# Screening and clinical evaluation of dominant peptides of centromere protein F antigen for early diagnosis of hepatocellular carcinoma

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Received June 20, 2017; Accepted November 22, 2017

DOI: 10.3892/mmr.2018.8372

**Abstract.** Tumor-associated antigens, such as centromere protein F (CENP-F), have been recognized as potential serological biomarkers for early diagnosis of hepatocellular carcinoma (HCC); however, the exact regions corresponding to the dominant peptides of CENP-F antigen remain to be explored. We aimed to screen and evaluate potential dominant peptides of CENP-F for early diagnosis of HCC. Dominant peptides of CENP-F were predicted by BioSun version 3.0, and the corresponding recombinant proteins were prepared. Enzyme-linked immunosorbent assays were conducted for initial screening of dominant peptides, and selected dominant peptides were subjected to further clinical evaluation. Eight dominant peptides of CENP-F antigens were predicted at amino acids (a.a) 121-220, 335-416, 1100-1265, 1670-1791, 1759-2093, 2075-2210, 2485-2592, and 2808-2960. Initial screening of the predicted peptides in samples of 47 HCC cases showed the highest diagnostic value for 121-220 a.a and 1670-1791 a.a peptides with area under the curve (AUC) values of 0.795 [95% confidence interval (CI), 0.706-0.884] and 0.809 (95% CI, 0.721-0.896), sensitivity of 58.3 and 85.4%, and specificity of 93.9 and 65.3%, respectively. Further evaluation of the two peptides in 405 samples comprised of 153 HCC, 126 liver cirrhosis and 126 healthy controls, presenting an AUC of 0.743 (95% CI, 0.674-0.812) for 121-220 a.a peptide in detecting early-stage HCCs. Specifically, the 121-220 a.a peptide showed a complementary effect in combination with α-fetoprotein (AFP) for the detection of early-stage HCC with increased AUC value of 0.840 (95% CI, 0.781-0.899), and sensitivity of 81.4% and specificity of 72.2%. In conclusion, our study identified the 121-220 a.a dominant peptide as the region of CENP-F antigen with the highest immunogenicity and demonstrated its value in combination with AFP for diagnosis of early-stage HCC.

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*Key words:* centromere protein F, dominant peptide, autoantibody, hepatocellular carcinoma, early diagnosis

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and the third leading cause of cancer-related death worldwide (1). Most patients with HCC are diagnosed at a later stage of disease, leading to poor prognosis with a 5-year survival rate of less than 16% (2). However, the 5-year survival rate increases to more than 50% for HCC detected at early stages (2). Therefore, searching for biomarkers for detecting early-stage HCC is critical in improvement of the overall prognosis of HCC.

At present, imaging techniques and determination of α-fetoprotein (AFP) levels are widely used as screening tools. Because of the high cost and radiation exposure, or insufficient sensitivity and specificity, imaging techniques have limitations in the screening of early HCC (3,4). AFP is currently the main serum biomarker used in the diagnosis of HCC, with low sensitivity of 46-59% and specificity of 87-93% for detection of early HCC (5). Other biomarkers reported in recent years include lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), des-γ-carboxyprothrombin (DCP), squamous cell carcinoma antigen (SCCA), and golgi protein 73 (GP73) (6-10), with area under the curves (AUCs) of 0.67 to 0.77, sensitivities

of 28.6-70.9%, and specificities of 74.9-92.7% for early-stage HCC (9-13).

In recent years, tumor-associated antigens (TAAs) with promising diagnostic value for tumors at early stages, including HCC, have been identified (14). Among them, serological autoantibody to centromere protein F (CENP-F) has been recognized to have potential value in the detection of early HCC (14,15). CENP-F is a kinetochore protein of 3,210 amino acids that plays a role in centromere formation and kinetochore organization during mitosis (16-21). Using protein microarray to evaluate the diagnostic value of CENP-F autoantibody in a large HCC cohort, our previous study showed that CENP-F antibody had better sensitivity for the detection of early-stage HCC compared with AFP and that combined autoantibody to CENP-F with AFP further improved the diagnostic capability for early HCC (22). However, much less has been known regarding the dominant epitopes of CENP-F antigen, and the exact regions corresponding to the dominant peptides of CENP-F antigen remain to be explored.

In the present study, we aimed to screen and evaluate potential dominant epitope peptides in the full-length CENP-F antigen protein with the aim of obtaining novel CENP-F antigens and improving the early diagnosis of HCC.

## Materials and methods

*Study population*. Screening group: for screening of antigens with the best serodiagnostic performance among eight individual antigens we collected serum samples including 47 cases of HCC (38 men and 9 women aged 44-80 years, with a median age of 57.0 years) and 48 healthy controls (21 men and 27 women aged 20-59 years, with a median age of 46.0 years). Validation group: For validation of the serodiagnostic performance of the selected antigen dominant epitopes we collected another set of 405 serum samples, including 153 cases of HCC with AFP data available (127 men and 26 women aged 0-81 years, with a median age of 57.0 years), of which 70 cases were early-stage HCC (57 men and 13 women aged 28-80 years, with a median age of 56.5 years); 126 cases of liver cirrhosis (95 men and 31 women aged 27-73 years, with a median age of 51.0 years); and 126 healthy controls (65 men and 61 women aged 20-70 years, with a median age of 47.0 years). Clinical characteristics of the samples are shown in Table I.

