



Identification of SEC61 β and its autoantibody as biomarkers for colorectal cancer

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ABSTRACT

Background: To identify novel serological biomarkers for human colorectal cancer (CRC), we analyzed CRC tissues using gel-assisted digestion and isobaric tags with related and absolute quantitation (iTRAQ) labeling mass spectrometry (MS). By comparing pairs of tumor tissues and matched normal tissues, we discovered the SEC61 β with expression changes 3.3-fold and a marginal statistical significance ($p=0.052$) previously.

Methods: SEC61 β expression in CRC tissues was further analyzed by western blotting and immunohistochemistry. We next assessed the putative diagnostic value of the SEC61 β autoantibody as a serum marker.

Results: Using western blotting analysis, SEC61 β expression was increased 1.9-fold in tumor tissues. Immunohistochemical analysis of 64 CRC specimens showed that SEC61 β was positively detected in 64% of the tumors, but weakly or not detected in >80% of the adjacent nontumor epithelial cells. Western blot analysis with plasma samples showed that the sensitivity and specificity of the SEC61 β autoantibody from patients with CRC were 79% and 75%, respectively. Importantly, the results of the SEC61 β autoantibody for early detection of colorectal cancer revealed a higher sensitivity of 77% than the carcinoembryonic antigen (CEA) assay.

Conclusions: Measurement of SEC61 β autoantibody levels may provide an alternative detection indicator for CRC, particularly among early-stage patients.

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1. Introduction

Colorectal cancer (CRC) is one of the most prevalent and fatal malignancies worldwide. The development of this cancer takes decades and involves multiple genetic events. Early detection of CRC is critical for successful patient therapy and lower risk of recurrence. The major symptoms of CRC are nonspecific, and many patients with early-stage CRC are asymptomatic. Screening methods currently available include digital rectal examination, fecal occult blood test (FOBT), sigmoidoscopy, and colonoscopy [1]. Their diagnostic value as a general screening tool is, however, limited because of poor sensitivity and a high false-positive rate (FOBT) [2,3], costs, risks, and inconvenience (colonoscopy)

[4,5]. To overcome these problems, the identification of novel biomarkers that can allow for the early detection of CRC is crucial.

Proteomic technology platforms are promising tools supporting the discovery of novel cancer biomarkers [6]. We previously applied gel-assisted digestion together with iTRAQ labeling MS to detect differences in protein expression profiles from tumors and adjacent normal mucosa from patients with CRC [7]. In this report, we have extended these findings in order to identify novel serological CRC biomarkers. For clinical application in screening, biomarkers should be detected in body fluids such as plasma, serum, or urine, meaning that tumor-associated secreted proteins or tumor-associated autoantibodies are of particular interest.

Identifying altered expression patterns of tumor-associated autoantibodies for use as diagnostic biomarkers has been of interest in the field of cancer research [8]. Although factors resulting in the production of autoantibodies in malignancy are not completely understood, autoantibodies have been used as reporters that identify abnormal cellular processes during tumorigenesis [9]. In addition, the antibody response to a particular tumor-associated antigen is

Abbreviations: CEA, carcinoembryonic antigen; CRC, colorectal cancer; FOBT, fecal occult blood test; iTRAQ, isobaric tags with related and absolute quantitation.

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generally undetectable in healthy individuals [10–13] and in various non-malignant diseases (benign colonic adenoma, familial adenomatous polyposis, Peutz–Jeghers syndrome, gastritis, alcoholic cirrhosis, and chronic viral hepatitis) [14]. Therefore, the use of autoantibodies as serological markers for cancer detection is feasible. Substantial evidence supports the presence of an immune response to cancer in humans, as demonstrated by the presence of autoantibodies in cancer patient sera. Autoantibodies have been reported in many cancers and include anti-survivin and anti-livin in lung cancer [15], anti-thyroglobulin in thyroid cancer [16], and anti-CENP-B and anti-SS-B in breast cancer [17]. Autoantigens altered before or during tumor formation elicit an immune response [18–24]. Because tumor-associated autoantibodies can be detected at early stages of cancer before cancer diagnosis, they are potential biomarkers [19,20,25].

Serological responses to CRC are heterogenic. Several studies have assessed the potential of various serum autoantibodies for the detection of CRC, including serum NCC-ST 439 [26], anti-DEAD-box protein 48 [12], anti-p53 [10,11,14] and anti-sFasL [13]. Although these serum biomarkers showed a specificity above 94% in all of these studies, sensitivities hardly reached 30%. These autoantibodies are present only in a limited proportion of patients, and the sensitivity of their detection is insufficient for use in routine diagnosis. Therefore, it is necessary to discover novel serological biomarkers with a high sensitivity that will be able to improve plasma-based diagnosis or monitoring of CRC.

