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Clinical significance of plasma anti-TOPO48 autoantibody and blood survivin-expressing circulating cancer cells in patients with early stage endometrial carcinoma

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Abstract

Purpose To examine the clinical significance of an autoantibody (AAb) against a novel tumor-associated antigen (TAA) derived from human DNA-topoisomerase I, termed as TOPO48 AAb, and peripheral blood survivin-expressing circulating cells (CCC) in patients with early stage endometrial cancer (EC).

Methods Blood samples were collected from 80 patients with early stage EC and 80 age-matched healthy subjects. Plasma levels of the TOPO48 AAb were measured with a specific antibody capture enzyme-linked immunosorbent assay (ELISA) and blood survivin-expressing CCC assessed with a reverse transcription-polymerase chain reaction products based on a hybridization-enzyme-linked immunosorbent assay (RT-PCR–ELISA). Sixty patients were followed up for 36 months after the initial assay test.

Results There were 75% and 60% samples with positive levels of the TOPO48 AAb and survivin-expressing CCC in the cancer patients, respectively. However, the cumulative positive rate of combination of the two markers was increased to 93.3% with 0.927 (95% CI 0.871–0.984) of area under the curve (AUC) in receiver operating characteristic (ROC) curve analysis. During the follow-up period, patients with positive TOPO48 AAb but negative surviving-expressing CCC had a higher survival rate and a longer survival time than those with negative AAb but positive CCC ($P=0.01$).

Conclusions The combination of TOPO48 AAb and survivin-expressing CCC may be used as a novel recipe to improve the efficiency of early diagnosis and provide more accurate prognostic prediction in patients with early stage EC.

Keywords Endometrial carcinoma · Early diagnosis · Prognosis · Anti-TOPO48 autoantibody · Survivin-expressing circulating cancer cells

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Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in developed countries. However, the incidence of EC is rapidly increasing worldwide and the age of incidence has become younger than before [1]. In China, it was estimated that there were 63,400 new cases with 21,800 deaths during 2015 [2].

Early stage EC generally has a favorable outcome, but up to 30% of EC patients are diagnosed at advanced stages with a relatively poor prognosis [3]. Thus, increasing early-stage diagnosis rate is an important way of improving outcome of EC patients. Moreover, patients who had undergone potentially curative surgeries retain the risk of recurrence that causes most cancer deaths [3, 4]. Therefore, it is necessary to find sensitive and specific methods for early predicting recurrence to increase overall survival rate in EC patients.

For early diagnosis, circulating autoantibodies (AAbs) against tumor-associated antigens (TAAs) represent one class of potentially useful biomarkers. This is so because increased levels of AAbs are found in very early stage of cancer patients while very low incidences of AAbs occur in healthy individuals [5, 6]. Moreover, growing evidence indicates that humoral immune response in the form of AAbs is present in patients with breast, gastric, colorectal, esophageal and lung cancer before clinical demonstration of disease [7–11].

Recently, we reported a novel TAA with a molecular weight around 48 KD [12]. We identified this novel TAA as a protein fragment derived from human DNA-topoisomerase I (TOPI). We also found that the novel TAA induced a specific AAb that was distinct from SCL-70 AAb, a hallmark of systemic sclerosis (SSC). As such, we termed the TAA as TOPO48 and its autoantibody as TOPO48 AAb. Furthermore, we have demonstrated that the AAb could be used as a novel biomarker for early diagnosis and favorable prognosis in patients with esophageal [13] and lung cancer [14]. Thereby, we speculate that detection of the AAb might be also applied as a potential biomarker for early diagnosis and prognosis in EC although almost no study was conducted on any cancer-relative AAb in EC.

For predicting the recurrence, a number of studies have demonstrated that detection of circulating cancer cells (CCC) could be used as a potential marker to predict the relapse of various types of cancer including EC [15–18]. Previously, we have developed a technique of reverse transcription-polymerase chain reaction products based on a hybridization-enzyme-linked immunosorbent assay (RT-PCR–ELISA) to measure survivin-expressing CCC in various types of cancer such as breast, lung, esophageal, gastric and colorectal cancer [19–22]. In those previous studies, we have demonstrated that survivin-expressing CCC were detectable in about 50% of peripheral blood samples from these patients, but not in the healthy volunteers that were used as controls, thus, could provide valuable information for predicting metastasis and recurrence in these patients. Our results have been verified by other studies in patients with blander, colorectal and gastric cancer [23–25].

