Int J Clin Exp Pathol 2017;10(2):2083-2088
www.ijcep.com /ISSN:1936-2625/IJCEP0045345

Original Article

Circulating IgG antibody to ABCC3 in gynecological cancers

Yao Wang[1], Tong Liu[2], Leiguang Ye[2], Hua Zhao[3], Yonglong Jin[4], Bao Liu[2], Baogang Liu[2], Xuan Zhang[4], Jun Wei[5]

[1]Department of Urology, China-Japan Union Hospital, Jilin University, Changchun, China; [2]Third Affiliated Hospital of Harbin Medical University, Harbin, China; [3]School of Basic Medicine, Jilin University, Changchun, China; [4]Second Hospital of Jilin University, Changchun, China; [5]Division of Health Research, University of The Highlands & Islands, Inverness, UK

Received November 29, 2016; Accepted November 30, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Recent work revealed an increase in circulating anti-ABCC3 antibodies in patients with lung cancer and esophageal cancer. To confirm whether the alteration of anti-ABCC3 antibodies occurs in other types of malignancies, the present work was undertaken to test circulating anti-ABCC3 antibodies in patients with gynecological cancers. An in-house enzyme-linked immunosorbent assay (ELISA) was applied to detect plasma anti-ABCC3 IgG among 148 patients with breast cancer (BC), 97 patients with cervical cancer (CC) and 109 control subjects. One-way analysis of variance (ANOVA) showed significant differences in plasma anti-ABCC3 IgG levels among BC patients, CC patients and control subjects ($F=7.75$, df=2, 351, $P=0.0005$). Binary logistic regression showed that anti-ABCC3 IgG levels were significantly lower in BC patients than CC patients (adjusted $P=0.009$) and control subjects (adjusted $P=0.0003$) but there was no significant difference in anti-ABCC3 IgG levels between CC patients and control subjects (adjusted $P=0.286$). Moreover, patients with ductal carcinoma but not those with lobular carcinoma had a decreased anti-ABCC3 IgG level compared with control subjects (adjusted $P=0.0006$); the levels of circulating anti-ABCC3 IgG appeared to decrease with stages of breast cancer. In conclusion, circulating anti-ABCC3 IgG may be a specific biomarker for prognosis of breast cancer although more tests are needed to confirm this initial finding.

Keywords: ABCC3 transporter, autoantibodies, breast cancer, cervical cancer, ELISA, tumor immunity

Introduction

Resistance of tumor cells to chemotherapy has been reported in a large body of publications and different tumor cells may display simultaneous resistance to multiple structurally unrelated antitumor agents, so-called multidrug resistance (MDR). Convincing evidence suggests that the development of MDR is likely to result from the overexpression of ATP-binding cassette (ABC) transporters [1-3]. There are 49 distinct genes identified to encode ABC transporters in humans. These ABC transporters are classified into 7 families (A to G), of which the roles of ABCB1, ABCC1-3 and ABCG2 in developing MDR have been frequently reported in study of therapeutic failure for many types of malignant tumors [1, 2]. These transporters function as an energy-driven pump to maintain intracellular drug concentrations below a toxic level. As a result, the tumor cells survive and chemotherapy becomes ineffective. It has been noted that the overexpression of some ABC transporters by tumors may trigger the secretion of anti-ABC antibodies based on the work on tumor-associated antigens (TAAs) [4]. Recent studies demonstrated that the levels of circulating antibodies to ABCC3 transporter were found to be significantly higher in patients with esophageal cancer and lung cancer than control subjects [5, 6]. To confirm whether circulating antibodies to ABCC3 may be a novel biomarker specific for other types of malignancies, the present work was undertaken to test circulating IgG antibodies to ABCC3 transporter in gynecological cancers, including breast cancer (BC) and cervical cancer (CC).