In a previous study, we found that transport protein Sec61 subunit beta (SEC61 β) was upregulated in CRC tissues by >2-fold with a marginal statistical significance (3.3-fold, $p=0.052$) by iTRAQ labeling MS [7]. We further successfully identified SEC61 β that could be detected at elevated levels in tumor tissues and conditioned media from CRC cell lines (Colo205). The SEC61 β was initially identified as a component required for the constitution of SEC61 translocon necessary for protein translocation in the endoplasmic reticulum [27]. However, its role other than protein translocation remains largely unknown. The SEC61 β had not been identified as overexpressed in CRC previously and is worthwhile to further estimate the valuable for a serological marker for CRC. In this study, significantly elevated serum levels in patients with CRC were demonstrated for SEC61 β autoantibody. Thus, we suggest that the SEC61 β autoantibody may be a candidate for use as a serological biomarker for CRC detection.

2. Materials and methods

2.1. Individuals and sample collection

2.1.1. Tissue specimens

A total of ten CRC tissue samples and adjacent normal tissues from patients with CRC were utilized to verify SEC61 β expression identified by proteomic analysis previously. Each of the CRC samples included >70% tumor cells, and patients who had received any chemo- and/or radio-therapeutic treatment before surgery were excluded from this study. Normal tissue was obtained from the distal edge of the resection at least 10 cm from the tumor. Fresh snap-frozen samples were obtained immediately at the time of surgery and stored at -80°C until use. Serial sections from formalin-fixed, paraffin-embedded blocks of 64 tissue samples from CRC patients admitted to the Chang Gung Memorial Hospital, Lin-Kou, Taiwan between 2006 and 2007 were applied to immunohistochemical evaluation.

2.1.2. Human plasma

Plasma samples from 86 patients with CRC and 72 healthy controls were collected for this study. The number of patients with CRC in pathological stages I, II, III, and IV were 10, 25, 35, and 16, respectively. Control individuals with autoimmune diseases and irritable bowel

diseases were excluded from this study. For the blood preparation, 3 ml of blood was collected in an EDTA tube, and the plasma was prepared as described by the HUPO Plasma Proteome Project [28]. Plasma samples were collected before surgery and stored at -80°C until use. Written informed consent from all patients was obtained before surgery in accordance with medical ethics. This study was approved by the Human Clinical Trial Committee at Chang Gung Memorial Hospital.

2.2. Cell cultures

CRC Colo205 (ATCC no. CCL-222), SW620 (ATCC no. CCL-227), and SW480 (ATCC no. CCL-228) cell lines were maintained in RPMI medium 1640 or Liebovitz's L-15 medium (GIBCO, Invitrogen Corporation, NY) supplemented with 10% FBS (Life Technologies, Grand Island, NY), penicillin (1 $\mu\text{g}/\text{ml}$), and streptomycin (1 $\mu\text{g}/\text{ml}$) at 37°C in 5% CO_2 . Conditioned media and cell extracts from the various cancer cell lines were collected and processed as described [29]. The protein concentrations of the various samples were determined using the BCA protein assay reagent from Pierce (Rockford, IL).

2.3. Western blotting

Each tissue sample was mixed with electrophoresis sample buffer containing 2% SDS and 5% 2-mercaptoethanol and boiled for 5 min. Proteins were separated by electrophoresis on 12% denaturing polyacrylamide gels and transferred to PVDF membranes (Pall Europe Ltd., Portsmouth, UK). The blots were blocked with 5% skim milk and then probed with rabbit anti-human SEC61 β polyclonal antibodies (LS-C10071; Lifespan Biosciences, Bio Pioneer Tech Co., Ltd, Taipei, Taiwan) at a dilution of 1:1000 for 2 h at room temperature, followed by incubation for 1 h with peroxidase-conjugated secondary antibody at room temperature. The blots were developed with enhanced chemiluminescence (ECL) western reagents and exposed to Kodak Biomax light films. The immunoblot images were acquired by Imagemaster (Amersham Pharmacia Biotech, Piscataway, NJ). The protein level of each band was quantified by densitometry and analyzed with Multi Gauge Version 2.0 software (Fuji PhotoFilm, Tokyo, Japan). Data were analyzed with a paired *t*-test using the statistical software SPSS/Windows 12.0 statistical package (SPSS, Inc., Chicago, IL). $P<0.05$ was considered statistically significant.

