cDNA library prepared from the mRNA of T.Tn cells (esophageal cancer cell line)[33,34] and a commercially available human fetal testis cDNA library (Uni-ZAP XR Premade Library; Stratagene, La Jolla, CA, USA) to screen for clones that were immunoreactive against serum IgG from patients with ESCC as previously described.[35] *Escherichia coli* XL1-Blue MRF' was infected with λZAP II or Uni-ZAP XR phage and the expression of resident cDNA clones was induced after blotting the infected bacteria onto NitroBind nitrocellulose membranes (Osmonics, Minnetonka, MN, USA). The membranes were pretreated with 10 mmol/L IPTG (Wako Pure Chemicals, Osaka, Japan) for 30 minutes. The membranes with bacterial proteins were rinsed 3 times with TBST (20 mmol/L Tris-HCl [pH 7.5], 0.15 mol/L NaCl, and 0.05% Tween-20), and nonspecific binding was blocked by incubating with 1% protease-free BSA (Nacalai Tesque Inc., Kyoto, Japan) in TBST for 1 hour. The membranes were exposed to 1:2000-diluted sera of patients for 1 hour. After 3 washes with TBST, the membranes were incubated for 1 hour with 1:5000-diluted alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Positive reactions were developed using 100 mmol/L Tris-HCl (pH 9.5) containing 100 mmol/L NaCl, 5 mmol/L $MgCl_2$, 0.15 mg/mL of 5-bromo-4-chloro-3-indolylphosphate, and 0.3 mg/mL of nitro blue tetrazolium (Wako Pure Chemicals).

Monoclonal phage cDNA clones were converted to pBluescript phagemids by excision in vivo using the ExAssist helper phage (Stratagene). Plasmid pBluescript containing cDNA was obtained from the *E. coli* SOLR strain after transformation by the phagemid. The sequences of cDNA inserts were evaluated for homology with identified genes or proteins within the public sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3 | Expression and purification of antigen proteins

The expression plasmids of GST-fused proteins were constructed by recombining the cDNA sequences into pGEX-4T-3 (GE Healthcare Life Sciences, Pittsburgh, PA, USA). The inserted DNA fragments were ligated into pGEX-4T-3 using Ligation Convenience Kits (Nippon Gene). Ligation mixtures were used for transforming ECOS-competent *E. coli* BL21 (DE3) (Nippon Gene), and appropriate recombinants were confirmed by DNA sequencing as well as

protein expression analyses. Treating the transformed *E. coli* with 0.1 mmol/L IPTG for 3 hours induced the expression of the GST-fusion proteins. The GST-fused recombinant proteins were purified by glutathione sepharose column chromatography in accordance with the manufacturer's instructions (GE Healthcare Life Sciences) and dialyzed against PBS as described in previous studies.[36,37]

2.4 | Amplified luminescence proximity homogeneous assay

Amplified luminescence proximity homogeneous assay (PerkinElmer Inc., Waltham, MA, USA) was carried out using 384-well microtiter plates (white opaque OptiPlate; PerkinElmer) containing 2.5 μL of 1:100-diluted sera and 2.5 μL GST or GST-fusion proteins (10 μg/mL) in AlphaLISA buffer (25 mmol/L HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/mL dextran-500, and 0.05% Proclin-300). The reaction mixture was incubated at room temperature for 6-8 hours. Next, anti-human IgG-conjugated acceptor beads (2.5 μL of 40 μg/mL) and glutathione-conjugated donor beads (2.5 μL of 40 μg/mL) were added and incubated further for 7-21 days at room temperature in the dark. The chemical emission was read on an EnSpire Alpha microplate reader (PerkinElmer) as previously described.[38-43] Selective reactions were calculated by subtracting Alpha values of GST control from the values of GST-fusion proteins.

