Cytokine 85 (2016) 157–164

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Y-box protein-1/p18 as novel serum marker for ovarian cancer diagnosis: A study by the Tumor Bank Ovarian Cancer (TOC)

Irena Rohr [a], Elena I. Braicu [a,*,1], Abdelaziz En-Nia [b], Michaela Heinrich [c], Rolf Richter [a], Radoslav Chekerov [a], Ralf Dechend [d], Harald Heidecke [e], Duska Dragun [f,g], Reinhold Schäfer [h], Xenia Gorny [b], Jonathan A. Lindquist [b], Sabine Brandt [b], Jalid Sehouli [a,*], Peter R. Mertens [b,*]

[a] Gynecological Tumor Center and European Competence Center for Ovarian Cancer, Charité Universitätsmedizin, Berlin, Germany
[b] Clinic of Nephrology and Hypertension, Diabetes and Endocrinology, Otto-von-Guericke University, Magdeburg, Germany
[c] Center for Chronic Sick Children, Charité Universitätsmedizin, Berlin, Germany
[d] Experimental and Clinical Research Center, a joint cooperation between the Max-Delbruck Center for Molecular Medicine and the Charité University Departments of Nephrology and HELIOS-Kliniken Berlin Buch, Berlin, Germany
[e] CellTrend, Luckenwalde, Germany
[f] Department of Nephrology and Intensive Care Medicine, Charité Universitätsmedizin, Berlin, Germany
[g] Center for Cardiovascular Research, Charité Universitätsmedizin, Berlin, Germany
[h] Laboratory of Molecular Tumor Pathology, Charité Universitätsmedizin, Berlin, Germany

ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 16 April 2016
Accepted 16 June 2016

Keywords:
Cold shock proteins
Epithelial ovarian cancer
Serum marker
Chemotherapy

ABSTRACT

Introduction: The cold shock Y-box binding protein-1 (YB-1) fulfills important roles in regulating cell proliferation and differentiation. Overexpression occurs in various tumor cells. Given the existence of extracellular YB-1 we set out to determine the diagnostic, predictive and prognostic role of serum YB-1/p18 for patients with primary epithelial ovarian cancer (EOC).
Methods: The protein fragment YB-1/p18 was quantified by sandwich ELISA in serum samples from 132 healthy female volunteers and 206 patients with histological diagnosis of primary EOC. The ELISA sensitivity and specificity to detect EOC were calculated using receiver operating curves. Survival data were calculated using Kaplan Maier curves.
Results: Median age at the time of diagnosis was 60 years and follow-up ended with a mean of 44.8 month. 188 (91%) patients were diagnosed at advanced stages (FIGO III/IV) and 188 patients (91%) suffered from high-grade serous ovarian carcinoma. YB-1/p18 levels were significantly decreased in older patients ($p = 0.021$). Significantly lower serum levels of YB-1/p18 were detected in the EOC cohort when compared to the control group ($p < 0.0001$, AUC = 0.827; 95% CI, 0.787–0.867). Using the expression of serum YB-1/p18 in early stages I and II cases these could be differentiated from control cases ($p < 0.0001$, AUC = 0.816; 95% CI 0.704–0.929). No other significant associations between clinical prognostic factors and YB-1/p18 serum levels were detected. Immunoblotting results with serum samples suggest that masking of epitopes by the YB-1/p18 fragment in multiprotein-complexes under non reducing conditions leads to the observed reduced ELISA readings in the EOC cohort.
Conclusions: The quantification of fragment YB-1/p18 derived from cold shock protein YB-1 in serum samples could be useful for the early diagnosis of EOC.

Abbreviations: CA125, carbohydrate antigen 125; CI, confidence interval; CSD, cold shock domain; EOC, epithelial ovarian cancer; FIGO, International Federation of Gynecology and Obstetrics; GCIC, Gynecological Cancer Intergroup; HE4, human epididymis protein 4; HGSOC, high-grade serous ovarian carcinoma; kDA, kilodalton; OS, overall survival; PFS, progression free survival; ROC, receiver operator characteristic; ROMA, Risk of Ovarian Malignancy Algorithm; TOC, The Tumor Bank Ovarian Cancer Network; YB-1, Y-box protein-1; YB-1/p18, YB-1 protein fragment p18.

* Corresponding authors at: Gynecological Tumor Center and European Competence Center for Ovarian Cancer, Charité Medical University, Augustenburgerplatz 1, Mittelallee 9, 13353 Berlin, Germany (E.I. Braicu and J. Sehouli). Clinic of Nephrology and Hypertension, Diabetes and Endocrinology, Otto-von-Guericke-University Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany (P.R. Mertens).
E-mail addresses: jalid.sehouli@charite.de (J. Sehouli), peter.mertens@med.ovgu.de (P.R. Mertens).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.cyto.2016.06.021
1043-4666/