Furthermore, survivin, an inhibitor of the apoptosis protein family, has been found to be expressed in tissues during fetal development and in many common human carcinomas such as EC [26]. However, it is rarely found in normal tissues [27]. By detecting survivin expressions in the primary lesion, several studies suggested that survivin might be an ideal tumor marker in the diagnosis and prognosis of EC [26–28]. Therefore, we hypothesized that detection of survivin-expressing CCC might be also used as a biomarker for predicting the recurrence of EC.

In the present study, we first test whether the detection of the TOPO48 AAb and the peripheral blood surviving-expression CCC could be used for early diagnosis and prediction of recurrence of EC, respectively. Second, we examined whether or not combination of the two biomarkers could improve the efficiency of early diagnosis and the accuracy of prognosis of EC. The combination was based upon evidence that there was relative high frequency of the blood survivin-expressing CCC in the EC patients and the TOPO48 AAb showed a favorable outcome while the CCC indicated an unfavorable prognosis in the course of the study.

Materials and methods

Study population and sample collection

A total of 80 patients with EC (aged 32–69 years old; median value: 53 years old) diagnosed and treated between 2007 and 2010 in Sichuan Provincial Peoples Hospital, Sichuan, Chengdu, China, were included in this study. Eighty age-matched healthy volunteers, who were confirmed to be cancer free with clinical and imaging examinations, were used as controls in this study. The protocol has been previously approved by the ethics committee of the hospital (No. 20070126, Chengdu, China), and the subjects were informed and gave their consent for the study.

All tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Out of the 80 EC patients, 60 (75.0%) were classified as being in stage I, and 20 (25%) were considered to be in stage II. The samples were also grouped by histological grade as follows: 43 were Grade I, 27 were Grade II and 10 were Grade 3. Furthermore, 59 patients were diagnosed

Table 1 Patient characteristics ($N = 80$)

Characteristics	N (%)
Age, Median (range) year	53 (32–69)
FIGO stage	
IA	34 (42.5)
IB	26 (32.5)
II	20 (25.0)
Histology	
Endometrioid	59 (73.7)
Non-endometrioid	21 (26.3)
FIGO grade	
1	43 (53.7)
2	27 (33.8)
3	10 (12.5)
Depth of myometrial invasion	
< 0.5	45 (56.2)
≥ 0.5	35 (43.8)

as an endometrioid histological type and 21 classified as a non-endometrioid histological type. The characteristics of the study population are listed in Table 1.

After the initial assay test, only 60 patients who were treated with similar adjuvant therapy regimens were available for follow-up for an average of 27.3 months (range 3–36 months).

A 2 ml sample of peripheral blood from all of the subjects was collected into test tubes containing sodium citrate, which were then sent to the laboratory for immediate processing in which the blood cells were collected for CCC determination and separated plasma were stored at − 80 °C until being analyzed for TOPO48 AAb.

All of the samples were obtained at the time of diagnosis before any curative resection in the patients. None of the patients had any pre-operative chemotherapy or radiotherapy. However, after surgery, the patients received a combination of radiotherapy and chemotherapy or hormone therapy.

TOPO48 AAb ELISA

We have previously described the method for a specific antibody capture ELISA in determination of plasma levels of the TOPO48 AAb [12]. Briefly, we added 1:100 plasma samples in phosphate buffer saline (PBS) at 100 µl per well in the plate that was coated with purified TOPO48 protein (2 µg/ml, 50 µl/well) and incubated the plates for 1 h at room temperature. We then washed the plates five times with washing buffer (0.01 M PBS-0.05% Tween-20, PBST) and further incubated the plate for 1 h at room temperature after the addition of 50 µl per well of 1:5,000 diluted rabbit anti-human IgG labeled with horseradish peroxidase (Sigma-Aldrich, Saint Louis, MO, USA). We again washed the plates five times with PBST, and developed color reaction by adding 100 µl per well of tetramethylbenzidine solution (TMB) (Sigma). Finally, after incubation in the dark for 15 min, we stopped the reaction by adding 50 µl per well of 1 M HCl. We measured the absorbance at 450/630 nm and represented the results as U/ml.