2.5 | Statistical analyses

All statistical analyses were undertaken using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) and R 3.5.1 statistical software (R Development Core Team, Vienna, Austria) . The Mann-Whitney *U* test was used for determining the significance of differences between 2 groups. The predictive values of markers for disease were assessed by ROC curve analysis. Cut-off values were determined by the values that maximize the sums of the sensitivity

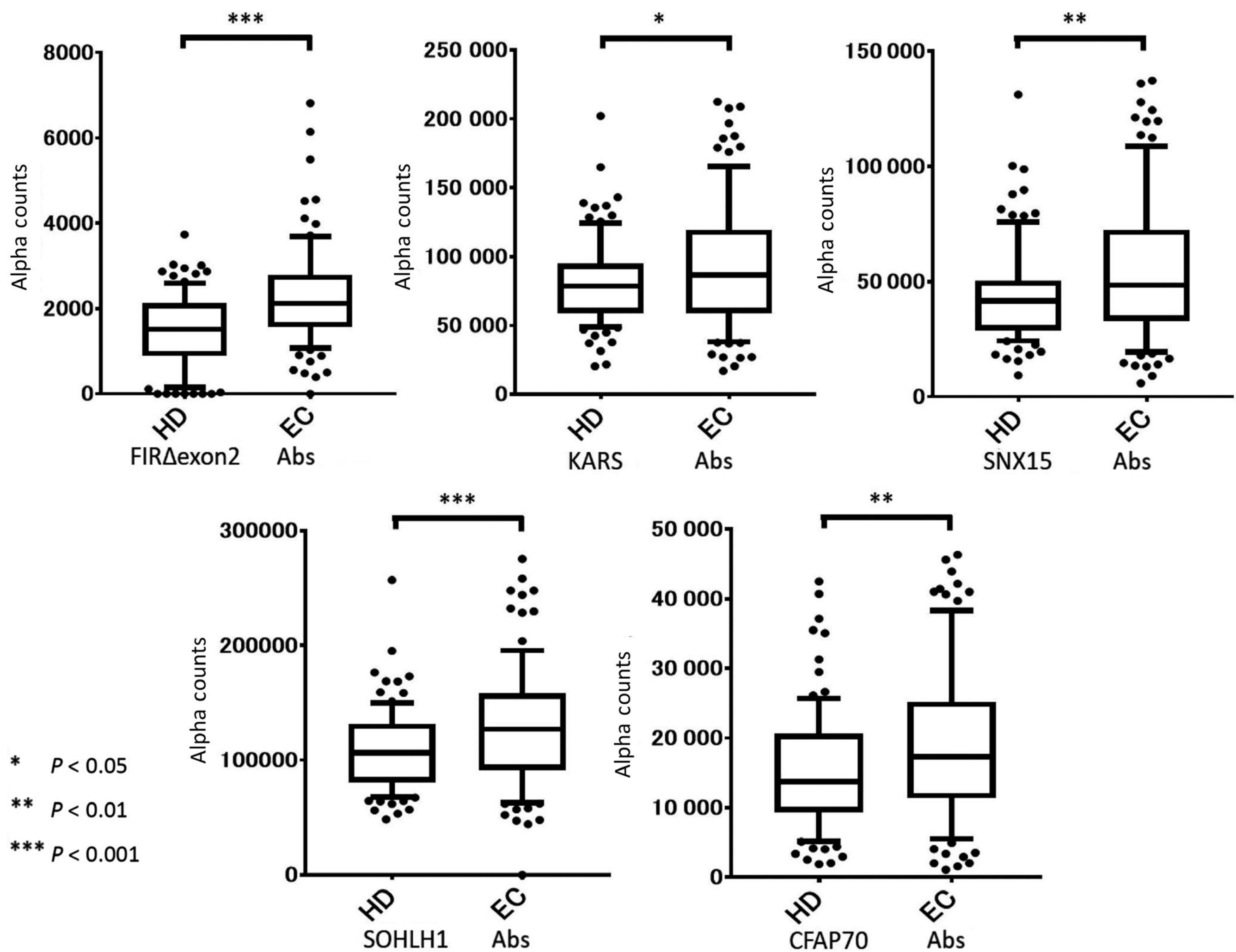

FIGURE 1 Comparison of levels of Abs against SEREX and FIRΔexon2 antigens in ESCC patients. The levels of Abs against FIRΔexon2, KARS, SNX15, SOHLH1, and CFAP70 Abs in healthy donors (HD) and patients with ESCC (EC) examined by AlphaLISA are shown. Serum Ab levels examined by AlphaLISA are shown using a box-whisker plot. The box plots display the 10th, 20th, 50th, 80th, and 90th percentiles. *P* values compared with the HD specimens are shown. *P* values were calculated using Mann-Whitney *U* test

and specificity. All tests were 2-tailed and a *P* value below 0.05 was considered significant. Antibody group-specific *Z*-scores were calculated for facilitating the comparison across all Ab groups. *Z*-score analysis was carried out after normalization to healthy donor mean values:

Z-score = [(control mean) − (individual value)]/(control standard deviation).[44,45]

The combined ROC analysis was undertaken by adding each *Z* score. To compare the significant differences among the single or combined ROCs, AUCs were calculated and examined by DeLong tests.[46-49] The following formula is used the DeLong test:

$$D = \frac{V^r(\theta^r) - V^S(\theta^S)}{\sqrt{S^r + S^S}}.$$

3 | RESULTS

3.1 | Identification of SEREX antigens in the sera of ESCC patients

Five SEREX antigens were identified in the sera of patients with ESCC by the expression cloning assay through λZAP II library construction, including FIRΔexon2 (Accession No. NM_001271099.1),[24,50] KARS (Accession No. NM_001130089.1),[51-53] SNX15 (Accession No. NM_013306.4),[54,55] SOHLH1 (Accession No. NM_001101677.1),[56,57] and cilia and flagella-associated protein 70 (CFAP70) (Accession No. NM_145170.3).[58] Recombinant proteins were expressed in *E. coli* as GST-fusion proteins and were purified by affinity-chromatography using glutathione sepharose column (Table S1).[29]

3.2 | Higher levels of autoantibodies were detected in the sera of ESCC patients than that of healthy donors

The levels of serum autoantibodies were analyzed by AlphaLISA using the sera of HDs (Table S2) and patients with ESCC obtained from Higashi Funabashi Hospital and from Chiba University Hospital, respectively. Results showed that the levels of FIRΔexon2, KARS, SNX15, SOHLH1, and CFAP70 Abs were significantly higher in patients with ESCC compared with HD (Figure 1). The cut-off value was determined as the average + 2 SDs of HD (95% confidence interval). The percentages of Ab-positive cases are as follows: FIRΔexon2 (17/95, 18%), KARS (14/95, 15%), SNX15 (17/95, 18%), SOHLH1 (12/95, 13%), and CFAP70 Abs (12/95, 13%) (Table 1). Clinical features of patients with ESCC are listed in Table 2.

3.3 | Anti-FIRΔexon2 Ab is an independent marker of ESCC

Spearman's rank correlation analysis was used for exploring whether a correlation exists between FIRΔexon2, KARS, SNX15, SOHLH1,

TABLE 1 Percentage of Ab-positive cases on AlphaLISA

		FIRΔexon2 Abs	%	*P* value	KARS Abs	%	*P* value	SNX15 Abs	%	*P* value	SOHLH1 Abs	%	*P* value	CFAP70 Abs	%	*P* value
Healthy subjects	(94)	1	(1)		3	(3)		5	(5)		2	(2)		5	(5)	
Esophageal cancer	(95)	17	(18)	<0.001	14	(15)	0.042	17	(18)	0.004	12	(13)	0.001	12	(13)	0.009

P values were calculated by Mann-Whitney *U* test.

Comparison of auto-Abs detected in the sera between ESCC patients and healthy subjects examined by AlphaLISA.

TABLE 2 List of clinical features of patients with esophageal squamous cell carcinoma