2.4. Immunohistochemical analysis

Serial sections from formalin-fixed, paraffin-embedded blocks of 64 tissue samples were applied to 3-aminopropyltriethoxysilane-coated slides (Sigma). Deparaffinization and rehydration were performed using xylene and alcohol. The sections were pretreated in a microwave oven for antigen retrieval. To block endogenous peroxidase activity, a 5-min incubation with blocking reagent (DAKO, Glostrup, Denmark) was carried out. Sections were then incubated for 30 min at room temperature with rabbit anti-human SEC61 β polyclonal antibodies (LS-C10071; Lifespan Biosciences) at a 1:200 dilution. Horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz Biotechnology, Inc., CA) and 3,3'-diaminobenzidine (DAKO) were used to visualize labeled proteins. Expression of the SEC61 β protein was categorized as positive or negative and was evaluated according to the simplified H score system [30], which is based on the percentage of cells stained (3, $\geq 90\%$; 2, 50–89%; 1, 10–49%; or 0, 0–9%) and the intensity of cell staining (3, strong; 2, moderate; 1, weak; or 0, no cell staining). The 2 scores were multiplied by each other and divided by 3 to obtain the final score. Positive staining was defined as a final score ≥ 1 .

2.5. Preparation of recombinant SEC61 β (r-SEC61 β)

SEC61 β cDNA was obtained by reverse transcription-PCR using total RNA isolated from Colo205 cells (ATCC no. CCL-222) and the following SEC61 β -specific primers, synthesized by MWG-Biotech (Seminole Drive, Huntsville, AL) and based on the SEC61 β gene sequence of GenBank accession number NM_006808: SEC61 β forward primer, 5'-CCGAA TTCCA GTGGC ACTAA CGTGG GATCC TC-3'; SEC61 β reverse primer, 5'-TGCTC GAGCG AACGA GTGTA CTTGC CCC-3'. The PCR amplification product containing nucleotides 94–364 of SEC61 β was subcloned into a pET30b (Novagen, EMD Chemicals Inc., Darmstadt, Germany) expression vector. Sequence analysis was performed using a T7 promoter primer to confirm the SEC61 β sequence. The SEC61 β expression plasmid was then transformed into *Escherichia coli* BL21 (DE3) pLysS for recombinant expression. Isopropyl β -D-thiogalactopyranoside (IPTG) was used to induce protein expression. The cells were harvested by centrifugation and disrupted by sonication in lysis buffer containing 0.2 mol/l Tris-HCl, pH 7.5, 20% (v/v) glycerol and 1 mmol/l phenylmethylsulfonyl fluoride (PMSF) at 4 °C. r-SEC61 β was purified using a HiTrapTM Chelating HP column (GE Healthcare, Munich, Germany). Expression and purification of the recombinant protein were confirmed with immunoblotting using a rabbit anti-human SEC61 β polyclonal antibody (LS-C10071, Lifespan Biosciences).

2.6. Detection of SEC61 β autoantibodies by western blotting

Purified r-SEC61 β (4 μ g) was boiled in loading buffer for 5 min, subjected to 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a PVDF membrane. The membrane was blocked overnight at 4 °C in PBS containing 50 g/l skim dry milk and 0.1% Tween-20. The membrane was then cut into strips, which were incubated separately with patient plasma (1:100 dilution each in PBS containing 50 g/l bovine serum albumin) or anti-His₆ mouse monoclonal antibody (Calbiochem, Darmstadt, Germany) for 1 h at 37 °C with agitation. After washing the strips 6 times with PBS containing 0.1% Tween-20, the strips were incubated with horseradish peroxidase-conjugated anti-human IgG (Santa Cruz Biotechnology) or horseradish peroxidase-conjugated bovine anti-mouse IgG (Santa Cruz Biotechnology) for 1 h at 37 °C. The strips were washed and color-developed in TMB (3,5,5 tetramethylbenzidine) membrane peroxidase substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The reaction was stopped by washing the strips with tap water. All plasma samples were analyzed in triplicate and mean values were calculated.

2.7. CEA antigen assay

The plasma CEA levels were assayed with a CEA ELISA kit (BioQuant, San Diego, CA).

2.8. Statistical analysis

Statistical analysis was conducted using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL). Descriptive statistics were expressed as means \pm standard deviation (SD). The differences of the serum levels of SEC61 β autoantibodies between groups were evaluated by the non-parametric Mann-Whitney test. The receiver operating characteristic curves (ROC) were constructed by plotting sensitivity versus (1-specificity), considering each observed value as a possible cut-off value. The final cut-off value was determined by obtaining the optimal Youden's index (sensitivity + specificity - 1). According to the final diagnosis, the following calculations will be made: sensitivity, specificity, positive predictive value and negative predictive value. Discriminative power was assessed using the area under a ROC curve (AUC). A $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Differential SEC61 β expression detected by western blotting