All samples were obtained from the Cancer Hospital, Chinese Academy of Medical Science, Beijing, China; Beijing Youan Hospital, Capital Medical University, Beijing, China; and Beijing Friendship Hospital, Capital Medical University (Beijing, China) from November 2013 to December 2016.

A diagnosis of HCC was based on the guideline for diagnosis and treatment of primary HCC (2012 version, China). Early-stage HCC was defined as a tumor at TMN stage I. Diagnosis of LC was based on ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) characteristics, laboratory indexes, and histopathology (3). Healthy controls were healthy examiners with normal liver biochemistry, no history of liver disease, and no malignant disease.

All serum samples were stored at -80˚C until testing. The study protocol was approved by the Clinical Research Ethics Committee of Beijing Friendship Hospital, Capital Medical University (Beijing, China).

*Bioinformatics analysis of dominant epitope peptides of CENP-F*. Candidate dominant epitopes of CENP-F protein were predicted using BioSun version 3.0 software developed by the Center of Computational Biology, Beijing Institute of Basic Medical Sciences (Beijing, China). Based on the epitope curve, peptides containing the dominant CENP-F epitopes with highest peak values were selected as the target peptides.

*Construction, expression, and purification of recombinant proteins*. Coding sequences of each dominant antigen peptide of eight single antigens (121-220 a.a, 335-416 a.a, 1100-1265 a.a, 1670-1791 a.a, 1759-2093 a.a, 2075-2210 a.a, 2485-2592 a.a, 2808-2960 a.a) with GST or His tags were chemically synthesized and inserted into the prokaryotic expression plasmid pET-6P with GST or 6-His tags (constructed in house) using specific endonuclease restriction sites *Bam*HI and *Xho*I. The recombinant plasmids were transformed into *Escherichia coli* BL21 or BL21 (DE3) and the fusion proteins were expressed following induction with 0.1 M isopropyl β-D-thiogalactoside at 16˚C for 12 h. The soluble expression of recombinant proteins were purified by affinity chromatography using GST-Sefinose resin or His-Sefinose resin (Sangon Biotech Co., Ltd., Shanghai, China). The purity of fusion proteins was analyzed by sodium alt-polyacrylamide gel electrophoresis (SDS-PAGE) and Gel-Pro Analyzer version 3.1.00.00 (Media Cybernetics, Inc., Silver Spring, MD, USA) and the protein concentration was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA).

*Enzyme-linked immunosorbent assay (ELISA) for screening and evaluation of dominant peptides*. For ELISA, 96-well microplates (Nunc A/S, Roskilde, Denmark) were coated with individual antigens at 5 $\mu$g/ml (100 $\mu$l/well) in coating buffer (0.05 M carbonate/bicarbonate, pH 9.6) and incubated at 4˚C overnight. The plates were washed once with phosphate-buffered saline (PBS) containing 0.05% Tween-20 and blocked by the addition of 200 $\mu$l of 10% newborn bovine serum (Life Technologies, Burlington, ON, Canada) and incubation at 37˚C for 2 h. Next, 100 $\mu$l of standard serum (in-house preparation) diluted 1:4 (1,000 $\mu$g/ml), 1:8 (500 $\mu$g/ml), 1:16 (250 $\mu$g/ml), 1:32 (125 $\mu$g/ml), 1:64 (62.5 $\mu$g/ml), 1:128 (31.25 $\mu$g/ml), 1:256 (15.625 $\mu$g/ml), and 1:512 (7.8125 $\mu$g/ml) in 10% newborn bovine serum or 100 $\mu$l of patient serum diluted 1:11 in PBS containing 10% newborn bovine serum was added to the wells and incubated for 1 h at 37˚C. The plates were washed five times and then 100 $\mu$l of a 1:8,000 dilution of rabbit anti-human IgG-peroxidase antibody (Sigma-Aldrich, St. Louis, MO, USA) was added and incubated for 30 min at 37˚C, followed by addition of 100 $\mu$l TMB HRP-Substrate (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and incubation for 10 min at 37˚C. The reaction was stopped by addition of 50 $\mu$l stop solution (Beijing Solarbio Science & Technology Co., Ltd.) and absorbance was immediately read at 450 nm or 630 nm using a microplate reader SpectraMax M3 (Molecular Devices, LLC, Sunnyvale, CA, USA).