Esophageal cancer	FIRΔexon2 Abs (positive rate %)		*P* value	CFAP70 Abs (positive rate %)		*P* value	KARS Abs (positive rate %)		*P* value	SNX15 Abs (positive rate %)		*P* value	SOHLH1 Abs (positive rate %)		*P* value
Gender															
Male (84)	15	(18)		11	(13)		14	(17)		16	(19)		12	(14)	
Female (11)	2	(18)	0.979	1	(9)	0.707	0	(0)	0.143	1	(9)	0.418	0	(0)	0.180
Age															
≤67 y (45)	7	(16)		4	(9)		3	(7)		5	(11)		3	(7)	
>67 y (50)	10	(20)	0.573	8	(16)	0.298	11	(22)	0.035	12	(24)	0.102	9	(18)	0.097
Stage															
0, I, II (38)	9	(24)		4	(11)		5	(13)		8	(21)		2	(5)	
III, IV (50)	6	(12)	0.149	6	(12)	0.829	7	(14)	0.909	8	(16)	0.543	9	(18)	0.074
N.D. (7)	2	(29)		2	(29)		2	(29)		1	(14)		1	(14)	
CEA															
Positive (18)	4	(22)		6	(33)		8	(44)		7	(39)		5	(28)	
Negative (75)	13	(17)	0.630	6	(8)	0.004	6	(8)	<0.001	8	(11)	0.004	7	(9)	0.036
N.D. (2)	0	(0)		0	(0)		0	(0)		1	(50)		0	(0)	
CYFRA															
Positive (32)	4	(13)		6	(19)		5	(16)		7	(22)		7	(22)	
Negative (60)	13	(22)	0.281	5	(8)	0.143	9	(13)	0.937	10	(12)	0.540	5	(7)	0.066
N.D. (3)	0	(0)		1	(33)		0	(0)		0	(0)		0	(0)	
p53-Abs															
Positive (29)	9	(31)		5	(17)		7	(24)		7	(24)		6	(21)	
Negative (64)	8	(13)	0.032	6	(9)	0.277	7	(11)	0.099	10	(16)	0.325	6	(9)	0.132
N.D. (2)	0	(0)		1	(50)		0	(0)		0	(0)		0	(0)	
eso-SCC															
Positive (36)	8	(22)		6	(17)		10	(28)		9	(25)		10	(28)	
Negative (56)	9	(16)	0.458	5	(9)	0.264	4	(7)	0.007	7	(13)	0.123	2	(4)	<0.001
N.D. (3)	0	(0)		1	(33)		0	(0)		1	(33)		0	(0)	

P-values were calculated by Pearson's χ^2 test.N.D., not determined.

or CFAP70 Abs and clinically used tumor markers. As indicated in Figure 2, the correlation coefficient between FIRΔexon2 Abs and clinically used tumor markers was not significant. KARS Abs were positively correlated with SNX15, SOHLH1, and CFAP70 Abs, but not with FIRΔexon2 Abs (Figure 2). Next, clinical features of patients with ESCC were explored.

Venn diagram analysis showed that Ab-positive cases, including 5 cases of FIRΔexon2, 3 cases of CFAP70, 1 case of KARS, 4 cases of SNX15, and 1 case of SOHLH1 Abs, were noncommon Ab-positive cases between the p53 Abs, CEA, and CYFRA comparison groups. In addition, 4 candidate markers, including FIRΔexon2, KARS, SNX15, and SOHLH1 Abs, were not common Ab-positive cases between the p53 Abs, CEA, and CYFRA comparison groups (Figure 3). These results indicate that FIRΔexon2 Abs have no relation with clinically used tumor markers over other candidate markers. In addition, there was no correlation between anti-FIRΔexon2 Abs and the 9 tumor markers analyzed, thereby suggesting that anti-FIRΔexon2 Abs are independent markers of ESCC.

3.4 | Values of AUCs were increased in the combined ROC analysis compared to the individual ROC analysis

Receiver operating characteristic curve analysis was carried out for evaluating the ability of these candidate markers for detecting ESCC. The highest AUC values were obtained for FIRΔexon2 Abs compared to other ESCC candidate markers (Figure 4A). The Ab group-specific *Z*-scores were calculated to facilitate the comparison across all Ab groups. Among the Abs combined with CEA, only the AUC of SOHLH1 decreased to 0.6245 in sera of patients with ESCC. However, the AUC of the remaining Abs increased. Furthermore, the AUC of FIRΔexon2 Abs increased to 0.7190 in the sera of patients with ESCC (Figure 4B). Among all the Abs combined with p53 Abs, FIRΔexon2 showed an AUC of 0.759 in ESCC. There were no Abs with an AUC larger than 0.750, however, the AUC values were increased in the combined ROC analysis compared with the individual ROC analysis for all Abs (Figure 4C). Among the Abs combined with the both p53 and CEA Abs,