By comparing pairs of tumor tissues and matched normal tissues with iTRAQ labeling LC-MS/MS, we identified the SEC61 β with expression changes 3.3-fold and a marginal statistical significance previously. To further validate the results obtained from the proteomic study, we examined the expression of SEC61 β using western blotting. To estimate antibodies available for western blotting, we tested their reactivity with colorectal cell lines and CRC samples as a means of verification. SEC61 β was detected in colorectal cell lines, including Colo205, SW480, and SW620. Moreover, the protein was detected in conditioned medium from the Colo205 cell line, thus may be excreted by tumor cells (Supplemental Fig. 1). The likely release and detection of SEC61 β in body fluids made this protein an ideal biomarker candidate. To test whether SEC61 β protein was truly overexpressed in the CRC tumor tissues, we examined the expression of SEC61 β proteins in 10 colorectal carcinomas using western blot analysis (Fig. 1). The SEC61 β proteins were expressed at significantly higher levels in tumor tissues, as compared with the matched normal colorectal tissues. The expression level showed a clear discriminatory value between tumor and normal samples and was increased by 1.9-fold (SEC61 β / β -actin ratio \pm S.D.: 3.34 ± 1.09 in nontumor tissues versus 6.24 ± 3.63 in cancer tissues, $p < 0.05$). Thus the immunoblot results were consistent with the LC-MS/MS results.

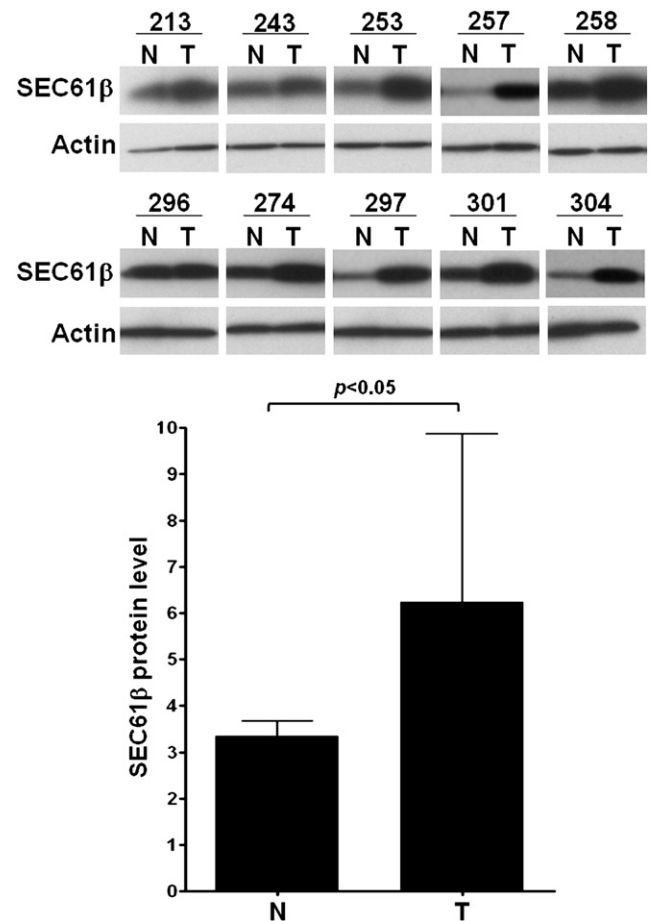


Fig. 1. Confirmation of the overexpression of SEC61 β by western blot analysis. Western blot analysis was performed on 10 pairs of CRC tissue and matched normal tissue samples. Actin was used as a loading control. Western blot images were quantified with densitometric scanning, and paired *t*-test analysis was performed after the intensity values were normalized to those of actin. N, normal; T, tumor.

Table 1
Analysis of 64 tissue specimens by immunohistochemistry with anti-SEC61 β .

Tissue type	Staining intensity	
	Negative	Positive
Non-tumor epithelium (n = 16)	13 (81%)	3 (19%)
Neoplastic colorectal tissues (n = 64)	23 (36%)	41 (64%)

3.2. Immunohistochemistry reveals overexpression of SEC61 β in tumor cells of CRC specimens

To further verify the elevated expression of SEC61 β protein in CRC, we examined the protein expression in 64 tissue sections with immunohistochemical staining with the SEC61 β antibody. Positive staining of SEC61 β was observed in 64% (41/64) of the tumors (Table 1). Three representative cases of positive SEC61 β staining are shown in Fig 2 (left panels). The antibody strongly stained the tumor cells but showed little staining of adjacent nontumor epithelial cells in most of the samples examined (Fig. 2, right panels). Among the 64 CRC tissue sections examined, 16 sections harbored nontumor epithelial cells; 81% of these (13 sections) were negative for SEC61 β expression, whereas two sections were weakly positive and one section was moderately

positive. The levels of SEC61 β in tumor tissues, as determined by immunohistochemistry or western blot analysis, have a tendency towards overexpression.