Subjects and methods

Subjects

A total of 148 female patients aged 50.2±9.1 years, who were newly diagnosed as having

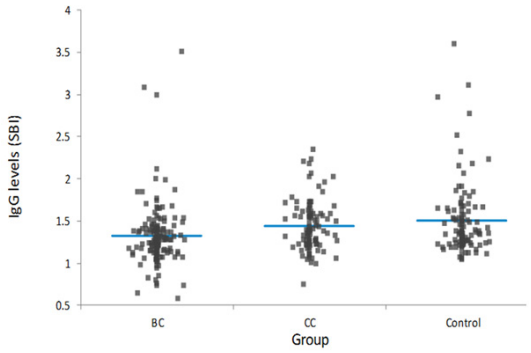

Figure 1. The differences in anti-ABCC3 IgG levels among patients with breast cancer, those with cervical cancer and control subjects. BC: breast cancer and CC: cervical cancer. The ANOVA test showed significant differences in circulating anti-ABCC3 IgG levels among BC patients, CC patients and control subjects (F=7.75, df=2, 298, *P*=0.0005). Binary logistic regression showed that anti-ABCC3 IgG levels were significantly lower in BC patients than CC patients (adjusted R^2=0.026, *P*=0.009) and control subjects (adjusted R^2=0.063, *P*=0.0003) but there was no significant difference in SBI between CC patients and control subjects (adjusted R^2=0.048, *P*=0.286).

breast cancer, were recruited for this study by the Third Affiliated Hospital of Harbin Medical University, Harbin, China. Of these 148 patients, 124 suffered from ductal carcinoma (DC) and 24 from lobular carcinoma (LC). Their diagnosis was made based on radiographic examination and histological confirmation with staging information. Blood samples were taken prior to any anticancer treatment. Ninety seven female patients aged 48.6±9.4 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. This study mainly included the patients at stages I and II, and those at stages III and IV were excluded. Pathological examination confirmed that of these 97 patients with cervical cancer, 84 had squamous cell carcinoma (SCC) and 13 had adenocarcinoma, adenosquamous carcinoma or small cell carcinoma. Plasma samples were taken prior to any anticancer treatment. One hundred nine healthy female subjects aged 52.6±7.8 years, were also recruited as controls from a local community. Clinical interview and radiographic examination were applied to rule out the control subjects who had history of breast cancer, cervical cancer or any other malignant tumors; the Pap smear was applied to rule out the control subjects who had suffered from cervical cancer. All the subjects were of Chinese Han origin and all gave written informed consent to participate in this study. This work was approved by the Ethics Committee of Second Hospital of Jilin University and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Autoantibody testing

Enzyme-linked immune-sorbent assay (ELISA) was developed in-house using two linear peptide antigens as described in previous studies [5-9]. In brief, these 2 linear peptide antigens were synthesized by a solid-phase chemical method. The synthetic peptides were dissolved in 67% acetic acid to obtain a concentration of 5 mg/ml as stock solution; 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 the ABCC3 antigen and to 20 μg/ml for the control antigen. Costar 96-Well Microtiter EIA Plates (ImmunoChemistry Technologies, USA) were half-coated in 0.1 ml/well of the ABCC3 antigen and half-coated in 0.1 ml/well of the control antigen. The antigen-coated 96-well plate was incubated at 4°C overnight. After the plate was washed 3 times with wash buffer made from Tris-buffered saline with Tween®20 (T9039, Sigma-Aldrich), 100 μl plasma sample diluted 1:150 in assay buffer (PBS containing 1.5% BSA) was added and 100 μl assay buffer was also added to the negative control (NC) wells. Following 2 hour incubation at room temperature, the plate was washed 3 times and 100 μl peroxidase-conjugated goat antibody to human IgG (A8667, Sigma-Aldrich) diluted 1:30000 in assay buffer was 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 minutes later by adding 50 μl Stop Solution (SS04, Life Technologies). The measurement of the optical density (OD) was completed within 10 minutes at 450 nm with a reference wavelength of 620 nm. To reduce the interference from a non-spe-

Table 1. The levels of circulating anti-ABCC3 IgG antibodies in breast cancer

Tomour	Patients (n)	Controls (n)	Adjusted R^2	*P**
DC	1.32±0.39 (124)	1.50±0.43 (109)	0.069	0.0006
LC	1.35±0.20 (24)	1.50±0.43 (109)	0.007	0.094
Combined	1.32±0.37 (148)	1.50±0.43 (109)	0.063	0.0003

The antibody levels are expressed as mean ± SD in SBI. *Adjusted *P* for age.

Table 2. The differences in circulating anti-ABCC3 IgG levels between staged cancer patients and controls

Stage	Patients (n)	Controls (n)	Adjusted R^2	*P**
I	1.39±0.43 (31)	1.50±0.43 (109)	0.063	0.217
II	1.28±0.23 (48)	1.50±0.43 (109)	0.067	0.0017
III	1.32±0.44 (61)	1.50±0.43 (109)	0.037	0.0083
IV	1.31±0.14 (8)	1.50±0.43 (109)	-0.001	0.219

The antibody levels are expressed as mean ± SD in SBI. *Adjusted *P* for age.