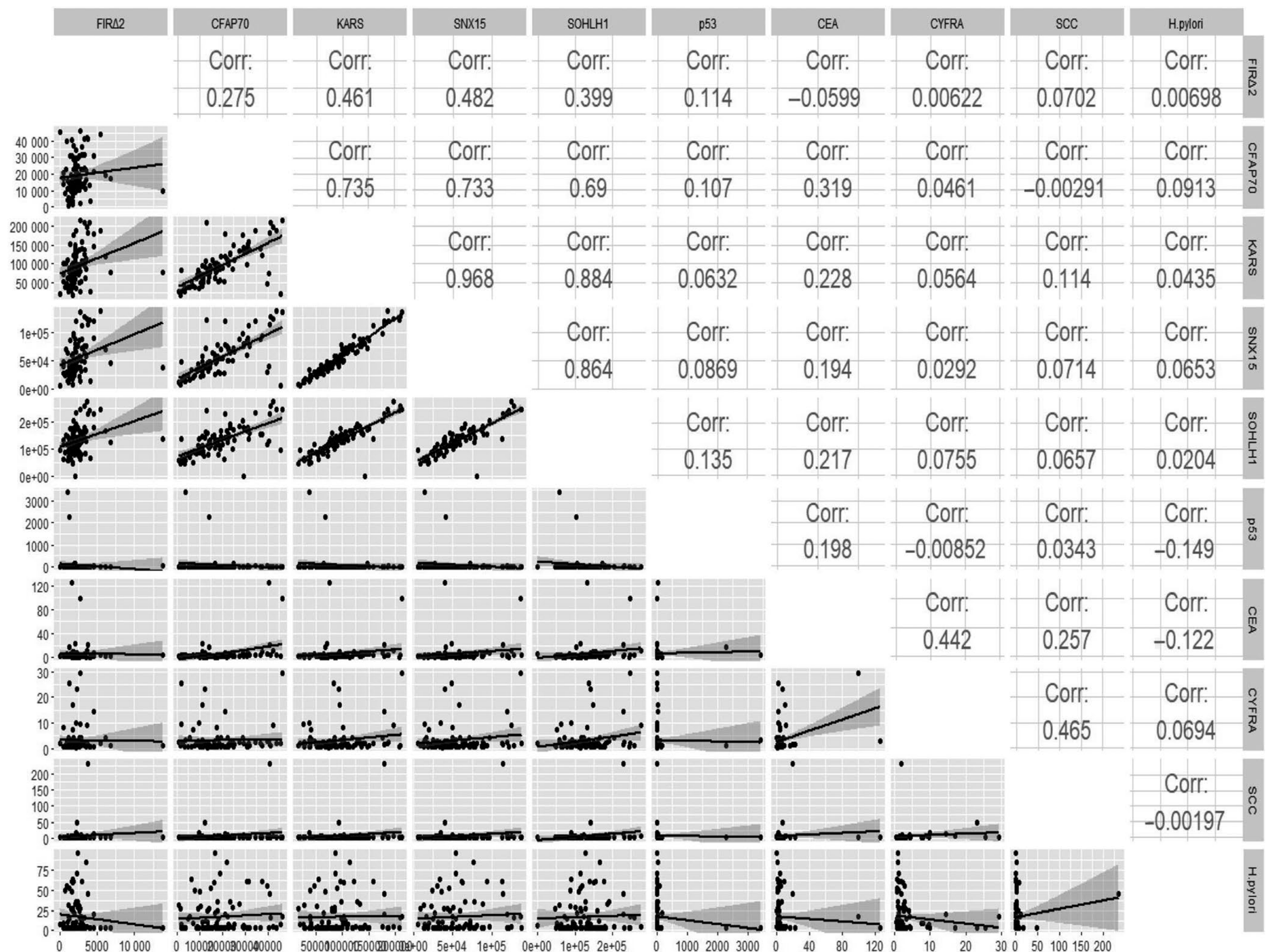

FIGURE 2 Correlation coefficients between candidate markers and clinically used tumor markers for detection of ESCC patients. The correlation (Corr) between the groups was assessed using Spearman's rank correlation coefficient. The lower triangular matrix shows the pairwise scatter plots between variables, whereas the upper triangular matrix shows Spearman's rank correlation coefficients among each paired measurement

FIRΔexon2 Abs showed an AUC of greater than 0.750 in the sera of patients with ESCC. There were no Abs with an AUC larger than 0.750, however, the AUC values were increased in the combined ROC analysis compared to the individual ROC analysis (Figure 4D). The highest AUC values were obtained for FIRΔexon2 + p53 Abs compared to all AUC values of ESCC.