3.3. Increased levels of SEC61 β autoantibody in plasma from patients with CRC

We next assessed the putative diagnostic value of the SEC61 β antigen and/or autoantibody as a serum marker. We examined the plasma level of SEC61 β and its autoantibody using western blot analysis. We first examined SEC61 β in plasma from patients with CRC; however, we could not develop an immunoassay with a satisfactory detection limit (data not shown). For SEC61 β autoantibody analysis, the r-SEC61 β protein was purified to near homogeneity (Supplemental Fig. 2), and its identity was verified by western blot analysis using commercial anti-SEC61 β (LS-C10071, Lifespan Biosciences). Plasma from 86 randomly chosen patients with CRC was examined for antibody reactivity against r-SEC61 β by western blotting. The levels of SEC61 β autoantibody were significantly increased in the plasma samples from patients with CRC (Fig. 3a,b) versus those from healthy controls (90.60 ± 160.20 versus 14.75 ± 69.55 , $p < 0.001$). The plasma level of SEC61 β was 6.1-fold higher in patients with CRC than in healthy controls (Fig. 3b). Although it is clear that the amounts of SEC61 β autoantibody were increased in patients with CRC as compared with healthy controls, the plasma levels

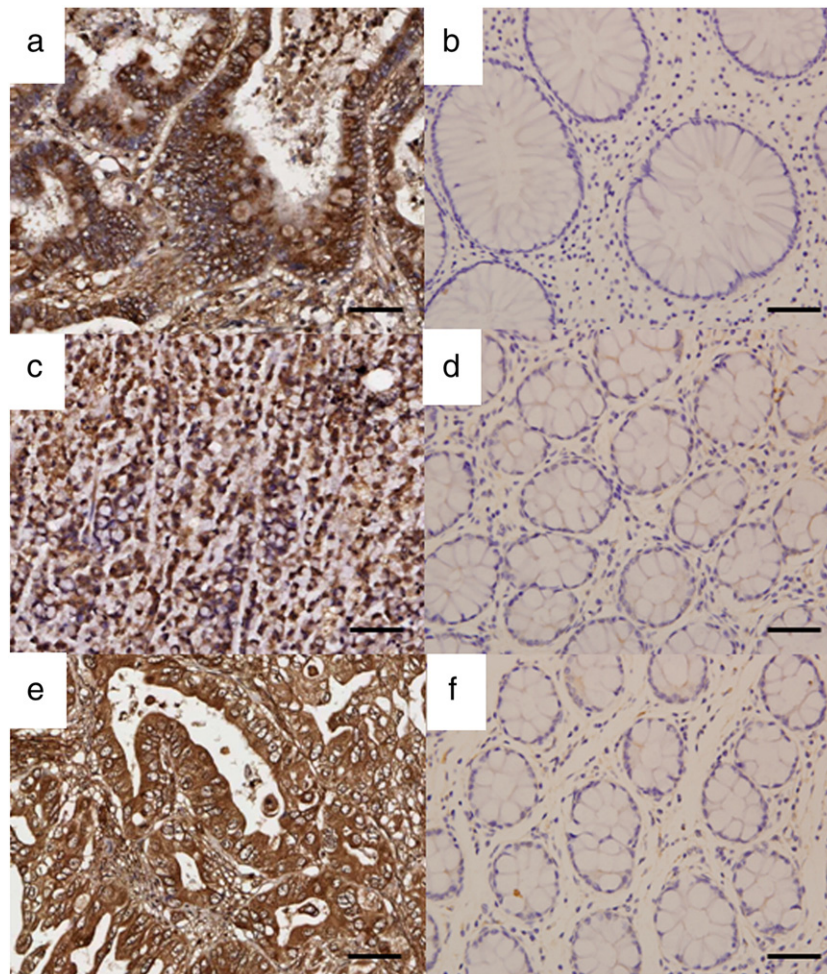


Fig. 2. Immunohistochemistry for SEC61 β in CRC tissues. Expression of SEC61 β was validated by immunohistochemistry using tissue sections from formalin-fixed, paraffin-embedded CRC tissue samples. Representative immunohistochemical images of carcinoma (a, c and e: from patients with CRC in stage III, II and IV), as well as adjacent benign epithelial cells (b, d and f). No positive staining was found in the benign epithelial cells (right panels: b, d and f). Expression of SEC61 β in carcinoma was expressed in most of the tumor cells (left panels: a, c and e). Scale bar: 50 μ m.