All samples were run in duplicates and randomly distributed on the plates. Serum samples from cancer patients and those from healthy controls were tested simultaneously. The ELISA was performed in a blinded manner. The validation of the ELISA including the specificity, sensitivity and reproducibility pleased to refer our recently publications [13, 14].

Detection of circulating cancer cells by RT-PCR–ELISA

Detailed procedure for measuring survivin mRNA in the peripheral blood cells using the RT-PCR–ELISA technique can be found in our previous reports concerning patients with breast cancer, non-small cell lung cancer, esophageal squamous cell carcinoma, gastric cancer or colorectal cancer [19–22]. The same procedures were utilized here to detect the CCC in patients with EC. The RT-PCR–ELISA technique is briefly summarized in the following.

First of all, we centrifuged the collected peripheral blood samples at 3,000 rpm in room temperature to obtain all blood cells. Then, we extracted the total RNA of whole blood cells with a Trizol™ Kit (Invitogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions and performed the reverse transcription in a thermocycler at 37 °C for 60 min. Second, we amplified the survivin mRNA fragment in the blood cells with 12.5 pM of fluorescein-labelled SUF and SUB primers to generate fluorescein-labelled PCR products [19]. We used a pQE-30UA/survivin plasmid diluted in serial concentrations ranging from 0 to 102.4 fg per tube as standards and amplified together with the samples in the same batch of assay. Third, we added an amount of 2.5 µl of each fluorescein-labelled PCR product in duplicates into probe-coated microtiter plates to make up a total volume of 10µl with 1 × PCR buffer. The fluorescein-labelled PCR product was initially denatured using 20 µl of 1 N NaOH/0.05% thymol blue solution at room temperature for 10 min and then hybridized to the coated probe in a hybridization buffer (0.95 M NaCl, 0.5 M NaH2PO3, 0.1 M sodium citrate, 1% block solution, pH 4.8) for 2 h at 50 °C. Last, after hybridization, we washed the plate four times with PBST, and subsequently added 50 µl per well of 1:1000 anti-fluorescein antibody–HRP conjugates (Roche, Basel, Switzerland) and incubated at room temperature for 1 h. Afterwards, we washed the plate for another four times with PBST and added a 100 µl of TMB. Following a 15-min incubation at room temperature, we stopped the color reactions by adding 50 µl per well of 1 M HCl, and read the

plate at 450/630 nm. Survivin mRNA levels in each of the samples were expressed in pg/ml.

The validation of the method including specificity and sensitivity of the RT-PCR process and the ELISA process as well as cell spiking experiments have been described detail in our previous report on breast cancer research [19]. Furthermore, we have demonstrated that the blood survivin mRNA detected by our method came from the CCC rather than normal blood cells even we did not use any upfront enrichment of peripheral blood leucocytes since, as described above, survivin is prominently expressed during embryonal development, absent in most normal, terminally differentiated tissues but upregulated in a variety of human cancers [19, 27].

Statistical analysis

Statistical analysis was performed using the SPSS software package (Abacus Concepts, Berkeley, CA, USA). Differences in blood cell survivin mRNA expression and plasma TOPO48 AAb between healthy controls and patients with stage I or II cancer were compared with the one-way analysis of variance. The one-way analysis of variance and the Student's *t* test were used to determine the association between survivin mRNA/TOPO48 AAb and various clinicopathological parameters. We used the Pearson chi-square test to compare the detection rates of plasma TOPO48 AAb and blood survivin-expressing CCC alone and combination with the two biomarkers. A receiver operating characteristic (ROC) curve analysis was performed to assess the feasibility of using TOPO48 AAb or survivin-expressing CCC or combination with the two markers for early diagnosis. The Kaplan–Meier method was introduced to estimate the recurrent and survival rates as a function of time with a log-rank test. The Cox proportional hazard model was used to identify prognostic factors for survival. A P value of < 0.05 was considered as statistically significant.

