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Study of circulating IgG antibodies to peptide antigens derived from BIRC5 and MYC in cervical cancer

Yangchun Xu [a,1], Yonglong Jin [a,1], Linlin Liu [a], Xuan Zhang [a,*], Yubing Chen [a,*], Jun Wei [b]

[a] The Second Hospital, Jilin University, Changchun 130041, China
[b] Division of Health Research, University of the Highlands & Islands, Centre for Health Science, Inverness IV2 3JH, UK

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ABSTRACT

The present study was undertaken to detect circulating IgG antibodies to peptide antigens derived from baculoviral IAP repeat-containing protein 5 isoform 2 (BIRC5) and myc proto-oncogene protein (MYC) in cervical cancer. A total of 107 female patients with cervical cancer of stages I and II, and 130 healthy female subjects were recruited for analysis of circulating IgG antibodies to BIRC5 and MYC. Student's *t*-test showed significant differences in circulating levels of anti-BIRC5 IgG ($t = -4.27$, df = 235, $P < 0.0001$) and anti-MYC IgG ($t = 3.51$, df = 232, $P = 0.0005$) between the patient group and the control group. Receiver operating characteristic (ROC) analysis showed an area under the ROC curve (AUC) of 0.67 with sensitivity of 23.4% against specificity of 90% for the anti-BIRC5 IgG assay and an AUC of 0.66 with sensitivity of 9.4% against specificity of 90.6% for the anti-MYC IgG assay. Analysis of quality control samples gave an inter-assay deviation of 8.9% in the anti-BIRC5 IgG assay and 9.0% in the anti-MYC IgG assay. This work suggests that anti-BIRC5 IgG could serve as a biomarker for early diagnosis of cervical cancer although a panel of such tumor-associated antigens is needed to develop a highly sensitive test.

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1. Introduction

Cervical cancer is a common malignant condition with cumulative risk of 0.9% in women and the fourth leading cause of cancer death in female subjects worldwide [6]. Etiologically, cervical cancer is a delayed consequence of human papillomavirus (HPV) infection. While HPV DNA testing could reduce the risk of developing cervical cancer, early diagnosis of this type of malignancy is still needed. Circulating autoantibodies have been suggested to serve as potential biomarkers for early diagnosis of cancer [13,17,8,5,11,12]. A successful test has been developed for early diagnosis of lung cancer [9,3,7]. It thus makes it possible to identify a panel of useful tumor-associated antigens (TAAs) for the development of antibody-based test for early diagnosis of cervical cancer. Increased expression of baculoviral IAP repeat-containing protein 5 isoform 2 (BIRC5) and myc proto-oncogene protein (MYC) have been reported in cervical cancer [1,10,20]; to our knowledge, it has not been documented whether antibodies against BIRC5 and MYC proteins are also increased in this malignant disease. Accordingly, the present work was undertaken to detect circulating IgG antibodies for BIRC5 and MYC among patients with cervical cancer and control subjects in a Chinese population.

2. Materials and methods

2.1. Subjects

A total of 107 female patients aged 48.8 ± 9.2 years, who were newly diagnosed as having cervical cancer, were recruited for this study by the Department of Gynecology and Obstetrics, Second Hospital of Jilin University, Changchun, China. Their diagnoses were made based on the Pap smear and histological confirmation and the tumors were staged by the International Federation of Gynecology and Obstetrics (FIGO) staging system. In this study,

Abbreviations: AUC, area under ROC curve; BIRC5, baculoviral IAP repeat-containing protein 5 isoform 2; HPV, human papillomavirus; IgG, immunoglobulin G; MYC, myc proto-oncogene protein; ROC, receiver operating characteristic curve; SBI, specific binding index; SCC, squamous cell carcinoma; TAAs, tumor-associated antigens

* Corresponding authors at: Department of Internal Medicine, Second Hospital of Jilin University, 218 Ziqiang Street, Changchun 130041, China. Tel.: +86 (0)431 88796862 (X. Zhang). Department of Radiotherapy, Second Hospital of Jilin University, 218 Ziqiang Street, Changchun 130041, China. Tel.: +86 (0)13664315885 (Y. Chen).

E-mail addresses: zhangxuankj@163.com (X. Zhang), ybchend@163.com (Y. Chen).

[1] These two authors contributed equally to this project.

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we included the patients with cervical cancer of stages I and II only, and those at stages III and IV were excluded. Pathological examination confirmed that of these 107 patients, 91 had squamous cell carcinoma (SCC) and 16 had adenocarcinoma, adenosquamous carcinoma or small cell carcinoma. Plasma samples were taken prior to any anticancer treatment. One hundred and thirty female subjects aged 50.9 ± 5.4 years, were also recruited as controls from a local community. Clinical interview and the Pap smear were applied to rule out those control subjects who had suffered from cervical cancer and any other malignant diseases. All the subjects were of Chinese Han origin and all gave written informed consent to attend this study as approved by the Ethics Committee of Jilin University Second Hospital.