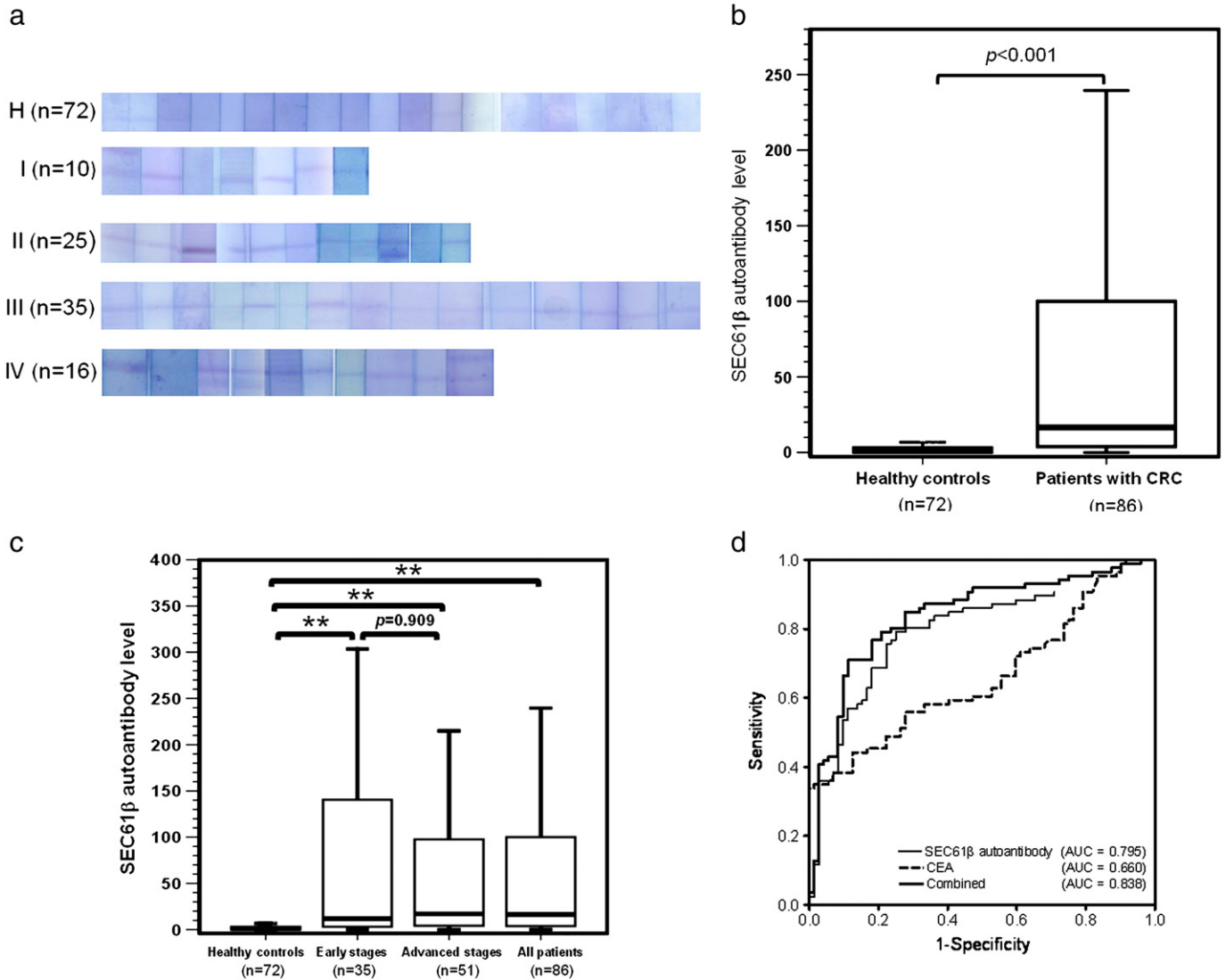


Fig. 3. SEC61 β autoantibody levels in plasma from healthy controls and patients with CRC. (a) Western blot analysis was performed for SEC61 β autoantibody with plasma samples from 86 patients with CRC and 72 healthy controls. All plasma samples were analyzed in triplicate. Abbreviations: H, healthy control; I–IV, patient with CRC in stage I–IV. (b) Western blot images were scanned, and their intensities were quantified with densitometry. Data represent mean values from three determinations. Data were presented as upper and lower quartile and range (box; 25 to 75 percentile), median value (horizontal line), and the middle 90% distribution (dashed line). A Mann–Whitney test was performed to compare patients with CRC with healthy controls. (c) CRC samples are divided into two groups (early stages, I and II; advanced stages, III and IV), and a Mann–Whitney test was performed to compare CRC in early stages, advanced stages, and stages I–IV (All patients) with healthy controls. ** was set at $p < 0.001$. (d) The relationship between the sensitivity and the specificity of SEC61 β autoantibody measurement for the detection of CRC is represented by ROC curves. The AUC values were 0.795 for SEC61 β autoantibody, 0.660 for CEA, and 0.838 for the 2 markers combined.

did not show a significant correlation ($p = 0.909$) with the tumor stage; levels of SEC61 β autoantibody were more than 4-fold higher in patients with CRC (8.5-fold in early stages and 4.6-fold in advanced stages) as compared with healthy donors, regardless of tumor stage (Fig. 3c).