Table I. Study population characteristics.

| Characteristics | HCC (N=153) | LC (N=126) | HC (N=126) |
|---|---|---|---|
| Age (mean ± SD) | 56.8±11.2 | 50.0±9.7 | 46.9±8.5 |
| Sex (n, %) | | | |
| Male | 127 (83.0) | 95 (75.4) | 65 (51.6) |
| Female | 26 (17.0) | 31 (24.6) | 61 (48.4) |
| HBV infection (n, %) | | | |
| HBV (+) | 107 (69.9) | 101 (80.2) | 0 (0.0) |
| HBV (-) | 44 (28.8) | 25 (19.8) | 126 (100.0) |
| Missing | 2 (1.3) | 0 (0.0) | 0 (0.0) |
| HCV infection (n, %) | | | |
| HCV (+) | 13 (8.5) | 0 (0.0) | 0 (0.0) |
| HCV (-) | 138 (90.2) | 126 (100.0) | 126 (100.0) |
| Missing | 2 (1.3) | 0 (0.0) | 0 (0.0) |
| TNM tumor stage (n, %) | | | |
| I | 70 (45.8) | - | - |
| >I | 83 (54.2) | - | - |
| Child-Pugh (n, %) | | | |
| A | 83 (54.2) | 57 (45.2) | - |
| B | 23 (15.0) | 33 (26.2) | - |
| C | 14 (9.2) | 23 (18.3) | - |
| Missing | 33 (21.6) | 13 (10.3) | - |
| AFP | | | |
| ≥20 ng/ml | 81 (52.9) | 22 (17.5) | 0 (0.0) |
| <20 ng/ml | 72 (47.1) | 104 (82.5) | 126 (100.0) |
| AST (U/l) | 54.2 (11.0-659.5) | 35.2 (13.9-393.7) | - |
| ALT (U/l) | 43.3 (8.2-837.4) | 30.6 (8.2-1328.0) | - |
| ALB (g/l) | 35.9 (22.1-63.4) | 36.2 (3.9-49.4) | - |
| TBIL ($\mu$mol/l) | 32.5 (6.9-945.0) | 23.9 (5.1-475.1) | - |
| DBIL ($\mu$mol/l) | 6.3 (0.9-243.0) | 5.6 (1.0-221.7) | - |

Continuous variables are expressed as the mean ± SD or the medians. Count data are described as frequency. HCC, hepatocellular carcinoma; LC, liver cirrhosis; HC, healthy controls; SD, standard deviation; HBV, hepatitis B virus; HCV, hepatitis C virus; TNM, tumor node metastasis; AFP, α-fetoprotein; AST, aspartate transaminase; ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin.

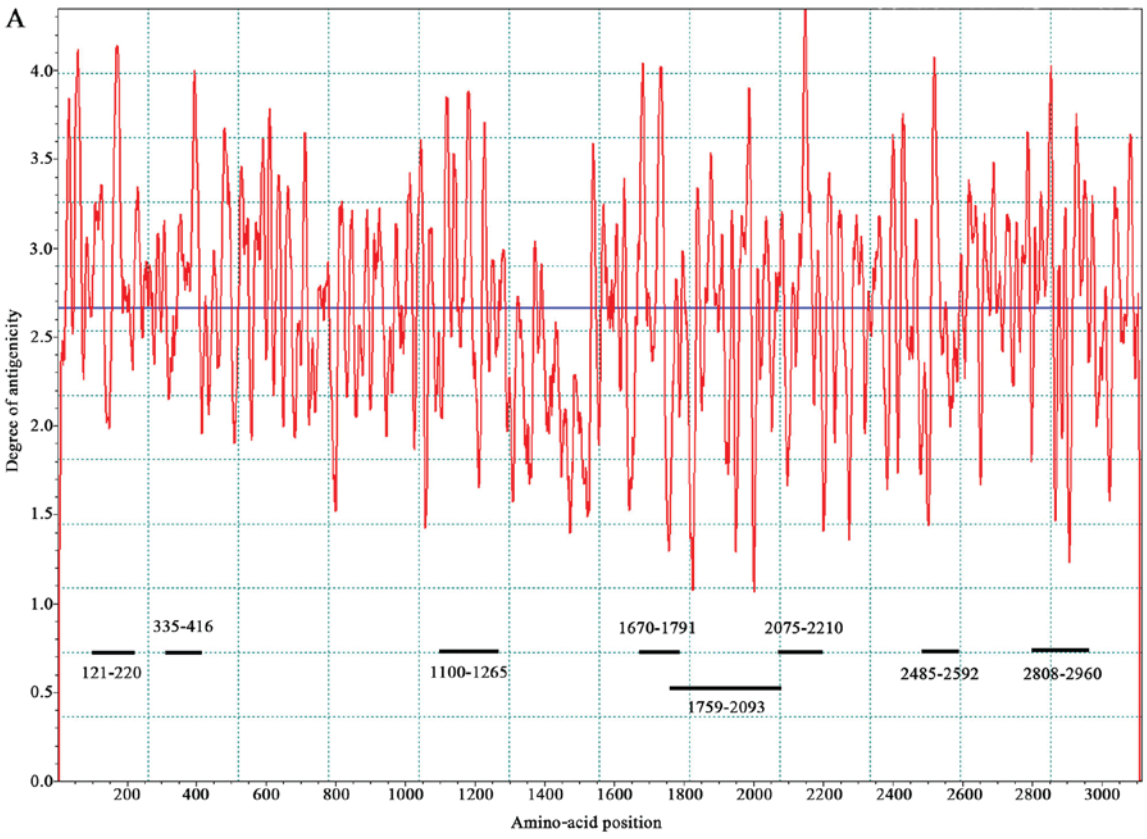
*Statistical analysis*. All statistical analyses were performed using SPSS (version 23.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 6.0c; GraphPad Software, Inc., La Jolla, CA, USA). Receiver operating characteristic (ROC) curves were plotted and the following diagnosis-related indicators, including sensitivity, specificity and AUCs along with 95% confidence intervals [95% confidence intervals (CIs)], were used to evaluate the diagnostic performance of individual biomarkers. The respective optimal cut-off values of individual biomarkers in detecting HCCs were determined by the Youden's index (sensitivity + specificity-1). We used the Chi-square ($\chi^2$) test to evaluate the correlation between the level of auto-antibody to CENP-F and TNM stage of HCC.