2.2. Antibody testing

Enzyme-linked immune-sorbent assay (ELISA) was developed in-house using linear peptide antigens derived from human BIRC5 and MYC proteins. The linear peptide antigens were designed according to the computational prediction of HLA-II epitopes [15,19] and their amino acid sequences are given in Table 1; a 28-mer peptide derived from a goat alpha-lactalbumin protein (Accession 1FKV_A) was used as the control antigen (Table 1). All peptide antigens were synthesized by a solid-phase chemical method and dissolved in 67% acetic acid to obtain a concentration of 5 mg/ml as stock solution stored at −20 °C. The working solution was made by diluting the stock solution with phosphate-buffered saline (PBS)-based coating buffer (P4417, Sigma–Aldrich) to 10 μg/ml for each of 2 human peptide antigens (hAg) and to 20 μg/ml for the control antigen. Costar 96-Well Microtiter EIA Plate (ImmunoChemistry Technologies, USA) was half-coated in 0.1 ml/well of each hAg and half-coated in 0.1 ml/well of the control antigen, and then incubated at 4 °C overnight. After the antigen-coated plate was washed at least 3 times with PBS containing 0.05% Tween-20 (PBS-T), 100 μl plasma sample diluted 1:200 in Assay Buffer (DS98200, Life Technologies) was added to the sample wells and 100 μl Assay Buffer was added to the negative control (NC) wells. Following 3 h incubation at room temperature, plates were washed at least 3 times and 200 μl peroxidase-conjugated goat antibody to human IgG (A8667, Sigma–Aldrich) diluted 1:30,000 in Assay Buffer were added to each well. After incubation at room temperature for an hour, color development was initiated by adding 100 μl Stabilized Chromogen (SB02, Life Technologies) and terminated 25 min later by adding 50 μl Stop Solution (SS04, Life Technologies). The measurement of the optical density (OD) was completed within 10 min at 450 nm with a reference wavelength of 620 nm. To reduce the interference from a non-specific signal produced by passive absorption of various IgG antibodies in plasma to the surface of 96-well microplate, a specific binding index (SBI) was used to express the levels of circulating antibodies to BIRC5 and MYC. Each sample was tested in duplicate and SBI was calculated as follows:

$$\mathrm{SBI} = (\mathrm{OD}_{\mathrm{hAg}} - \mathrm{OD}_{\mathrm{NC}})/(\mathrm{OD}_{\mathrm{control\ antigen}} - \mathrm{OD}_{\mathrm{NC}})$$

Table 1
Information of peptide antigens used for development of ELISA antibody test.

Antigen	Sequence (N → C)	NCBI accession	Working solution (μg/ml)
BIRC5	H-dflkdhristfknwlhhfqglfpgatslpv-OH	NP_001012270	10
MYC	H-rvkldsvrvlrqisnnrkcfellptpplsps-OH	NP_002458	10
Control	H-vfqklkdlkdyggvslpewvkiafhtsg-OH	1FKV_A	20

2.3. Data analysis

The experimental data were expressed as mean ± standard deviation (SD) in SBI. IBM SPSS Statistics 21.0 software was used to perform the Student's *t*-test for the differences in SBI between the patient group and the control group, and the receiver operating characteristic (ROC) analysis to work out the area under the ROC curve (AUC) with 95% confidence interval (CI) and the sensitivity of the ELISA antibody test against a specificity of >90%. To minimize intra-assay deviation, the ratio of the difference between duplicate sample OD values to their sum was used to assess the assay accuracy. If the ratio was found to be >10%, the test of this sample was treated as being invalid and would not be used for data analysis. The inter-assay deviation was estimated using pooled plasma samples, namely quality control (QC) samples, which were randomly collected from >200 healthy subjects and tested on every 96-well plate.

3. Results

Student's *t*-test showed significant differences in circulating levels (SBI) of the anti-BIRC5 IgG ($t = -4.27$, df = 235, $P < 0.0001$) and the anti-MYC IgG ($t = 3.51$, df = 232, $P = 0.0005$) between the patient group and the control group; SCC mainly contributed to the significant chance of circulating IgG levels (Table 2). ROC analysis showed that the anti-BIRC5 IgG assay produced an AUC of 0.67 (95%CI 0.60–0.74) with sensitivity of 23.4% against specificity of 90% and the anti-MYC IgG assay produced an AUC of 0.66 (95%CI 0.59–0.73) with sensitivity of 9.4% against specificity of 90.6% (Table 3 and Fig. 1). Analysis of QC samples gave an inter-assay deviation of 8.9% for anti-BIRC5 IgG assay and 9.0% for anti-MYC IgG assay.