The relationship between the specificity and the sensitivity of SEC61 β autoantibody measurement for the purpose of CRC detection was represented by a receiver operating characteristics (ROC) curve

(Fig. 3d). The area under the ROC curve (AUC) was 0.795 for the SEC61 β autoantibody. To evaluate the diagnostic value of the SEC61 β autoantibody, we measured the levels of CEA in the same set of plasma samples. The AUC value was 0.660 for CEA. Thus, SEC61 β autoantibody has more diagnostic efficacy than CEA. When cutoff values of 5 ng/ml for CEA and 2.644 (band intensity; determined by obtaining the optimal Youden's index) for SEC61 β autoantibody were

Table 2
Detection of SEC61 β autoantibodies, CEA and both markers combined in plasma samples from CRC patients and healthy controls.

	No.	Anti-SEC61 β antibodies			CEA			Anti-SEC61 β + CEA		
		Positive	Negative	p^a	Positive	Negative	p^a	Positive	Negative	p^a
Patients	86	68 (79%)	18 (21%)	<0.001	34 (40%)	52 (60%)	<0.001	61 (71%)	25 (29%)	<0.001
Healthy controls	72	18 (25%)	54 (75%)		9 (13%)	63 (87%)		8 (11%)	64 (89%)	

^a The calculated p values represent statistical differences in the CRC patients versus healthy controls.

applied, the diagnostic sensitivity of the SEC61 β autoantibody was 79% and the specificity was 75%, whereas for CEA, these values were 40% and 87%, respectively (Table 2).

Combined detection using CEA and SEC61 β autoantibodies produced better sensitivity (71%) and specificity (89%) as compared with CEA sensitivity (40%) or SEC61 β autoantibody specificity (75%) alone (Table 2). Importantly, the combination of the presence of CEA and SEC61 β autoantibodies showed a higher diagnostic capacity than either marker alone (AUC = 0.838; 95% CI, 0.774–0.903; Fig. 3d).

Notably, when a cutoff value of 2.644 was chosen for SEC61 β autoantibodies, 42 of 52 patients with CRC with CEA levels <5 ng/ml could be distinguished from healthy individuals (Fig. 4).

The positive predictive value (PPV) and negative predictive value (NPV) was 79% and 75% in our test for SEC61 β autoantibody and 79% and 55% for CEA. Notably, the PPV and NPV in our test for combination of SEC61 β autoantibody and CEA was 88% and 72%. These results collectively indicate that SEC61 β autoantibody might be a potentially useful serum biomarker for CRC, especially when used together with CEA.

When the diagnostic parameters were calculated for early-stage carcinoma only, the sensitivity of CEA was 31%, and for SEC61 β autoantibody, the sensitivity was 77% (Table 3). The results of the SEC61 β autoantibody for early detection of colorectal cancer revealed a higher sensitivity of 77% than the CEA assay. Detection of anti-SEC61 β response provides a novel serological biomarker for detecting patients with CRC, especially for early stage.

4. Discussion

In this study, we show increased expression of SEC61 β in CRC when compared with matched normal tissues. Differential expression of the protein was previously identified using a proteomics approach based on LC-MS/MS and performed a qualification test of SEC61 β for its potential as a detection marker. SEC61 β was highly expressed in CRC tissues when examined by western blotting and immunohistochemistry. The best cancer biomarkers are likely to be secreted proteins or the corresponding autoantibody. We first examined SEC61 β in serum from patients with CRC; however, we could not develop an immunoassay with a satisfactory detection limit (data not shown). This problem may be due to antigen release levels and/or its half-life in body fluids. In contrast, SEC61 β autoantibody was successfully analyzed by relatively noninvasive techniques and therefore has the potential for greatly enhanced acceptance as a biomarker for screening.