Table 3. The levels of IgG autoantibodies to ABCC3 transporter in patients with cervical cancer

Type	Patient (n)	Control (n)	Adjusted R^2	*P**
Squamous	1.43±0.30 (84)	1.50±0.43 (109)	0.040	0.218
Others	1.52±0.29 (13)	1.50±0.43 (109)	0.044	0.810
Combined	1.44±0.30 (97)	1.50±0.43 (109)	0.048	0.286

The antibody levels are expressed as mean ± SD in SBI. *Adjusted *P* for age.

cific 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 anti-ABCC3 IgG antibodies. Each sample was tested in duplicate and SBI was calculated as follows:

$$SBI=(OD_{sample}-OD_{NC})/(OD_{control}-OD_{NC})$$

To minimize an 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 >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) sample, which were randomly collected from >100 healthy subjects and tested on every 96-well plate.

Data analysis

All the data were expressed as mean ± standard deviation (SD) in SBI. One-way analysis of variance (ANOVA) was applied to examine the differences in circulating IgG levels between patients with breast cancer, those with cervical cancer and control subjects. Binary logistic regression was applied to examine the differences in SBI between different groups that were classified based on health conditions, types of tumors and clinical stages, with adjustment for age.

Results

ANOVA showed significant differences in plasma anti-ABCC3 IgG levels among patients with breast cancer, those with cervical and control subjects (F=7.75, df=2, 351, *P*=0.0005). As shown in **Figure 1**, binary logistic regression showed that plasma anti-ABCC3 IgG levels were significantly lower in BC patients than CC patients (adjusted R^2=0.026, *P*=0.009) and control subjects (adjusted R^2=0.063, *P*=0.0003) but there was no significant difference in SBI between CC patients and control subjects (adjusted R^2=0.048, *P*=0.286).

As shown in **Table 1**, patients with ductal carcinoma were more likely to have a decreased level of anti-ABCC3 IgG as compared with control subjects (adjusted *P*=0.006) but anti-ABCC3 IgG levels did not show a significant change in patients with lobular carcinoma (adjusted *P*=0.094). Plasma anti-ABCC3 IgG levels appeared to be decreased with stages of breast cancer (**Table 2**), and patients at stage I did not show a significant low level of anti-ABCC3 IgG as compared with control subjects (adjusted *P*=0.217), but a significant low level of this antibody was observed in those at stages II (adjusted *P*=0.0017) and III (adjusted *P*=0.0083). While patients at stage IV also had a lower anti-ABCC3 IgG level than control subjects (**Table 2**), this did not achieve a statistical significance due to a small sample size (adjusted *P*=0.219).

Of 97 patients with cervical cancer, 84 suffered from SCC), neither patients with SCC nor those with other types of CC showed significant

Table 4. The effect of stages in levels of IgG autoantibodies to ABCC3 transporter in cervical cancer

Stage	Patient (n)	Control (n)	Adjusted R^2	*P**
I	1.44±0.28 (38)	1.50±0.43 (109)	0.088	0.579
II	1.42±0.29 (45)	1.50±0.43 (109)	0.023	0.245
Unknown	1.53±0.43 (14)	1.50±0.43 (109)	-0.013	0.814

The antibody levels are expressed as mean ± SD in SBI. *Adjusted *P* for age.

changes in plasma anti-ABCC3 IgG levels as compared with control subjects (**Table 3**). As shown in **Table 4**, there was no significant change in plasma anti-ABCC3 IgG levels among patients at stage I (adjusted *P*=0.579) and those at stage II (adjusted *P*=0.245) as compared with control subjects.

Discussion

Breast cancer is the most common malignancy diagnosed in women worldwide [10]. Based on population-based cancer registration data collected by the National Central Cancer Registry in China, 248620 females were diagnosed as having breast cancer in 2011 [11]. Identification of powerful biomarkers for early diagnosis can help reduce morbidity of this malignancy. Despite several screening modalities have been applied for clinical diagnosis of breast cancer, there is still an urgent need to develop an alternative modality of screening for early diagnosis [12]. Circulating antibodies to TAAs are likely to serve as biomarkers for early diagnosis and prognostic evaluation as they can be detectable several years before identification of tumors by radiographic detection or incidence screening [13-16].