1. Introduction

YB-1 is a cold shock protein that fulfills pleiotropic functions in cell homeostasis by regulating cell cycle proteins and proliferation [1]. It acts as an RNA and DNA binding protein with specific functions in gene transcription, mRNA splicing as well as mRNA storage in ribonucleoprotein complexes [2]. YB-1 has biological functions in the cytoplasm and nucleus [3,4]. Within the cancer field YB-1 has attracted considerable attention given its involvement in oncogenic cell transformation, multi-drug resistance, and dissemination of tumor cells [2]. A number of studies have demonstrated that elevated cellular YB-1 levels correlate with an increased risk to develop epithelial neoplasms, such as lung cancer, bladder cancer and colorectal cancer [5–7]. Ample protein-protein interactions have been elucidated, that may explain the pivotal role for cell transformation, such as negative regulation of the tumor suppressor gene TP53 [8]. It is also described that YB-1 activates several signaling molecules of the RAS/Raf/MEK/ERK pathway and transcriptionally activates genes encoding EGFR and ERBB2 cell-surface receptors [8]. Elevated nuclear YB-1 expression is associated with poor survival of patients with breast cancer, high recurrence rates and response to adjuvant chemotherapy [9–12]. *In vitro* studies have shown that increased nuclear levels of YB-1 are associated with cisplatin [13] and paclitaxel resistance in EOC [14]. Recently a fragment of YB-1 with a relative molecular weight of 18 kDa was identified in serum samples of cancer patients from different origins by Western blotting. This polypeptide may serve as an independent biomarker for patients with malignant diseases [15]. Maldi-TOF analysis and application of different antibodies directed against different parts of the protein showed that the 18 kD fragment of YB-1 (YB-1/p18) contained the adjacent C-terminal domains and the cold shock domains (CSD) 1–3 of the full-length protein [15]. Extracellular addition of recombinant YB-1 and domains thereof resulted in a strong mitogenic effect in different tumor cells and increased the migratory capacity [16]. However, the role of YB-1/p18 in ovarian cancer has not been evaluated.

Ovarian cancer is the fifth leading cause of cancer death in women and it is the leading cause of gynecological cancer-related death. Primary treatment of EOC involves surgery and platinum-based chemotherapy [17]. The most important prognostic factors are FIGO stage, residual mass after surgery and response to platinum-based chemotherapy [18,19]. Despite treatments combining aggressive cyto-reductive surgery with platinum and taxane-based chemotherapy, the rates of long-term survival among patients in advanced FIGO stages (III or IV) are only 10–30%, compared with patients in early stages (FIGO-stage I or II), in whom the survival rates are 80–95% [20]. Currently, there exist no reliable screening or early diagnostic tests for EOC. Therefore it is pertinent to establish easy to perform detection systems for early diagnosis of ovarian cancer to reduce ovarian cancer mortality and morbidity. The most encountered subtype of ovarian cancer is the high grade serous ovarian cancer (HGSOC), which is mostly detected in advanced FIGO stages and has a very good response to chemotherapy. However, most patients relapse and subsequently develop resistance and die from this disease. HGSOC is characterized by genetic instability. The only known driver mutations are TP53 (encountered in 96% of cases), and BRCA1 and 2 (encountered in 20–30% of HGSOC patients). Until now the treatment with targeted therapies achieve an improvement in PFS, without translation into a better OS. Therefore HGSOC remains a deadly disease and there is an unmet need for early diagnosis and identification of new targets [21].

In the current study we detected and quantified extracellular YB-1/p18 in serum samples in a well characterized cohort of ovarian cancer patients enrolled within the Tumor Bank Ovarian Cancer (www.TOC-network.de). This allowed us to correlate YB-1/p18 serum levels with clinical outcome and compare them with those expressed in serum samples from healthy donors. We report on the predictive and prognostic role of YB-1/p18 for detection of EOC and for clinical outcome.

2. Patient cohorts and methods

2.1. Study design

206 patients were enrolled in this study, all diagnosed with primary EOC between 2000 and 2011 at the Department of Gynaecology at the Virchow Campus Clinic, Medizinische Universität Berlin. Only patients with histologically confirmed EOC were included. The clinical data was prospectively collected and included in an established online data bank provided by the TOC network (http://www.TOC-network.de) [22]. As a control group, 132 specimens from healthy asymptomatic women were collected at the Universität Göttingen. Ethical approval was obtained from the Ethical Committee Charité Medical University, Berlin (no. 207/2003) and Medical University Göttingen (no. EK22/2/04), written informed consent was provided by the patients before enrolment and serum sample collection.

All patients with primary EOC underwent cytoreductive surgery with the intention of optimal tumor reduction, in terms of no macroscopically detectable residual tumor mass. Follow-up was monitored for all patients. Within follow-up gynaecological examination, systematic ultrasound of the abdomen, CA125 and imaging were performed every three months within the first three years after diagnosis, afterwards twice a year. CA125 elevation alone was not considered as indication of relapse.

2.2. Clinical definitions

Overall survival (OS) is defined as the time from diagnosis until tumor-associated death or last contact. Progression-free survival (PFS) is the time during and after treatment without disease progression. Regarding definitions for treatment response and progression the clinical trials RECIST 1.1 criteria were applied [23]. The status of "platinum-resistance" was classified when the disease recurred within 6 month after platinum-based chemotherapy and "platinum-sensitive" as disease recurrence >6 months [24]. Residual tumor mass after cytoreduction was defined as no macroscopically detectable residual tumor mass, tumor mass <0.5 cm, <1 cm, 1–2 cm and >2 cm, respectively. Tumors were graded as G1 (low grade), G2 (intermediate grade) or G3 (high grade). For staging the FIGO system 1988 was used [25].

2.3. Collection of serum specimens

Serum samples from patients with primary EOC were collected at the Department of Gynaecology at the Virchow Campus Clinic, Medizinische Universität Berlin, before or during surgery. After collection and centrifugation, serum aliquots were stored at −80 °C until further analysis. The samples from 132 healthy female volunteers were collected at the Universität Göttingen, the samples being handled equally and stored at −80 °C until further processing.

2.4. Detection of YB1-P18 by solid-phase immunoassay

YB-1/p18 was quantified with a sandwich ELISA (combination of monoclonal and polyclonal antibodies, CellTrend GmbH Luckenwalde, Germany). The microtiter 96-well polystyrene plates were coated with a polyclonal affinity-purified rabbit anti-YB-1-p18 specific antibody (Eurogentech [36]). To maintain the conformational epitopes of the antigen 1 mM calcium chloride was added