Results

Significance of plasma TOPO48 AAb in early diagnosis of EC

No significant association of TOPO48 AAb with various clinicopathological parameters such as age, FIGO stage, histology, tumor grade, and myometrial invasions was found (Table 2). However, the levels of TOPO48 AAb in the cancer patients were significantly higher than the healthy controls ($P = 0.001$, Fig. 1). When we evaluated the potential of TOPO48 AAb in early diagnosis of EC using ROC curve analysis, the area under the ROC curve (AUC) was found to be 0.828 (95% CI 0.743-0.913) for healthy controls vs. stage I EC (Fig. 1). The specificity and sensitivity of the TOPO48 AAb in diagnosis of stage I EC were 100% and 74.5%, respectively.

Table 2 Relationship between clinicopathological features and plasma TOPO48 AAb levels or blood survivin mRNA concentrations

	N	Anti-TOPO48 autoantibody (U/ml) Mean ± SE	*P*	Blood Survivin mRNA (pg/ml) Mean ± SE	*P*
Age					
< 53	42	1.38 ± 0.06	0.429	1.40 ± 0.11	0.403
> 53	38	1.46 ± 0.08		1.26 ± 0.13	
FIGO					
I	60	1.45 ± 0.04	0.149	1.37 ± 0.12	0.173
II	20	1.41 ± 0.06		1.24 ± 0.18	
Tumor type					
Type I	59	1.41 ± 0.05	0.650	1.36 ± 0.14	0.667
Type II	21	1.45 ± 0.07		1.40 ± 0.22	
Tumor grade					
Grade I	43	1.45 ± 0.06	0.547	1.37 ± 0.14	0.593
Grade II	27	1.42 ± 0.06		1.34 ± 0.17	
Grade III	10	1.38 ± 0.06		1.36 ± 0.15	
Myometrial invasion					
< 0.5	45	1.43 ± 0.05	0.487	1.45 ± 0.11	0.097
≥ 0.5	35	1.40 ± 0.06		1.28 ± 0.17	

Significance of blood cell survivin-expressing CCC in predicting recurrence of EC

Similar to plasma TOPO48 TAA, no significant association of blood cell survivin-expressing CCC with various clinicopathological was found (Table 2) while the levels of the CCC in the cancer patients were significantly higher than the healthy controls ($P = 0.001$, Fig. 2a). During the entire follow-up period, 8 out of the 60 follow-up patients suffered from a relapse. Of the 60 patients, 48 were in disease stage I and 12 were in stage II. Survivin-expressing CCCs were detected in the peripheral blood samples of 36 out of the 60 patients at the time of the initial assay. Regarding the 36 cases with the positive CCC, 7 eventually suffered a relapse within the follow-up period (19.4%). By contrast, for the 24 patients who did not show the presence of the CCC in their peripheral blood samples, only one suffered from a relapse (4.2%). In the entire cohort, the cumulative recurrence rate for patients with positive survivin expressions was significantly higher than those with negative expressions ($P = 0.015$, log-rank test; Fig. 2b).