In addition, we further evaluated the diagnostic potential of multiple biomarkers using logistic regression models. The predicted probabilities were used to conduct ROC analyses, and diagnosis-related indicators were therefore calculated and reported.

## Results

*Prediction of peptides containing dominant epitopes of CENP-F*. Based on the epitope curve (Fig. 1A), eight peptides containing CENP-F dominant epitopes with higher peak values for each antigen were determined as follows: 121-220 a.a peptide, 335-416 a.a peptide, 1100-1265 a.a peptide, 1670-1791 a.a peptide, 1759-2093 a.a peptide, 2075-2210 a.a peptide, 2485-2592 a.a peptide, and 2808-2960 a.a peptide. The eight peptides covered all functional domains except for the domain responsible for 2x96 AA approximate tandem repeats (Fig. 1B). Recombinant proteins of the eight predicted dominant peptides were prepared, including 1759-2093 a.a peptide with his tag, and other seven peptides with GST tag (Fig. 2).

*Serological reactivity of the eight individual antigen peptides*. We used an indirect ELISA to evaluate the diagnostic




| | Motif | Amino acid region |
|---|---|---|
| | Interaction with SNAP25 and required for localization to the cytoplasm | 1-481 |
| | 2 × 96 AA approximate tandem repeats | 1435-1626 |
| | Interaction with NDE1 and NDEL1 | 2122-2447 |
| | 2 × 177 AA tandem repeats | 2207-2568 |
| | Sufficient for centromere localization | 2488-3113 |
| | Sufficient for self-association | 2488-2925 |
| | Sufficient for nuclear localization | 2927-3113 |

Figure 1. (A) Epitope curves for the eight predicted dominant peptides, and (B) schematic presentation of CENP-F domains and sequence motifs. CENP-F, centromere protein F.

performance of the individual peptides by the analysis of anti-CENP-F level in serum of 47 cases of HCC and 48 healthy control. The AUC value and sensitivities and specificities of the eight individual antigen peptides are shown in Table II. The scatter plots showing the eight antigens of CENP-F between HCC and HC groups are presented in Fig. 3, and the ROC curves of the eight individual CENP-F antigens in discriminating between HCC and healthy controls (HC) are shown in Fig. 4. The sensitivities of the eight individual peptide antigens ranged from 25.50 to 85.40%, and the specificities ranged from 59.20 to 93.30%. Among them, two peptides of CENP-F, 121-220 a.a and 1670-1791 a.a, had the highest diagnostic value for HCC with AUC values of 0.795 and 0.809, respectively. Specificity was better for peptide 121-220 a.a (93.9%) but sensitivity was better for peptide 1670-1791 a.a (Figs. 3 and 4, Table II).

*Validation of the diagnostic performance of the 121-220 a.a and 1670-1791 a.a antigen peptides*. A total of 405 serum samples from HCC, LC, and HC were used to evaluate the

Table II. Diagnostic performance of the eight predicted dominant peptides of CENP-F antigen evaluated by ELISA analysis of samples of screening group.

| Dominant peptides | AUC value | 95% CI | Sensitivity (%) | Specificity (%) | Cut-off value |
|---|---|---|---|---|---|
| 121-220 a.a | 0.795 | 0.706-0.884 | 58.3 | 93.9 | 154 |
| 335-416 a.a | 0.711 | 0.606-0.815 | 54.2 | 87.8 | 225 |
| 1100-1265 a.a | 0.614 | 0.501-0.727 | 60.4 | 63.3 | 250 |
| 1670-1791 a.a | 0.809 | 0.721-0.896 | 85.4 | 65.3 | 458 |
| 1759-2093 a.a | 0.513 | 0.397-0.629 | 25.0 | 85.7 | 793 |
| 2075-2210 a.a | 0.630 | 0.520-0.740 | 22.9 | 98.0 | 441 |
| 2485-2592 a.a | 0.682 | 0.576-0.788 | 68.8 | 59.2 | 276 |
| 2808-2960 a.a | 0.656 | 0.547-0.766 | 72.9 | 59.2 | 163 |

a.a, amino acid; AUC, area under curve; CI, confidence interval; CENP-F, centromere protein F; ELISA, enzyme-linked immunosorbent assay.

