The prognostic and predictive value of ERCC-1, p53, bcl-2 and bax in epithelial ovarian cancer

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Summary

Aim: To evaluate the expression of ERCC-1 in patients with epithelial ovarian cancer (EOC) and to correlate it with the expression of p53, bcl-2 and bax. *Materials and Methods:* Tumor samples from 60 patients with EOC were immunohistochemically investigated for the expression of ERCC1, p53, bcl-2 and bax. *Results:* ERCC-1 expression was significantly decreased in serous and endometrioid compared to clear cell carcinomas. P53 expression was significantly increased in serous compared to clear cell carcinomas. Bax expression was significantly increased in serous carcinomas as compared to MMTs. High disease stage was correlated with low ERCC-1 and high bcl-2 expression. ERCC-1 expression was associated with increased disease-free interval. *Conclusion:* ERCC-1 status seems to be correlated with disease-free interval, stage and tumor histologic subtype in patients with EOC. Nevertheless, our results indicate that single-gene expressions may be unreliable and thus caution is needed when used as potential prognostic or predictive markers.

Key words: Ovarian cancer, ERCC-1, p53, bcl-2, bax.

Introduction

Epithelial ovarian cancer (EOC) is the most common cause of death among women who develop gynecologic cancer [1].The current management of EOC includes cytoreductive surgery followed by combination chemotherapy for all patients with FIGO Stage ≥ IB [2]. Yet, the majority of women with advanced EOC will develop recurrences and will die of their disease as chemotherapy drug resistance leads to uncontrolled cancer growth [3]. Therefore, the determination of parameters that could identify those patients who would or not benefit from platinum-based chemotherapy could be of clinical significance. Important candidates that could be characterized as biological predictors for response of EOC to chemotherapy include the excision repair cross-complementation group 1 (ERCC1) enzyme and the apoptosis-related proteins p53, bcl-2 and bax.

In recent years, studies on mechanisms of chemotherapy resistance have focused on the identification of molecular markers involved in critical pathways through which the antineoplastic action of the drug is exerted [4]. Unfortunately, some cancer cells are able to circumvent drug action through increased DNA-repair capacity. ERCC1 is a rate limiting DNA repair protein in the nucleotide excision repair (NER) pathway that is specifically in charge of removing DNA platinum compounds [5].

Drug resistance to chemotherapy may also be the result of resistance to and escape from apoptosis, a process modulated by various oncogenes and tumor-suppressor genes, such as the previously mentioned p53, bcl-2 and bax. Deregulation of all three genes represents a crucial step in EOC carcinogenesis [6]. The aim of the present study was to analyze by immunohistochemistry ERCC1 expression in patients with EOC and to correlate the results with the immunohistochemical expression of p53, bcl-2 and bax, as well as with other clinicopathological data (histology, grade, FIGO stage, response to chemotherapy).

Materials and Methods

Patient selection

Sixty patients with EOC who were operated on from 1999 through 2007 at "Iaso" Women's Hospital were included in this analysis. Data extracted from the records included information regarding demographic details, initial stage, grade, and histological type of the carcinoma. Uniform optimal surgical staging and treatment according to FIGO guidelines were performed in all cases.

Immunohistochemistry

Sections 4-μm thick were cut from one representative paraffin block of each case. Antibodies used were ERCC-1 (clone 8F1, 1:200 dilution, Neomarkers, Fremont, CA, USA), p53 (Dako, Denmark, 1:30), bcl2 (Dako, Denmark 1:50) and bax monoclonal (clone B-9, 1:80, Santa Cruz, Biotechnology Inc, Santa Cruz, CA, USA). Immunoreactivity was evaluated by combining the staining intensity and the percentage of positively stained cells. Staining intensity for all four antibodies was scored as follows: 0 = none, 1 = weak, 2 = moderate and 3 = strong. The positively stained cells were expressed as the percentage on the whole tissue section and scored as follows:

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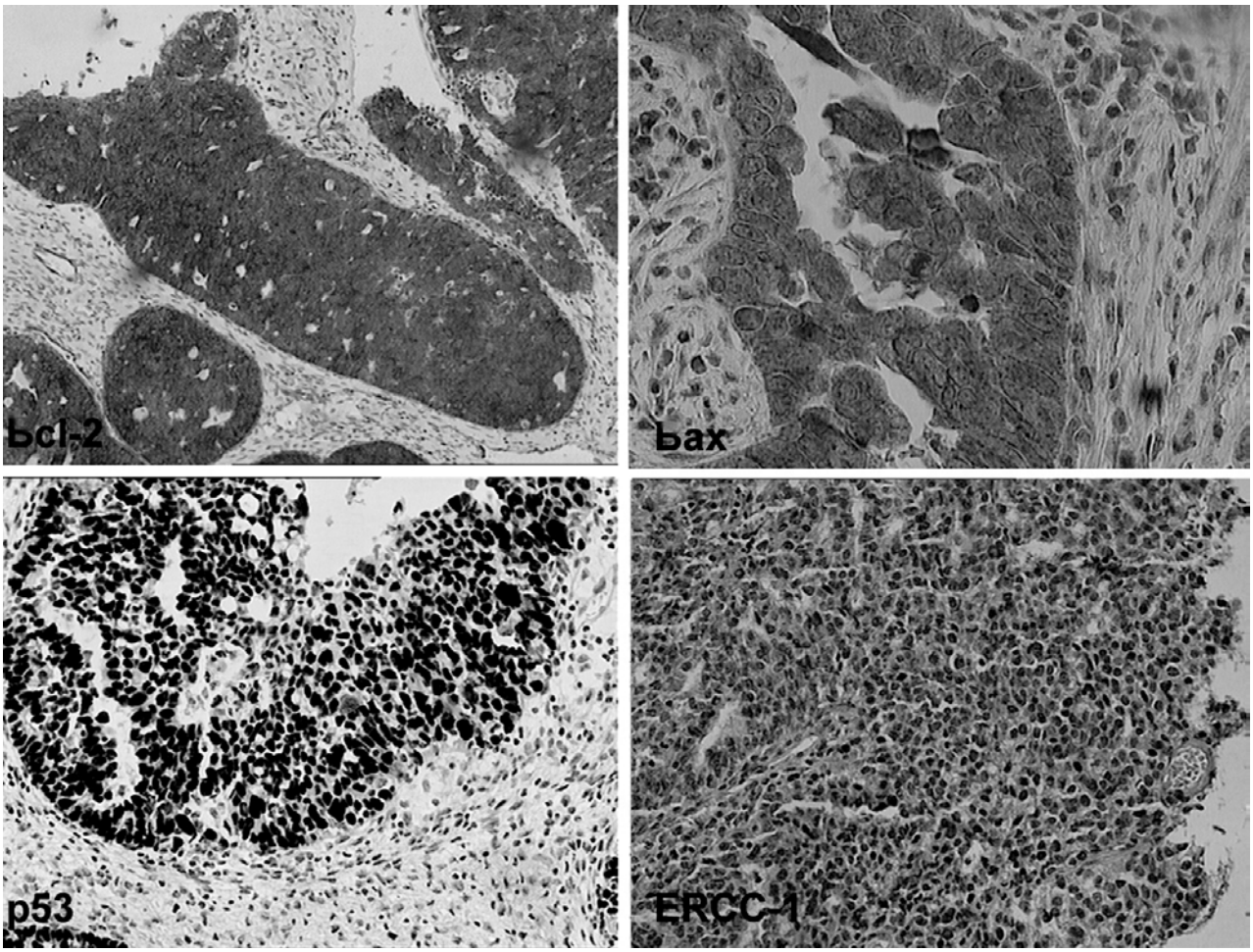

Figure 1. — Immunohistochemistry performed on epithelial ovarian cancer cases. Representative microphotographs indicating strong immunoreactivity for bcl-2, bax, p53 and ERCC-1.