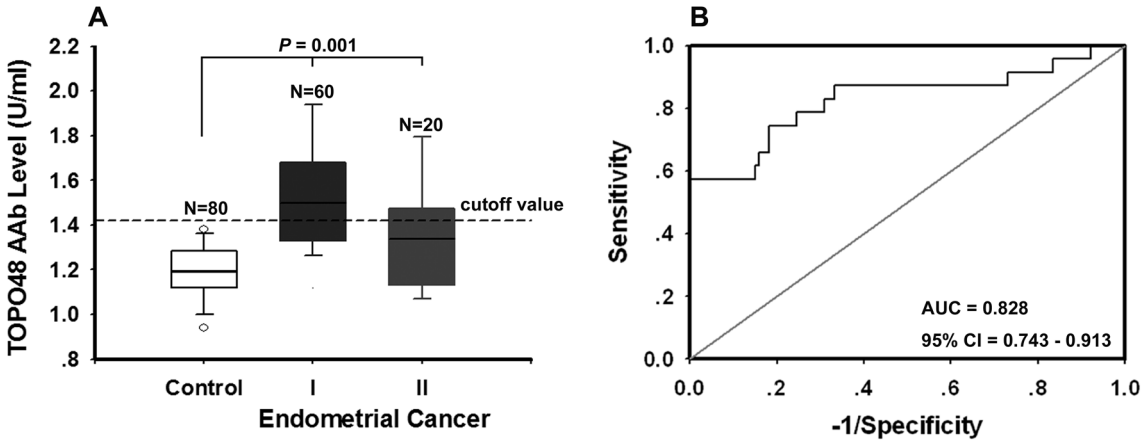

Fig. 1 Significance of plasma TOPO48 AAb in early diagnosis in EC patients. **a** Comparison of TOPO48 AAb among healthy controls ($n=80$), patients with stage I ($n=60$) and stage II ($n=20$) of EC patients. The mean level (range) was 0.88 U/ml (0–1.42 U/ml) in healthy controls, 1.45 U/ml (0.88–1.98 U/ml) in stage I EC and 1.41 U/ml (1.23–1.96 U/ml) in stage II EC. Horizontal line and outliers in each box indicated the median and the percentage of 5th/95th percentile for each group, respectively. A cutoff value of 1.42 U/ml was determined when specificity was set to 100% according to receiver operating characteristic curves (ROC) analysis (please see **b**). **b** ROC analysis for TOPO48 AAb levels for early EC detection (healthy controls vs. stage I EC)

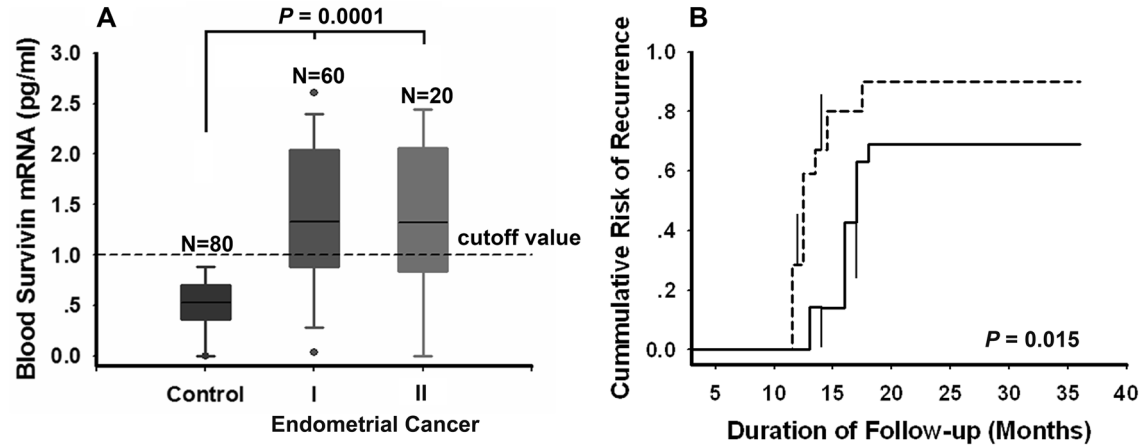

Fig. 2 Significance of peripheral blood cell survivin-expressing CCC in predicting recurrence in early stage patients. **a** Comparison of blood cell survivin mRNA concentrations as determined by RT-PCR–ELISA for healthy controls ($n=80$), stage I ($n=60$) and stage II EC patients ($n=20$). The mean concentrations (range) of survivin mRNA were 0.530 pg/ml (0–0.98 pg/ml) in the healthy controls, 1.37 pg/ml (0.02–2.61 pg/ml) and 1.24 pg/ml (0–2.44 pg/ml) in stage I and II EC, respectively. Horizontal line and outliers in each box indicated the median and the percentage of 5th/95th percentile for each group, respectively. A cutoff value of 0.98 pg/ml was determined when the specificity was set to 100% according to ROC curve analysis (please see Fig. 3c). **b** Kaplan–Meier estimates of the overall 36-months recurrence curves for EC patients with positive (- - - -) and negative (—) detection of survivin-expressing CCC on the entire cohort, $P=0.015$

Significance of combination of the two biomarkers in early diagnosis of EC

As shown in Fig. 3a, 41 samples (68.3%) from stage I patients were found either positive for TOPO48 AAb or survivin mRNA while only 14 cases (23.3%) had both positive and 5 cases (8.3%) were both negative for the two markers. Thus, the cumulative prevalence of TOPO48 AAb and survivin-expressing CCC reached 93.3% in stage I EC patients, which was statistically significant when compared to detection of TOPO48 AAb or survivin-expressing CCC alone ($P=0.001$, Fig. 3b).