The AUC values obtained for cancer classified as early or advanced stage are shown in Figure 5. The highest AUC values were obtained for FIRΔexon2 Abs in both early and advanced stage cancers when compared with AUC values of other ESCC candidate markers. The AUC values of early stage cancers were as follows: FIRΔexon2, 0.696; KARS, 0.598; SNX15, 0.632; SOHLH1,

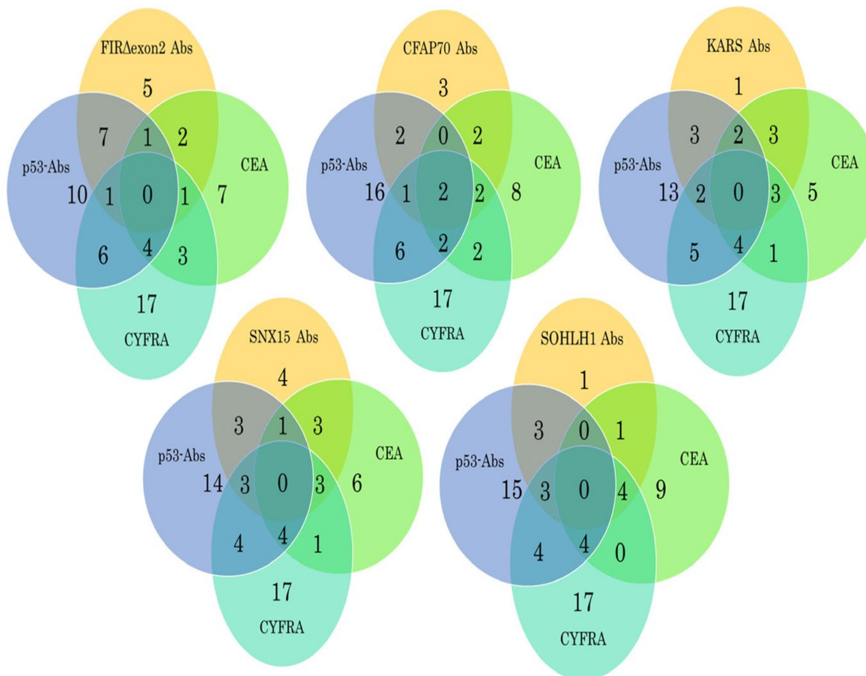

FIGURE 3 Venn diagram analysis among candidate markers and clinically used tumor markers. Results of Venn diagram analysis for differentially detected markers identified from patients of esophageal squamous cell carcinoma.