Cervical cancer is the third leading cause of cancer death in females worldwide [10]. While this type of cancer is radiosensitive, it is resistant to chemotherapy such as platinum anticancer drugs [17, 18]. The ABC transporter system may be involved in mediating the efflux of anticancer drugs from cervical cancer cells [17]. However, the present work failed to show a significant change of circulating anti-ABCC3 IgG levels in cervical cancer. Possibly, such antibody is not a specific biomarker for cervical cancer.

ABCC3 is one of the MDR-associated proteins encoded by ABC transporter genes and is highly expressed in several tumor types, including breast cancer [19-21]. A recent study revealed that 28.1% of HER2-positive tumor samples from patients with primary breast cancer overexpressed ABCC3 transporter compared with 7.3% of HER2-negative tumor samples [21], suggesting that ABCC3 may be a novel biomarker for breast cancer. With respect to circulating IgG antibodies to ABCC3, two previous studies made analyses in lung cancer [5] and esophageal cancer [6]; they showed that the levels of circulating antibodies to ABCC3 transporter were significantly higher in patients with these two types of thoracic cancer. However, data from our study showed that the individuals with breast cancer had a significant low level of anti-ABCC3 IgG antibody, especially those with ductal carcinoma (**Table 2**). These results suggest that circulating anti-ABCC3 IgG may be a useful biomarker for prognosis of breast cancer.

Theoretically, the overexpression of ABCC3 in primary breast tumors may result in elevated level of circulating anti-ABCC3 IgG antibody as reported in thoracic cancers. It raises a question of why circulating anti-ABCC3 IgG antibody is decreased in patients with breast cancer. One possible explanation is the antigen-specific immunologic high-dose tolerance. A previous report showed a decrease in immune response to TAAs as the malignancy became disseminate [22]. In addition, it has been reported that insufficiency in immune response is often correlated with poor prognosis [23]. This observation is consistent with our data showing that plasma anti-ABCC3 IgG levels were decreased with stages of breast cancer (**Table 2**). Moreover, it was considered that sex hormones may significantly affect the humoral immune responses to TAAs [24]. Therefore, we speculate that ABCC3-specific immunologic high-dose tolerance may occur in patients with breast cancer.

In conclusion, our study provided evidence that circulating anti-ABCC3 IgG may have prognostic values for breast cancer but not for cervical cancer, although further validation remains needed to confirm this initial finding.

Acknowledgements

We thank the patients and healthy volunteers for their support and participation. This work

was supported by the Young Scientist Research Award funded by the Natural Science Foundation of Heilongjiang Province (Grant number QC2014C092) and by the Research Fund granted by the Health Bureau of Heilongjiang Province (Grant number 2013070). All the subjects gave written informed consent to participate in this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Baogang Liu, Third Affiliated Hospital of Harbin Medical University, Harbin 150040, China. Tel: +86-13904303985; E-mail: liubaoganghum@gmail.com; Dr. Xuan Zhang, Department of Internal Medicine, Second Hospital of Jilin University, 218 Ziqiang Street, Changchun 130041, China. Tel: +86-431 88796862; E-mail: zhangxuankj@163.com

References

[1] Calcagno AM, Ambudkar SV. Molecular mechanisms of drug resistance in single-step and multi-step drug-selected cancer cells. Methods Mol Biol 2010; 596: 77-93.

[2] Gatti L, Cossa G, Beretta GL, Zaffaroni N, Perego P. Novel insights into targeting ATP-binding cassette transporters for antitumor therapy. Curr Med Chem 2011; 18: 4237-49.

[3] Marzac C, Garrido E, Tang R, Fava F, Hirsch P, De Benedictis C, Corre E, Lapusan S, Lallemand JY, Marie JP, Jacquet E, Legrand O. ATP Binding Cassette transporters associated with chemoresistance: transcriptional profiling in extreme cohorts and their prognostic impact in a cohort of 281 acute myeloid leukemia patients. Haematologica 2011; 96: 1293-301.

[4] Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci U S A 1997; 94: 1914-18.

[5] Cheng Y, Xu J, Guo J, Jin Y, Wang X, Zhang Q, Liu L. Circulating autoantibody to ABCC3 may be a potential biomarker for esophageal squamous cell carcinoma. Clin Transl Oncol 2013; 15: 398-402.