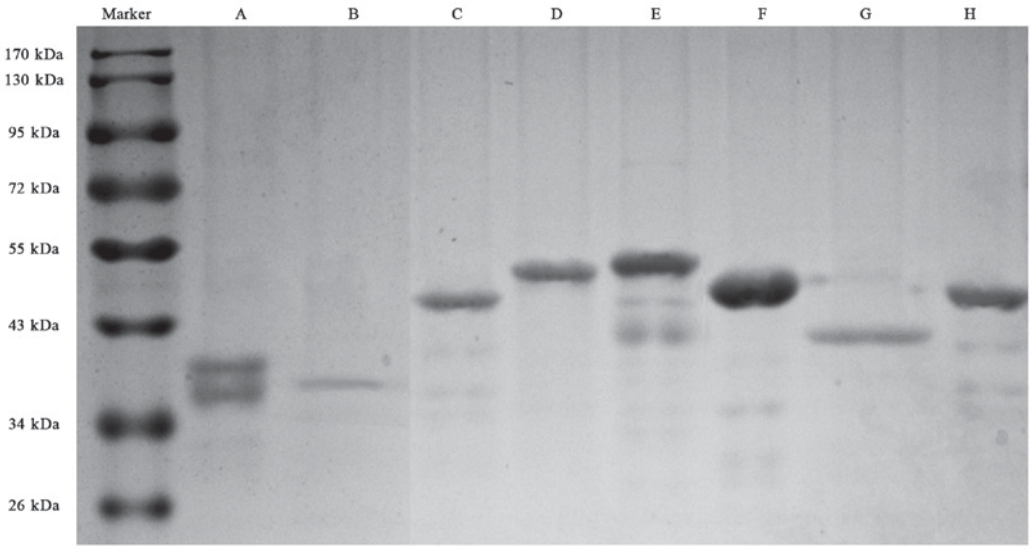



Figure 2. SDS-PAGE electrophoresis for the eight recombinant fragments of CENP-F protein. (A), 121-220 a.a with GST tag, with a degraded band (with CENP-F antigenicity); (B), 335-416 a.a with GST tag; (C), 1759-2093 a.a with his tag; (D), 1100-1265 a.a with GST tag; E, 1670-1791 a.a with GST tag; F, 2075-2210 a.a with GST tag; G, 2485-2592 a.a with GST tag; H, 2808-2960 a.a with GST tag. SDS-PAGE, sodium alt-polyacrylamide gel electrophoresis; CENP-F, centromere protein F; a.a, amino acid.

diagnostic value of the two antigen peptides 121-220 a.a and 1670-1791 a.a, as well as the effect of combined antigen peptide with AFP, for the detection of HCC.

As shown in Table III, the 121-220 a.a peptide gave results consistent with the data obtained in the screening group; the AUC value for the discrimination of HCC from HC was 0.749 (95% CI, 0.692-0.807) with sensitivity of 68% and specificity of 72.2%, and the AUC for discrimination of early HCC from HC was 0.743 (95% CI, 0.674-0.812) with sensitivity of 68.6 and specificity of 72.2%, but there was no significant difference between HCC or early HCC and LC. However, the 1670-1791 a.a peptide showed lower performance compared with the results in the screening group; the AUC value for the discrimination of HCC from HC was 0.628 (95% CI, 0.563-0.693) with sensitivity of 62.1% and specificity of 61.9%, and the AUC for discrimination of early HCC from HC was 0.584 (95% CI, 0.500-0.668) with sensitivity of 72.9% and specificity of 50.0%, with no significant difference between HCC or early HCC and LC.

Specifically, the combination of AFP, for which a serum level greater than 20 ng/ml was defined as positive, and auto-antibody to 121-220 a.a dominant peptide of CENP-F antigen improved the ability to distinguish HCC from the healthy controls, with the AUC (95% CI), sensitivity, specificity of 0.875 (95% CI, 0.835-0.914), 75.2 and 84.9%, respectively, better than AFP solely (Table IV). Meanwhile, improved diagnostic performance for detection of early-stage HCC was also observed for the combination, with AUC of 0.84, higher than AFP (0.72) solely (Table IV).

## Discussion

Autoantibody to CENP-F has been recognized as a potential serological biomarker for the early diagnosis of HCC (22). As CENP-F is a high molecular weight protein of 3210 a.a, the immunogenicity of CENP-F antigen is critical for the sensitivity and specificity of detection of autoantibody to CENP-F. In the present study, we screened the predominant epitopes within

Table III. Diagnostic value of 121-220 a.a and 1670-1791 a.a dominant peptides of CENP-F evaluated by ELISA analysis of samples of validation group.

| Dominant peptides | Cases | AUC value | 95% CI | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| | HCC vs. HC | | | | |
| 121-220 a.a | | 0.749 | 0.692-0.807 | 68.0 | 72.2 |
| 1670-1791 a.a | | 0.628 | 0.563-0.693 | 62.1 | 61.9 |
| | HCC vs. LC | | | | |
| 121-220 a.a | | 0.559 | 0.491-0.627 | 51.0 | 61.9 |
| 1670-1791 a.a | | 0.541 | 0.474-0.609 | 68.0 | 43.7 |
| | Early-stage HCC vs. HC | | | | |
| 121-220 a.a | | 0.743 | 0.674-0.812 | 68.6 | 72.2 |
| 1670-1791 a.a | | 0.584 | 0.500-0.668 | 72.9 | 50.0 |
| | Early-stage HCC vs. LC | | | | |
| 121-220 a.a | | 0.465 | 0.383-0.546 | 92.9 | 15.1 |
| 1670-1791 a.a | | 0.518 | 0.435-0.602 | 75.7 | 38.1 |

a.a, amino acid; CENP-F, centromere protein F; ELISA, enzyme-linked immunosorbent assay; HCC, hepatocellular carcinoma; LC, liver cirrhosis; HC, healthy controls; AUC, area under curve; CI, confidence interval.

