Gynecologic Oncology 119 (2010) 325–331

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Excision repair cross-complementation group 1 protein overexpression as a predictor of poor survival for high-grade serous ovarian adenocarcinoma

Stefanie Scheil-Bertram [a,*,1], Patricia Tylus-Schaaf [a,1], Andreas du Bois [b], Philipp Harter [b], Matthias Oppitz [a], Nina Ewald-Riegler [b], Annette Fisseler-Eckhoff [a]

[a] Institute of Pathology and Cytology, HSK, Dr. Horst Schmidt Klinik, Academic Teaching Hospital of University of Mainz, Wiesbaden, Germany
[b] Department of Gynecology & Gynecological Oncology, HSK, Dr. Horst Schmidt Klinik, Academic Teaching Hospital of University of Mainz, Wiesbaden, Germany

ARTICLE INFO

Article history:
Received 14 April 2010
Available online 21 August 2010

Keywords:
ERCC1
Ovarian carcinoma
Survival

ABSTRACT

Objective. The excision repair cross-complementation group 1 (ERCC1) expression is a predictor of survival after surgical treatment for several malignancies. Its overexpression has been reported as a marker of platinum resistance in lung cancer. However, the relevance of ERCC1 expression in ovarian cancer (OC) is the subject of controversy, both as a predictive parameter for platinum resistance and because of its association with poor prognosis. Therefore, we performed a retrospective study investigating ERCC1 expression and its correlation with patients' survival in OC.

Methods. We analyzed the ERCC1 protein expression using four different ERCC1 antibodies (clone 8F1) with different staining protocols. Immunohistochemistry was performed on multi-tissue microarrays (77 patients with primary serous ovarian cancer treated between 1999 and 2004; median age at diagnosis 67 years; range 32 to 88 years; 90% FIGO III + IV). In all cases cytoreductive surgery was followed by platinum-based chemotherapy.

Results. The Kaplan–Meier analysis revealed that the survival of patients with ERCC1-negative OCs (n = 45; 62%) was significantly better (median survival 50.0 months) compared with the ERCC1-positive group (n = 32; 38%; 20 months; p = 0.004). Furthermore, ERCC1 expression was of prognostic relevance (p = 0.002) in the case of negative expression in patients with residual tumor, where a higher survival rate was observed (median survival 30 months compared to 7.8 months in the ERCC1-positive group).

Conclusions. ERCC1 protein overexpression may act as a prognostic marker for poor survival of high-grade OC even in patients operated with residual disease.

Introduction

The overall 5-year survival rate for ovarian cancer patients is approximately 40% [1]. If the tumor presents with widespread metastatic disease, the 5-year survival rate drops to 10–20% [1]. Cytoreductive surgery plays the most important role in advanced ovarian cancer [2]. In addition to bulky residual disease, the histological subtype, FIGO stage, grading of the tumor and age of the patient at diagnosis are important prognostic markers in ovarian cancer [2–6]. Recently, a retrospective analysis of 3126 patients recruited in prospectively randomized trials and homogeneously treated with platinum and taxanes was published [6]. An interaction between the extent of cytoreduction and biologic tumor prognosticators was described, e.g. histological type and grading [6]. Based on their observations, the authors recommended analysis of potential biological prognosticators in datasets stratified for tumor residuals [6].

DNA repair mechanisms are of great importance to platinum resistance. Nucleotide excision repair (NER) is the pathway that repairs damage to cellular DNA [7,8]. In the early 1990s Dabholkar and co-workers concluded that increased mRNA levels of excision repair cross-complementation group 1 (ERCC1) in clinically platinum-resistant tumors were correlated with platinum-sensitive cases (n = 15) [9]. Many studies on the mRNA-level of ERCC1 expression have since been published [9–17]. During the last decade, monoclonal antibodies specific to ERCC1 have become available and are highly specific for ERCC1 [18–23]. In all of the above-mentioned studies, the clone 8F1 was used on archived formalin-fixed and paraffin-embedded tissue [18–23]. Immunohistochemical studies demonstrated that high levels of ERCC1 are associated with a good prognosis in NSCLC as well as being an important marker of platinum resistance [18,22]. In contrast, some authors suggest ERCC1 immunohistochemistry is not a predictive parameter in ovarian cancer (OC) [21,23]. Today, the ERCC1 expression can be studied independently from its mRNA expression, which may be degraded during the embedding

* Corresponding author. Institute of Pathology and Cytology, Dr. Horst Schmidt Clinic Wiesbaden, Academic Hospital of University of Mainz, Ludwig Erhard Strasse 100, D-65199 Wiesbaden Germany. Fax: +49 611 43 3102.
E-mail address: scheil-bertram@pathologie-wiesbaden.de (S. Scheil-Bertram).
[1] Authors contributed equally to this work.

0090-8258/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2010.07.018

procedure. Furthermore, a single nucleotide polymorphism in codon 118 or C8092A of the ERCC1 gene is thought to affect mRNA levels and shows an association with better overall survival [20,24–26]. An ERCC1 splicing variant may have a transcriptional modulatory function in ovarian cancer [27].