Further ROC curve analysis showed that the AUC was 0.927 (95% CI 0.871–0.984) for healthy controls vs. stage I EC when the two markers were combined, that was obviously higher than TOPO48 AAb or survivin-expressing CCC alone (Fig. 3c).

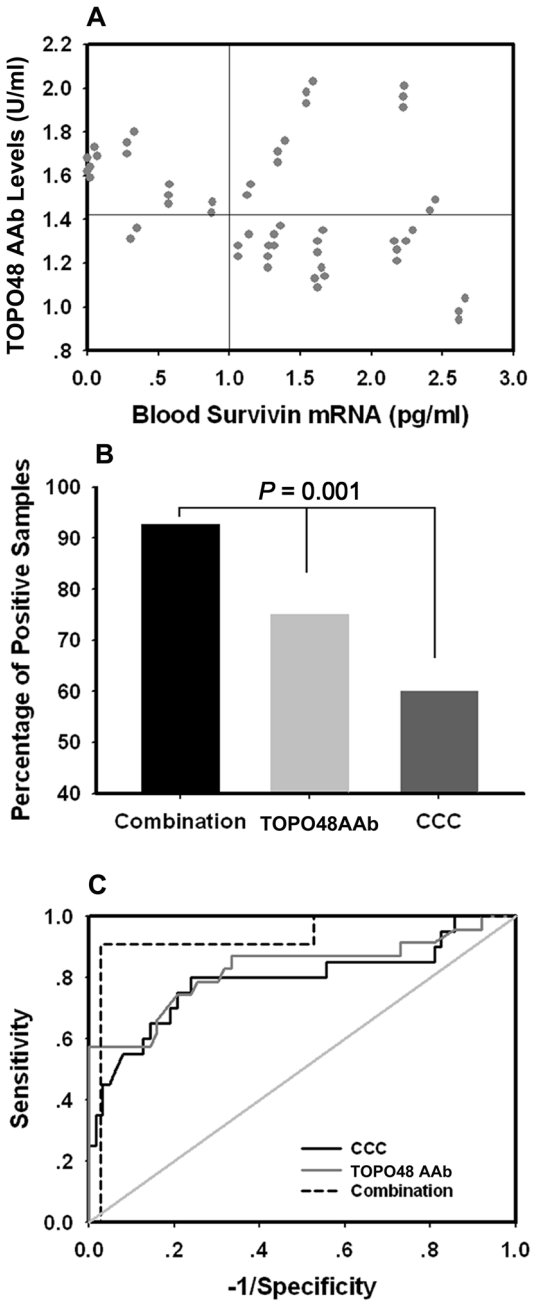

Fig. 3 Significance of combined plasma TOPO48 AAb with blood survivin-expressing CCC in early diagnosis of stage I EC. **a** Simultaneous detection of TOPO48 AAb by ELISA in plasma and survivin-expressing CCC in peripheral blood samples from stage I EC patients ($n=60$). The horizontal and vertical lines indicate the cutoff value for positivity (1.42 U/ml for TOPO48 AAb and 0.98 pg/ml for survivin-expressing CCC). **b** Comparison of positive rate of combination of TOPO48 AAb and survivin-expressing CCC with TOPO48 AAb or survivin-expressing CCC alone at stage I EC. **c** Comparison of ROC curves for early diagnosis of EC: the AUC was 0.927 (95% CI 0.871–0.984) for healthy controls vs. stage I EC when the two markers were combined while the AUC was 0.826 (95% CI 0.743–0.913) and 0.791 (95% CI 0.657–887) for healthy controls vs. stage I EC, respectively, when TOPO48AAb and survivin-expressing CCC were detected alone