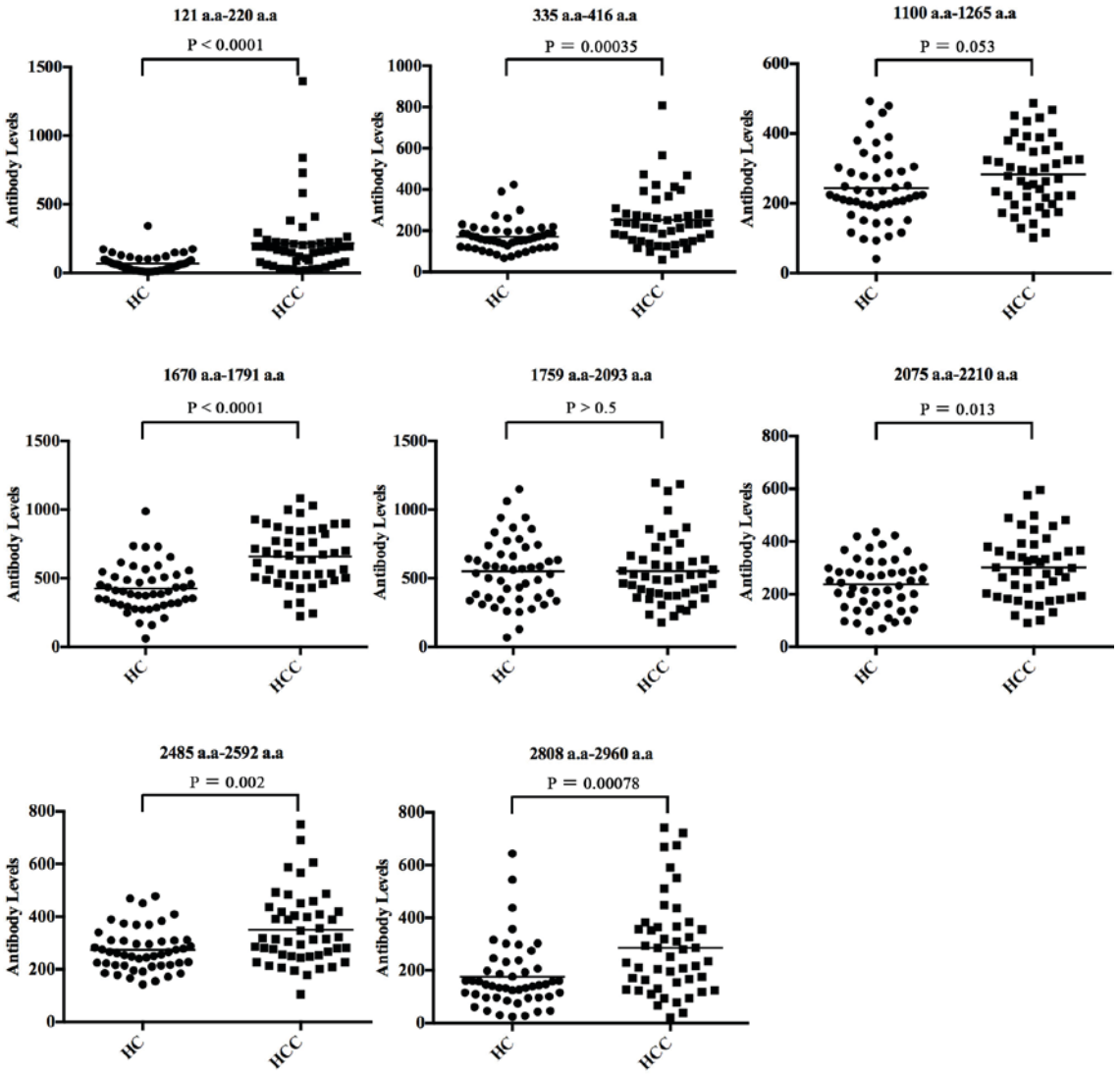



Figure 3. Scatter plot of the eight antigen peptides of CENP-F for discrimination between patients with hepatocellular carcinoma and healthy controls. Black horizontal lines indicate means. a.a, amino acid; CENP-F, centromere protein F.