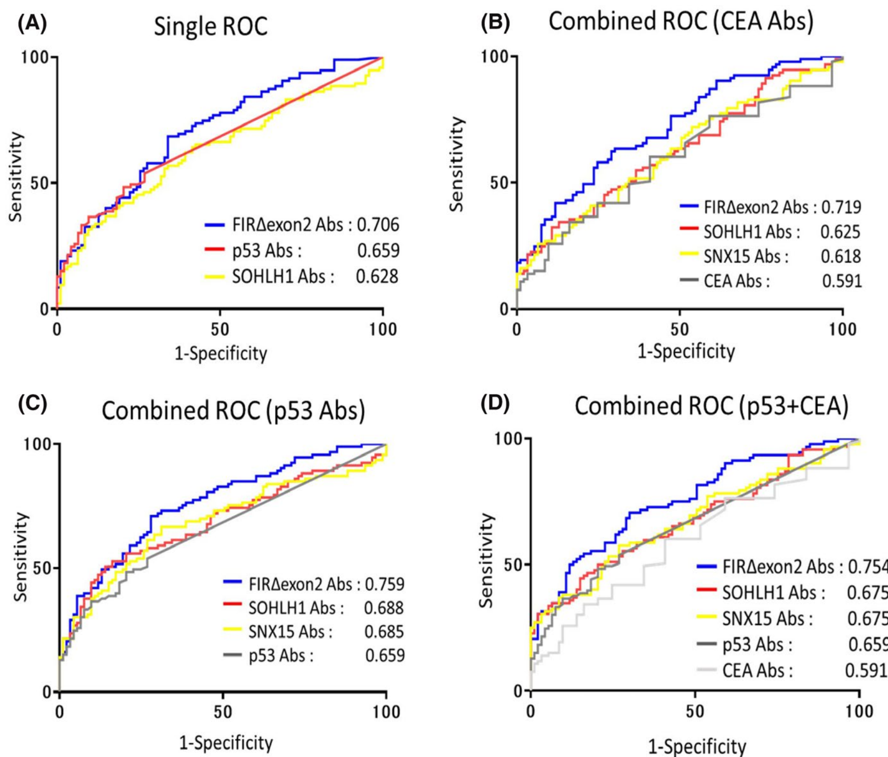

FIGURE 4 Comparison of combined ROCs in ESCC patients. A, Overall diagnostic efficiency of seven Abs was evaluated by comparing ROC curves. AUC values were calculated using GraphPad Prism 7. Values are shown in descending order of AUC. B-D, ROC analysis for the combination of candidate markers examined by the basis of *Z* score data normalized to SD of the quantified alpha count data of 95 patients with ESCC and 94 healthy subjects. Values are shown in descending order of AUC

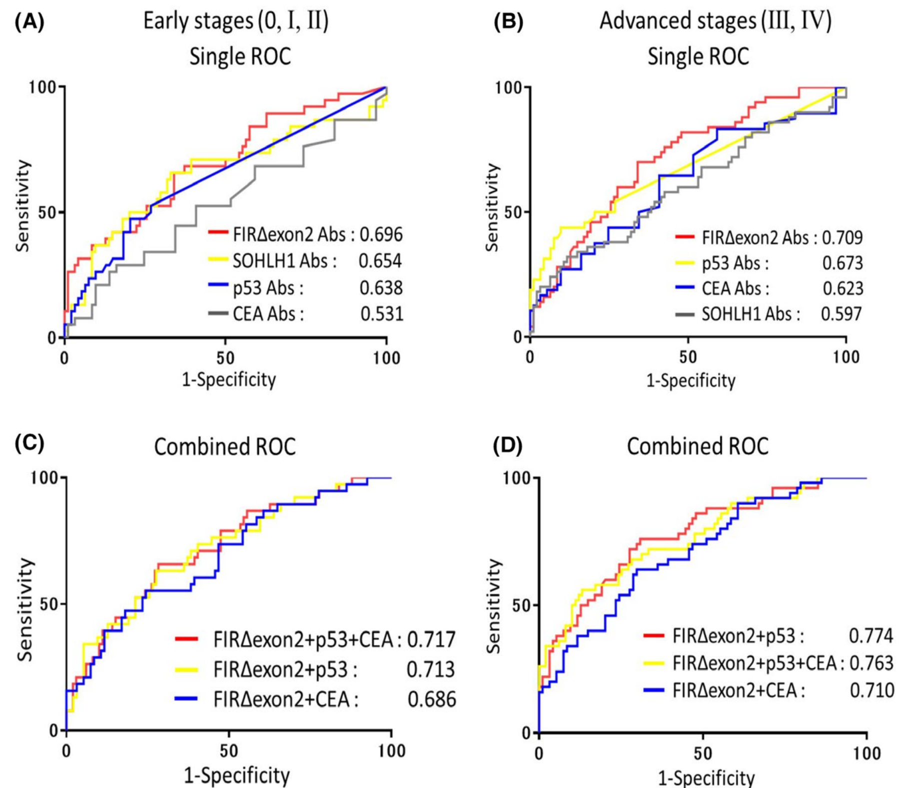

FIGURE 5 The Receiver operating characteristic (ROC) ROC curve analysis depicting the diagnosis efficiency of FIRΔexon2 Abs in combination with SOHLH1, CEA, and p53 Ab markers in ESCC patients. A, Area under the ROC curve (AUC) values of candidate markers for early stage ESCCs. B, AUC values of candidate markers for advanced stage ESCCs. C, ROC analysis depicting the diagnosis efficiency of FIRΔexon2 Abs in combination with CEA/p53 Ab markers for early stage ESCCs. D, ROC analysis depicting the diagnosis efficiency of FIRΔexon2 Abs in combination with CEA/p53 Ab markers for advanced stage ESCCs