4. Discussion

The present work recruited patients with early stage cervical cancer and revealed a significant increase in circulating antibodies to peptide antigens derived from BIRC5 and MYC proteins (Table 2). However, ROC analysis suggests that anti-BIRC5 IgG instead of anti-MYC IgG could serve as a biomarker for early diagnosis of this malignancy as the anti-MYC IgG assay had a low sensitivity (Table 3). Based on the analysis of QC samples, the

Table 2
The levels of IgG antibodies to BIRC5 and MYC in cervical cancer.

Antibody[1]	Patient (*n*)	Control (*n*)	*t*	*P*
BIRC5				
Squamous	1.30 ± 0.22 (91)	1.17 ± 0.20 (130)	4.51	<0.0001
Others	1.22 ± 0.23 (130)	1.17 ± 0.20 (130)	0.95	0.344
Combined	1.29 ± 0.22 (107)	1.17 ± 0.20 (130)	4.27	<0.0001
MYC				
Squamous	1.42 ± 0.28 (90)	1.28 ± 0.27 (128)	3.57	0.0004
Others	1.36 ± 0.23 (16)	1.28 ± 0.27 (128)	1.02	0.310
Combined	1.41 ± 0.28 (106)	1.28 ± 0.27 (128)	3.51	0.0005

[1] Data were expressed as mean ± SD in SBI.

Table 3
ROC analysis of circulating IgG to BIRC5 and MYC in cervical cancer.

Antibody	AUC	95%CI	SE	Sensitivity (%)	Specificity (%)
BIRC5	0.67	0.60–0.74	0.036	23.4	90.0
MYC	0.66	0.59–0.73	0.036	9.4	90.6

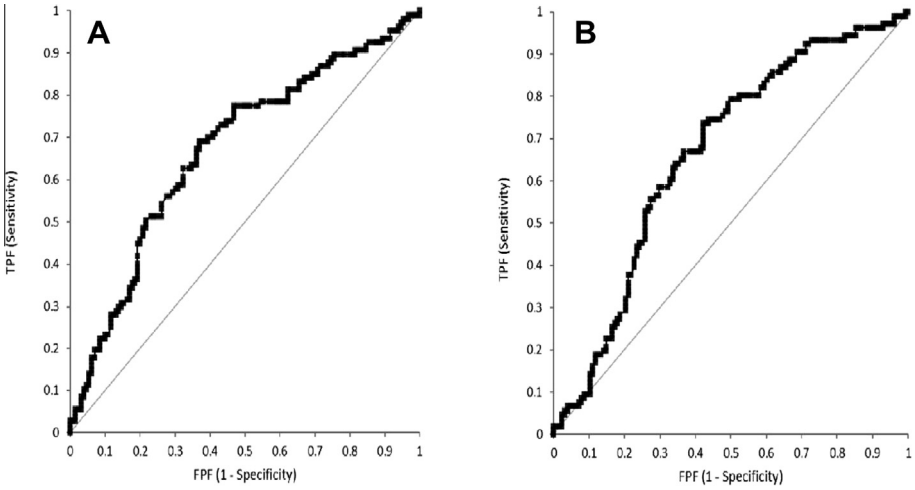

Fig. 1. The area under the ROC curve of circulating IgG autoantibodies to BIRC5 and MYC in patients with cervical cancer. (A) The anti-BIRC5 IgG assay produced an AUC of 0.67 (95%CI 0.60–0.74) with sensitivity of 23.4% against specificity of 90%. (B) The anti-MYC IgG assay produced an AUC of 0.66 (95%CI 0.59–0.73) with sensitivity of 9.4% against specificity of 90.6%.

anti-BIRC5 IgG assay gave an inter-assay deviation of 8.9% and the anti-MYC IgG assay gave that of 9.0%, suggesting that this antibody test had a very good reproducibility.

BIRC5 is the inhibitor of apoptosis (IAP) family member, which plays a crucial role in mitosis and in protecting against apoptotic cell death. The BIRC5-knockout mice showed an extensive overexpression of the proteins related to cellular maintenance, organization and protein synthesis [2]. It is possible that increased circulating anti-BIRC5 IgG may affect apoptosis of many types of cells in the body and facilitate the transformation of normal cells to malignant cells. The mechanism behind an increase in anti-BIRC5 IgG levels is unknown, but it has been proposed that humoral immune responses to TAAs may result from a self-immunization process linked to increased immunogenicity of TAAs during malignant transformation, including antigen mutation [16], overexpression [4], altered antigen folding [14] and aberrant degradation [18]. Further investigation will clarify the complicated mechanism involved in triggering the secretion of anti-BIRC5 IgG antibodies in cervical cancer.

5. Conclusion

Circulating IgG antibodies to the linear peptide antigens derived from the BIRC5 protein could serve as a biomarker for early diagnosis of cervical cancer although a panel of such tumor-associated antigens is needed to develop a highly sensitive test.

Conflict of interest statement

All the authors declared that they had no conflict of interest.

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