Therefore, we investigated 77 primary serous ovarian carcinomas, using ERCC1 immunohistochemistry. The aim of our retrospective study was to investigate a spectrum of ERCC1 antibodies within stratified cohorts of serous OC classified by amount of residual tumor (microscopic versus macroscopic) and its correlation with patients' survival.

Material and methods

Patients

Seventy-seven consecutive patients, who had been operated between 1999 and 2004 and had been treated with platinum-based chemotherapy (Table 1), were included in our retrospective study of primary ovarian serous adenocarcinoma. Independently of each other, the tumors were histologically graded and classified by three pathologists according to the World Health Organization classification and recommendations given by the Kurman group [28]. The tissue areas for multi-tissue array (TMA) were defined after selecting a representative high-grade tumor area in the corresponding H&E-stained section of each tumor block by the pathologists. All tumors were classified as primary high-grade ovarian serous adenocarcinoma. The median age at surgery was 67 years (range 32 to 88 years). The tumors were classified according to FIGO: 3 IA–C, 6 IIA–C, 51 IIIB–C, and 17 IV. Twenty-eight cases were graded as G2 and 49 cases were graded as G3. While 45 patients had no residual disease, there was residual tumor in 32 patients. Residual disease was defined as macroscopically visible tumor.

Immunohistochemistry

We studied the ERCC1-protein expression using different monoclonal antibodies specific for ERCC1 protein with the same clone 8F1. Analysis of ERCC1 expression was performed as previously described by Olaussen and co-workers [18]. We analyzed a TMA with an area of 1.2 up to 2.4 mm^2 using immunohistochemistry. According to Olaussen and co-workers the endothelial cells of tonsillar gland tissue were used as a positive control, referred to as a staining intensity of grade 2 [18]. In all cases, immunohistochemistry was performed with monoclonal mouse antibodies to ERCC1. Four different antibodies were used: clone 8F1 from ABR, USA (ERCC1-A), the clone 8F1 from Diagnostics BioSystems, USA (ERCC1-B), the clone 8F1 from Dianova, Germany (ERCC1-C), and the clone 8F1 from Neomarkers (ERCC1-D; for details see Table 2). After deparaffinization, the arrays of formalin-fixed tissue sections (3 μm thick) were rehydrated, immersed in preheated target retrieval solution pH9 (Dako, #S2367) and treated with heat for 20 min in a steamer. The slides were stained with the monoclonal anti-ERCC1 antibody (clone 8F1) as described above, using the Dako autostainer. The different detection systems we used are shown in Table 2. In case of antibody B, two different pre-treatment temperatures were used, e.g. 37 °C (see B37 in Table 2) and 55 °C (see B55 in Table 2).

Evaluation of ERCC1 expression

Two investigators who were unaware of the clinical data independently evaluated ERCC1 staining under a light microscope in a high power field (0.62 mm field diameter). Discordant cases were reviewed. This evaluation was decisive for the final score. The samples were scored according to recommendations by Olaussen and co-workers [18]. We modified the scoring system slightly as follows: negative cases were all cases with less than 50% positive nuclei and no or weak staining intensity (<score 2 according to Olaussen et al.). Positive cases were all cases with moderate or high staining intensity in 50% or more of tumor cell nuclei (staining intensity grade 2 and 3 according to Olaussen et al.) [18,22].

Statistical analysis

Survival function estimates were calculated using Kaplan–Meier analysis, differences in the probability of survival were assessed using the log-rank test. Time to effect ("progression-free survival") was calculated as time between surgery and recurrence, or between surgery and last contact. Multivariate Cox's proportional hazard regression analysis was performed to examine the association between residual tumor disease, ERCC1 expression alone, and ERCC1 expression in patients without residual disease, and period of PFS. All statistical tests including cross tables were evaluated with the SPSS statistics 17.0 advanced model module (SPSS, Munich, Germany).

Results

ERCC1 immunohistochemistry

Seventy-seven primary ovarian serous adenocarcinomas were analyzed. All lesions were stained with four different antibodies

Table 1
Clinical characteristics of 77 patients and statistical analysis.

	Kaplan–Meier				Cox regression			
	n	PFS median (month)	p value	CI 95%	p value	Hazard ratio	p value	Hazard ratio
			Log-rank test	Univariate	Univariate	Univariate	Multivariate	Multivariate
Tumor rest	77		<0.0001		<0.0001	3659	0.001	2903
yes	45	56.3		45.904–66.765				
no	32	20.0		12.054–27.891				
Age	77		0.253		0.265	1524	0.157	1662
<60 years	24	43.1		31.060–55.105				
>60 years	53	39.2		28.667–48.432				
FIGO stage	77		0.004		0.071	27,156	0.964	ND
I, II	9	53*		ND				
III, IV	68	16*		ND				
ERCC1	77		0.004		0.006	2353	<0.0001	2532
Negative	48	50.0		39.912–60.158				
Positive	29	20.1		12.631–27.219				

ND, there was a significantly longer survival in the Kaplan–Meier analysis of FIGO stage I and II (n = 9) compared to FIGO stage III and IV (n = 68) cases (p = 0.004), but the groups were too unequal, so that no reliable data on median survival are available.

* Median of progression-free survival (PFS) was calculated manually due to the small number of FIGO stage I and II (9/77).