0.654; CFAP70, 0.627; p53, 0.638; and CEA, 0.531 (Figure 5A). For advanced stage cancers, AUC values were as follows: FIRΔexon2, 0.709; KARS, 0.525; SNX15, 0.568; SOHLH1, 0.597; CFAP70, 0.567; p53, 0.673; and CEA, 0.623 (Figure 5B). The AUC values of FIRΔexon2 Abs were higher in advanced stage cancers compared to early stage (Figure 5C,D). However, in early stage cancers, the combined AUC showed increased values. Furthermore, in early stage cancers, the early diagnosis efficiency increased when FIRΔexon2 Abs were combined with p53 Abs (AUC 0.713) or with p53 and CEA Abs (AUC 0.717). In advanced stage cancers, the diagnosis efficiency increased when FIRΔexon2 Abs were combined with p53 Abs (AUC 0.774).

4 | DISCUSSION

In the present study, we identified potential novel diagnostic markers for ESCC using SEREX screening.[10] Serum Ab markers were detected using purified GST-fusion proteins as antigens. One hundred and eighty-nine patients with various cancers were evaluated for the presence of several Abs. Patients with confirmed ESCC showed significantly higher levels of Abs against most SEREX antigens. Additionally, all 5 SEREX antigen markers identified were significantly higher in patients with ESCC compared to HDs. Similar results were obtained by ROC analysis. The AUC values were greater than 0.5912 for all markers, with the exception of KARS Abs when compared with CEA (Table 1). Furthermore, AUC values greater than 0.700 were observed for FIRΔexon2 Abs in patients with ESCC. It is conceivable that FIRΔexon2 Abs are common markers for ESCCs.[23,31,32]

The combined ROC analysis of candidate Abs with anti-p53 Abs and CEA showed increased AUC values in the sera of patients with ESCC. Specifically, the combination of anti-p53 and FIRΔexon2 Abs was shown to improve the diagnostic efficiency, thus aiding in the early detection of ESCC (Figure 4 and Table 2). Furthermore, the significance of ROCs among single or combined markers was examined through comparing the AUC by DeLong tests.[46,49] In all stages of ESCCs, FIRΔexon2 Abs + p53 Abs or FIRΔexon2 Abs + CEA or FIRΔexon2 Abs + CEA + p53 Abs were significantly higher than that of CEA alone (Table S3, middle column). Similarly, there was significance in early or advanced stages of ESCCs (Table S4). Therefore, FIRΔexon2 Abs with anti-p53 Abs or CEA improves the specificity and sensitivity for screening ESCCs. Further prospective multi-institutional studies comparing the sensitivity and specificity of this combinational detection approach will be required.[28]

Amplified luminescence proximity homogeneous assay is an excellent method for measuring Ab levels compared with ELISA because of its low variation, stable background, and high specificity. It does not involve plate-washing steps; however, it involves mixing antigens with Abs in sera followed by the addition of donor and acceptor beads. For instance, Figure 1 showed highly reproducible results, including distributions, *P* values, and positive rates despite using different sets of sera from healthy donors and patients. AlphaLISA is a novel, recently developed method. After examining suitable AlphaLISA conditions in this study, we concluded

that the incubation for 7-21 days is the best to obtain specific antigen-Ab reaction as well as to reduce noise background. The precise measurement offered by AlphaLISA might enable establishment of Ab markers, although most of the existing tumor diagnosis methods involved antigen markers, with the exception of the p53 marker. The measurement of Abs was more sensitive in comparison to the measurement of the antigen levels owing to the stability of IgG proteins and their amplification by repeated exposures to antigenic proteins.[38] Prior to development, highly malignant tumors can induce necrosis, leading to the exposure of intracellular antigenic proteins to plasma. Therefore, using combinational Ab detection approaches could allow for the precise early detection of tumors.[28] In this study, we explored and examined 5 SEREX antigen markers for improved efficiency in diagnosing ESCC. To the best of our knowledge, our study is the first to suggest that FIRΔexon2, KARS, SNX15, SOHLH1, and CFAP70 Abs are candidate markers for ESCC and offer promise for the future selection of potential diagnostic markers.

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DISCLOSURE

The authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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