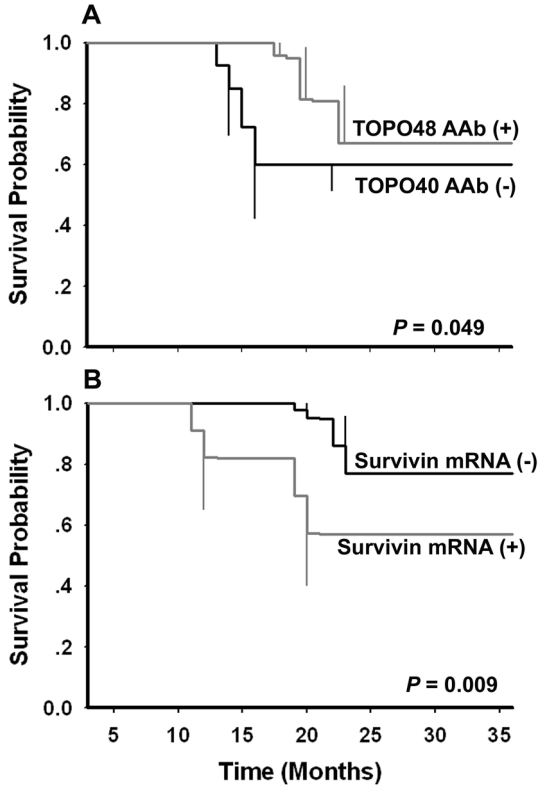

Fig. 4 **a** Kaplan–Meier estimates of the overall 36-month survival curves for EC patients with positive and negative of TOPO48 AAb on the entire cohort. $P=0.049$ was determined by log-rank test. **b** Kaplan–Meier estimates of the overall 36-month survival curves for EC patients with and without detection of survivin-expressing CCC on the entire cohort. $P=0.009$ was determined by log-rank test

Significance of combination of the two biomarkers in prediction of patient survival

Within the follow-up period of 36 months, there were 11 cancer-related deaths in the total follow-up population. The effects of the plasma levels of the TOPO48 AAb and the survivin-expressing CCC on the survival of patients are shown in Fig. 4a, b, respectively. The overall survival rate and mean survival time for patients with negative levels of the TOPO48 AAb were significantly lower and shorter when compared to those patients with positive levels of the TOPO48 AAb ($P=0.049$). By contrast, the overall survival rate and mean survival time for patients with negative survivin-expressing CCC were significantly higher and longer when compared to those patients with positive survivin-expressing CCC ($P=0.009$).

This rose an interesting question what prognosis would be if the two biomarkers were both positive and negative or TOPO48 AAb was negative but survivin-expressing CCC was positive or opposite? Table 3 shows that cases with positive TOPO48 AAb but negative survivin-expressing CCC had the highest survival rate and the longest mean

Table 3 Comparison of survival rate and time among patients with various status of plasma TOPO48 AAb and blood survivin-expressing CCC

	N	Survival rate (%)	Mean for survival time (month) Mean ± SE (95% CI)	*P*
TOPO48 AAb (+), survivin-expressing CCC (−)	22	90.9	32.8 ± 1.7 (29.8 − 35.8)	
TOPO48 AAb (−), survivin-expressing CCC (+)	19	73.6	22.8 ± 2.1 (18.6 + 27.0)	
TOPO48 AAb (+), survivin-expressing CCC (+)	14	78.6	24.7 ± 2.3 (20.1 − 29.3)	
TOPO48 AAb (−), survivin-expressing CCC (−)	5	80.0	28.9 ± 2.4 (24.0 − 33.8)	0.01

survival time, followed by cases with both negative TOPO48 AAb and survivin-expressing CCC and cases with positive TOPO48 AAb but negative survivin-expressing CCC. The cases with negative TOPO48 AAb but positive survivin-expressing CCC had the lowest survival rate and the shortest survival time ($P < 0.01$).

Univariate survival analysis was performed to investigate any possible prognostic impact of the TOPO48 AAb and survivin-expressing CCC on EC patients. As evident in Table 4, the presence of the TOPO48 AAb was correlated with an improvement in the survival probability while the survivin-expressing CCC was correlated with a poor prognosis ($P < 0.05$). These were also confirmed by a multivariate survival analysis that included tumor histology.

Discussion

In the present study, we have first demonstrated that the detection of plasma TOPO48 AAb and blood cell survivin-expressing CCC may be used as potential biomarkers for early diagnosis and recurrent prediction of early stage EC, respectively, as we and other studies found in esophageal, lung, gastric, colorectal, breast and blander cancer [13, 14, 19–25].