Table IV. Diagnostic value of the combination of AFP and 121-220 a.a or 1670-1791 a.a dominant peptides of CENP-F antigen.

| Dominant peptides | Cases | AUC value | 95% CI | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| | HCC vs. HC | | | | |
| 121-220 a.a + AFP | | 0.875 | 0.835-0.914 | 75.2 | 84.9 |
| 1670-1791 a.a + AFP | | 0.827 | 0.779-0.876 | 63.4 | 93.7 |
| AFP | | 0.768 | 0.712-0.824 | 53.6 | 100.0 |
| | HCC vs. LC | | | | |
| 121-220 a.a + AFP | | 0.702 | 0.641-0.763 | 53.6 | 82.5 |
| 1670-1791 a.a + AFP | | 0.700 | 0.638-0.761 | 55.6 | 82.5 |
| AFP | | 0.681 | 0.618-0.744 | 53.6 | 82.5 |
| | Early-stage HCC vs. HC | | | | |
| 121-220 a.a + AFP | | 0.840 | 0.781-0.899 | 81.4 | 72.2 |
| 1670-1791 a.a + AFP | | 0.779 | 0.706-0.852 | 51.4 | 93.7 |
| AFP | | 0.721 | 0.639-0.804 | 44.3 | 100.0 |
| | Early-stage HCC vs. LC | | | | |
| 121-220 a.a + AFP | | 0.626 | 0.543-0.709 | 42.9 | 84.9 |
| 1670-1791 a.a + AFP | | 0.639 | 0.557-0.721 | 48.6 | 80.2 |
| AFP | | 0.634 | 0.383-0.546 | 44.3 | 82.5 |

AFP, α-fetoprotein; a.a, amino acid; CENP-f, centromere protein F; AUC, area under curve; CI, confidence interval; HCC, hepatocellular carcinoma; LC, liver cirrhosis; HC, healthy controls.

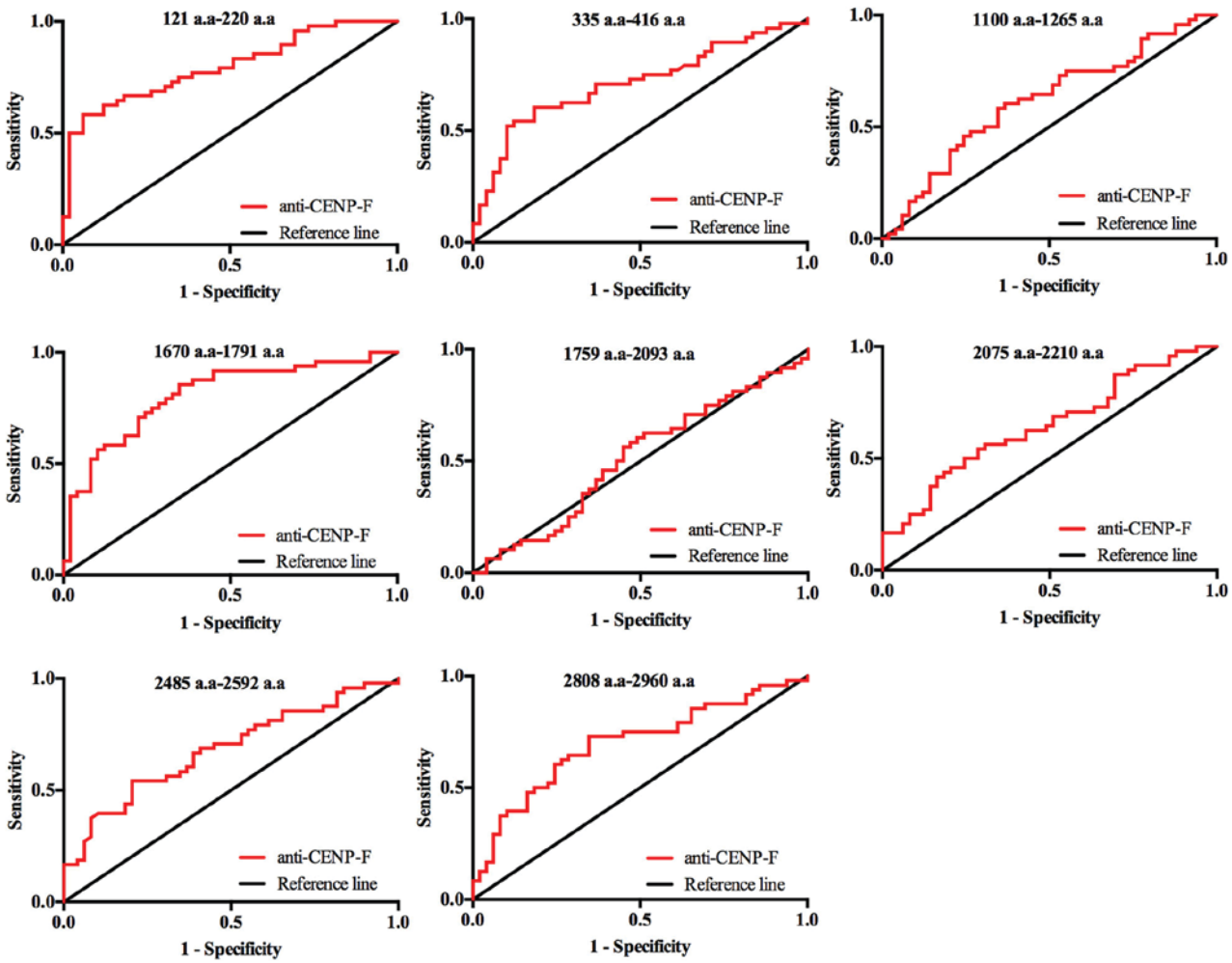



Figure 4. ROC curves of the eight individual CENP-F antigens for discriminating between patients with hepatocellular carcinoma and healthy controls. ROC, receiver operating characteristic; a.a, amino acid; CENP-F, centromere protein F.