[6] Liu L, Liu N, Liu B, Yang Y, Zhang Q, Zhang W, Yu P, Jin Y, Guo J, Guan S, Sun S, Miao L, Wei J. Are circulating autoantibodies to ABCC3 transporter a potential biomarker for lung cancer? J Cancer Res Clin Oncol 2012; 138: 1737-42.

[7] Xu Y, Jin Y, Liu L, Zhang X, Chen Y, Wei J. Study of circulating IgG antibodies to peptide antigens derived from BIRC5 and MYC in cervical cancer. FEBS Open Bio 2015; 5: 198-201.

[8] Ye L, Wang W, Chen C, Meng Q, Yu Y. Study of circulating IgG antibodies to BIRC5 and MYC in non-small cell lung cancer. FEBS Open Bio 2015; 5: 809-12.

[9] Chen C, Wang W, Meng Q, Wu N, Wei J. Further study of circulating IgG antibodies to CD25-derived peptide antigens in non-small cell lung cancer. FEBS Open Bio 2016; 6: 211-5.

[10] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 66: 87-108.

[11] Chen W, Zheng R, Zeng H, Zhang S, He J. Annual reprot on status of cancer in China, 2011. Chin J Cancer 2015; 27: 2-12.

[12] Weigelt B, Geyer FC, Reis-Filho JS. Histological types of breast cancer: how special are they? Mol Oncol 2010; 4: 192-208.

[13] Gagnon A, Kim JH, Schorge JO, Ye B, Liu B, Hasselblatt K, Welch WR, Bandera CA, Mok SC. Use of a combination of approaches to identify and validate relevant tumor-associated antigens and their corresponding autoantibodies in ovarian cancer patients. Clin Cancer Res 2008; 14: 764-71.

[14] Liu W, Peng B, Lu Y, Xu W, Qian W, Zhang JY. Autoantibodies to tumor-associated antigens as biomarkers in cancer immunodiagnosis. Autoimmun Rev 2011; 10: 331-5.

[15] Piura E, Piura B. Autoantibodies to tailor-made panels of tumor-associated antigens in breast carcinoma. J Oncol 2011; 2011: 982425.

[16] Tan HT, Low J, Lim SG, Chung MC. Serum autoantibodies as biomarkers for early cancer detection. FEBS J 2009; 276: 6880-904.

[17] Zhu K, Chen L, Han X, Wang J, Wang J. Short hairpin RNA targeting Twist1 suppresses cell proliferation and improves chemosensitivity to cisplatin in HeLa human cervical cancer cells. Oncol Rep 2012; 27: 1027-34.

[18] Okada T, Murata K, Hirose R, Matsuda C, Komatsu T, Ikekita M, Nakawatari M, Nakayama F, Wakatsuki M, Ohno T, Kato S, Imai T, Imamura T. Upregulated expression of FGF13/FHF2 mediates resistance to platinum drugs in cervical cancer cells. Sci Rep 2013; 3: 2899.

[19] Kool M, van der Linden M, de Haas M, Scheffer GL, de Vree JM, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Elferink RP, Baas F, Borst P. MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc Natl Acad Sci U S A 1999; 96: 6914-19.

[20] O'Brien C, Cavet G, Pandita A, Hu X, Haydu L, Mohan S, Toy K, Rivers CS, Modrusan Z, Amler LC, Lackner MR. Functional genomics identifies ABCC3 as a mediator of taxane resistance in HER2-amplified breast cancer. Cancer Res 2008; 68: 5380-9.

[21] Partanen L, Staaf J, Tanner M, Tuominen VJ, Borg A, Isola J. Amplification and overexpression of the ABCC3 (MRP3) gene in primary breast cancer. Genes Chromosomes Cancer 2012; 51: 832-40.
[22] Pardridge DH, Wang BS, Mannick JA, Deckers PJ. Specificity of immune paralysis at large tumor volumes. Surg Forum 1975; 26: 118-20.
[23] Zavadova E, Vocka M, Spacek J, Konopasek B, Fucikova T, Petruzelka L. Cellular and humoral immunodeficiency in breast cancer patients resistant to hormone therapy. Neoplasma 2014; 61: 90-8.
[24] Weber-Mzell D, Kotanko P, Hauer AC, Goriup U, Haas J, Lanner N, Erwa W, Ahmaida IA, Haitchi-Petnehazy S, Stenzel M, Lanzer G, Deutsch J. Gender, age and seasonal effects on IgA deficiency: a study of 7293 Caucasians. Eur J Clin Invest 2004; 34: 224-8.