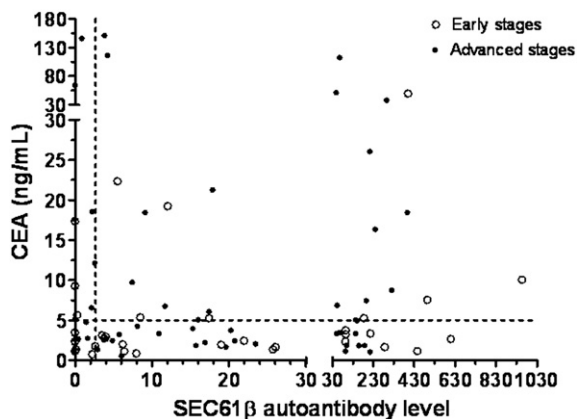


Fig. 4. Analysis of SEC61 β autoantibody levels and preoperative CEA concentrations in plasma from patients with CRC ($n=86$). The vertical and horizontal dashed lines indicate cutoff values for SEC61 β seropositivity (arbitrary units = 2.644) and CEA concentration (5 ng/ml), respectively.

Table 3

Comparison of the sensitivity of diagnosing patients with CRC using SEC61 β autoantibodies, CEA, and the two markers combined.

Stages	Number	CEA seropositive	SEC61 β autoantibody seropositive	Two markers combined
I	10	0 (0%)	8 (80%)	7 (70%)
II	25	11 (44%)	19 (76%)	18 (72%)
I + II	35	11 (31%)	27 (77%)	25 (71%)
III	35	12 (34%)	29 (83%)	26 (74%)
IV	16	11 (69%)	12 (75%)	10 (63%)
III + IV	51	23 (45%)	41 (80%)	36 (71%)
Total	86	34 (40%)	68 (79%)	61 (71%)

The autoimmune response may be induced by tumor proteins such as p53, HER2, NY-ESO1, or MUC1 that are affected by specific point mutations, misfolding, overexpression, aberrant glycosylation, truncation, or aberrant degradation [21,31–35]. Several studies have suggested that detection of autoantibody responses against tumor-associated antigens may be useful for early-stage cancer diagnosis. For example, serum p53 antibodies are detected before clinical detection of lung cancer [36] and oral cancer [37], and HER-2/*neu* antibodies are detected in serum from patients with early-stage breast cancer [38]. Although several colorectal tumor-associated autoantibodies have been identified, none of them has sufficient sensitivity for practical usage in screening patients with early-stage CRC [39,40].

In this regard, the discovery of SEC61 β autoantibody as a serological marker is likely to have a great impact because the increase in autoantibody in the plasma of patients with CRC was highly significant (Fig. 3). This increase was higher than that in earlier studies that showed that in patients with CRC, the percentage of serum samples with antibodies against p53 is 13–32% [10,41–43]; the percentage with survivin autoantibodies is 8.2% (4/49) [44]; and the percentage with autoantibodies against MUC5AC, a secreted mucin aberrantly expressed by colorectal polyps and carcinomas, is 27.3% (6 of 22) in healthy volunteers, 45% (9 of 20) of patients with polyps, and 60% (18 of 30) of patients with CRC [45].

The potential clinical value of SEC61 β autoantibody may be best discussed in view of the properties of CEA, which is probably the most widely used and the best current single tumor marker for CRC [46,47]. Nevertheless, CEA testing is not recommended for early detection of CRC because of a lack of sensitivity and specificity. On the contrary, in comparison to the control group, SEC61 β autoantibody levels showed a remarkable change from the early stages of cancer, even in CEA-seronegative patients, implying the diagnostic value for early detection of CRC. Using a concentration of 5 ng/ml CEA as the cutoff for detection, CEA seropositivity is 3%, 25%, 45%, and 65% in patients with Dukes' stages A, B, C, and D CRC, respectively [48]. For screening purposes, we believe it will be necessary to demonstrate the expression of serum antibodies against SEC61 β in patients with early-stage CRC and to determine how these responses overlap with the serum CEA biomarker. We found that the seropositivity of SEC61 β autoantibody was 77% in patients with early-stage CRC and that the presence of SEC61 β autoantibody could further distinguish between healthy individuals and 81% (42/52) of patients with CRC with CEA levels <5 ng/ml. The results of the SEC61 β autoantibody for early detection of colorectal cancer revealed a higher sensitivity of 77% than the CEA assay. Detection of anti-SEC61 β response provides a novel serological biomarker for detecting patients with CRC, especially for early stage. The application of multiple biomarkers is generally considered preferable for increasing the diagnostic performance [39,49]. The diagnostic performance of the SEC61 β autoantibody may be improved by using more specific candidate biomarkers in the future. Detection of this disease at an earlier stage by mass screening and subsequent intervention reduces the risk of CRC-associated fatalities [50,51].

In summary, we found that patients with CRC overexpress SEC61 β , which had not been previously identified as an overexpressed protein in CRC. Moreover, we showed that SEC61 β autoantibody is detected in plasma from patients with CRC at significantly higher levels as compared with that from healthy controls, suggesting that SEC61 β autoantibody may represent a new serum marker for CRC, especially for early stage.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.cca.2011.01.012.

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