the full-length protein by bioinformatics analysis followed by ELISA detection, and selected two peptides (121-220 a.a and 1670-1791 a.a) for further clinical evaluation in a large cohort of HCC cases. Among the eight peptides of CENP-F tested, peptide 121-220 a.a demonstrated the best diagnostic value.

The CENP-F protein contains several motifs including tandem repeats that are sufficient for centromere, cytoplasm, or nuclear localization and for self-association (21) (Fig. 1B). Rattner *et al* (23) reported that the C-terminal end of CENP-F is especially antigenic; however, the exact regions were not determined. In other studies, Welner *et al* (24) evaluated the 1882-2153 a.a peptide of CENP-F antigen by indirect ELISA using overlapping 20-mer peptides of CENP-F spanning the amino acid sequence from 1882 to 2153 and two independent monoclonal antibodies to CENP-F in serum samples and found several peptides with potentially good immunogenicity. They further showed that approximately 50% of patients who were clinically tested for antinuclear antibody (ANA) and expressed antibodies to CENP-F were diagnosed with various kinds of cancer, confirming that such antibodies may function as circulating tumor markers.

In the present study we first predicted antigen epitope peptides and screened dominant epitopes through bioinformatics analysis, identifying eight candidate epitopes of CENP-F peptides. In subsequent evaluation of the serological responses of these eight antigens by indirect ELISA, two of the candidate epitopes showed better diagnostic value for HCC; the 121-220 a.a peptide of CENP-F had good specificity whereas the 1670-1791 a.a peptide had better sensitivity (Table III) suggesting that combined use of both peptides of CENP-F in further studies would enhance both the sensitivity and the specificity. The 121-220 a.a peptide is located in the N-terminal of the CENP-F protein, which is quite different from the antigen reported previously, whereas the 1670-1791 a.a peptide is located close to the 1882-2153 a.a peptide of CENP-F reported in other studies (23,24). Finally, we conducted clinical evaluation in a large cohort of cases to validate the diagnostic value of the two candidate epitopes. Our results confirmed the promising diagnostic value of the 121-220 a.a peptide of CENP-F in the detection of early HCC (Table III); however, the 1670-1791 a.a peptide had lower diagnostic performance in the validation group compared with the screening group suggesting that further study with more cases is essential to understand the diagnostic value of this peptide of CENP-F.

CENP-F has already been reported as a potential biomarker for early-stage HCC (15,22). Through high-throughput microarray analysis in large-scale cohorts of HCC and early-stage HCC cases, our previous study confirmed the diagnostic performance of anti-CENP-F in the detection of early HCC. In the present study, we evaluated the clinical significance of the 121-220 a.a peptide of CENP-F by indirect ELISA in 405 serum samples including patients with early-stage HCC, advanced HCC, and LC. The results showed that anti-CENP-F antibody had promising diagnostic performance in the detection of HCC and, moreover, could complement AFP leading to improved diagnosis of HCC or early HCC. However, the results also revealed the limited value of anti-CENP-F antibody in the discrimination of HCC and LC, consistent with our previous studies (22).

According to the ROC curve, we defined cases with antibody level of more than 125 ng/ml as positive for auto-antibody to CENP-F (121-220 a.a). The results showed that 48 of 70 HCC cases (68.6%) with TNM stage I, and 56 of 83 HCC cases (67.5%) with TNM stage II or III, are positive for auto-antibody to CENP-F (P=0.512), suggesting CENP-F auto-antibody level is not related with the stage of HCC. However, the CENP-F had the highest prevalence of autoantibody positivity in HCC cases with TNM stage I implies that auto-antibody to CENP-F may have value in detection of early HCC.

There are some other limitations in our study. Although two candidate epitope peptides of CENP-F were identified, the exact structure of the antigen and the underlining mechanism remain unknown. In addition, the numbers of early-stage HCC cases were still limited, and further studies with a larger sample sizes would further warrant the findings in our study. Finally, as the pathology analysis of liver biopsy is the golden standard for diagnosis of HCC, but in the present study only a part of HCC cases had pathology results. In our future study, we will use more pathologically confirmed HCC cases to further evaluate the diagnostic value of the CENP-F antibody.

In conclusion, through bioinformatics analysis and clinical evaluation, we identified the 121-220 a.a peptide as the peptide with highest immunogenicity in the CENP-F antigen. It also showed promising diagnostic value in detecting early-stage HCC and could therefore be a complement to AFP in early diagnosis of HCC.

## Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (grant nos. 81071973 and 81602032), and Capital Foundation of Medical Developments (2016-2-2025) and State Key Projects Specialized on Infectious Diseases (2017ZX10201201-007-002).

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