One of characteristics of EC is that approximately 72% of cases at stage I could be diagnosed using clinical conventional diagnostic methods [29]. Although positive percentage of TOPO48 AAb samples at stage I EC were found to be 73.3% that was similar to that we observed in esophageal and lung cancer [13, 14], the sensitivity seemed not to be in the great ascendant compared to clinical conventional diagnostic methods for this type of carcinoma. However, it is interesting that 70% samples of the stage I patients were found either positive for TOPO48 AAb or survivin-expressing CCC. Thus, the sensitivity of early diagnosis could be dramatically increased to 93.3% when we combined the AAb with the blood CCC without any change in specificity. So, the present study has established a novel recipe could be applied for early diagnosis of stage I EC that should be better than conventional diagnostic methods.

Previously, we found that the positive rate of blood survivin-expressing CCC was increased from early stages to advanced disease in breast, lung, esophageal, gastric and colorectal cancer [19–22]. Relative high frequency of the blood survivin-expressing CCC found in stage I EC should be noticed. Using immunohistochemistry, Yilmaz and his colleagues reported that there was higher positive rate of survivin expression in stage I EC tissue samples than that in later stages [28]. It may partially explain the relative high positive rate of survivin-expressing CCC in stage I EC in the present study. However, further studies with larger population with the disease are needed to confirm the phenomenon.

The present study also revealed that patients with positive TOPO48 AAb had a favorable prognosis while positive survivin-expressing CCC showed a poor outcome in the stage I EC. The results were similar to our previous findings in breast, esophageal, gastric, colorectal, and lung cancers [13, 14, 19–25].

What's new in the present study? We further analyzed and found that the patients with the positive AAb but the negative CCC had the highest survival rate and the longest survival time while the lowest survival rate and the shortest survival time were discovered in the patients with the

Table 4 Univariate and multivariate analysis: cox proportional hazard model

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (< 53 vs. > 53 years old)	0.97	0.55–1.701	0.909			
Tumor grade (Grade I vs. Grade II vs. Grade III)	1.36	0.81–2.19	0.205			
FIGO stage (I vs. II)	1.58	1.20–2.09	0.197			
Tumor type (endometrioid vs. non-endometrioid)	1.42	1.00–2.01	0.048	1.173	0.75–1.76	0.439
Myometrial invasion (< 0.5 vs. ≥ 0.5)	0.69	0.39–1.23	0.209			
TOPO48 AAb (positive vs. negative)	0.38	0.210–0.70	0.002	0.49	0.23–0.90	0.031
Survivin-expression CCC (positive vs. negative)	3.71	2.02–6.82	0.001	3.05	1.50–6.21	0.002

negative AAb but positive CCC among the four different conditions. As we well know, the prognosis estimation of EC is mainly dependent on FIGO staging [3, 30], and the histological classification of EC also seems to be very useful for prediction of possible prognosis [31]. This study revealed that the combination of plasma TOPO48 AAb and blood survivin-expressing CCC could further distinguish which patients with stage I EC had a favorable prognosis or take more risks for a poor outcome.

However, EC seems to be more heterogeneous since there is a subtype of tumors that share mutual features of endometrioid and serous cancers. Furthermore, a new molecular classification of the tumor based upon studies by The Cancer Genome Atlas Research Network (TGCA) may complement or even replace the classical classification of EC [32]. Therefore, the role of the combination of the two biomarkers for EC prognosis in the molecular classification of EC needs further investigations in the future.

In summary, in the present study we have developed a novel recipe of combination of the TOPO48 AAb and the survivin-expressing CCC to early diagnose the EC and to more accurately estimate the prognosis of these early stage EC patients.

Author contributions XHJ, ZYY and XH: data collection, experimental performs, data analysis, manuscript draft writing; KX and JZ: clinical patients and control support; JBZ, JC and MC: experimental support; SMY, project development, data collection, manuscript writing and review

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving were in accordance with the ethical standards of the institutional research committee of the Sichuan Provincial People's Hospital (No. 20070126,) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Availability of data and materials We have full control of all primary data and that we agree to allow the Journal to review our data if requested.

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