0 = none, 1 = 0-25%, 2 = 26-50%, and 3 = 51-100%. The sum of those two scores was defined as follows: 0 = negative, 2 or 3 = weak, 4 = moderate and 5 or 6 = strong. The staining pattern was nuclear for p53, cytoplasmic for bcl2, membranous and cytoplasmic for bax and nuclear for ERCC1. Cases were grouped as either negative ≤ 3 or positive > 3.

Statistical considerations

Correlation analysis was performed by applying the Spearman test or by evaluating the gamma co-efficient. Differences in immunohistochemical (IHC) scores between histological groups were assessed by the Mann-Whitney test. In the current study, the effect of p53, bcl-2, ERCC-1 and bax expression on disease-free survival (DFS) was analyzed using the Kaplan-Meier method. Differences between groups were evaluated by applying the log-rank test. All analyses were performed using the SPSS v18.0 (SPSS Inc, USA).

Results

The main clinicopathological characteristics of the sample under study and the distribution of the immunohistochemical scores are presented in Table 1. Positive immunohistochemical expression for p53, bcl-2, bax and ERCC-1 is shown in Figure 1.

There was a statistically significant correlation between the immunohistochemical expression of ERCC-1 and bcl-2 and stage of disease. High stage disease was related to decreased ERCC-1 expression and to increased bcl-2 expression. There was no correlation between the expression of p53 and bax and stage of disease or between any of the molecules under investigation and grade of the tumor (Table 2). No significant correlation was found among the molecules of study.

The immunohistochemical expression of ERCC1 was significantly reduced in serous and endometrioid carcinomas when compared to clear cell carcinomas ($p = 0.007$ and $p = 0.031$, respectively). There was a significantly increased p53 expression in serous carcinomas as compared to clear cell carcinoma ($p = 0.032$), and a significantly increased expression of bax in serous carcinomas as compared to MMTs ($p = 0.041$). Further pairwise comparison of the several histological types regarding IHC scoring, revealed no statistically significant differences.

Table 1. — *Clinicopathologic features of the sample under study*.

		n	(%)
Histology			
Serous		25	41.67
Endometrioid		17	28.33
Clear		8	13.33
Mucinous		3	5.00
Mixed epithelial tumor		5	8.33
MMT		2	3.33
Grade			
G1		13	21.67
G2		19	31.66
G3		28	46.66
FIGO stage			
Ia		9	15.00
Ib		3	5.00
Ic		15	25.0
IIa		16	26.67
IIb		2	3.33
IIc		1	1.67
IIIa		7	11.67
IIIb		1	1.67
IIIc		3	5.00
IV	3	5.00	

Immunohistochemistry scores					
	n	(%)		n	(%)
p53			*ERCC-1*		
0	3	5.00	0	16	26.67
1	0	0.00	1	0	0.00
2	2	3.33	2	0	0.00
3	4	6.67	3	10	16.67
4	4	6.67	4	12	20.00
5	21	35.00	5	15	25.00
6	26	43.33	6	7	11.67
	n	(%)		n	(%)
bcl-2			*bax*		
0	27	45.00	0	7	11.67
1	0	0.00	1	0	0.00
2	2	3.33	2	13	21.67
3	4	6.67	3	8	13.33
4	3	5.00	4	21	35.00
5	8	13.33	5	7	11.67
6	16	26.67	6	4	6.67

No statistically significant difference in DFS was found between groups considered negative or positive for p53, for bcl-2 and for bax. On the contrary, irrespective of histological type, ovarian carcinomas considered positive for ERCC-1 had a significantly higher DFS than those considered negative for ERCC-1 (82.89 ± 8.09 vs 42.13 ± 7.27 months respectively, $p = 0.021$) (Figure 2).

Discussion

Epithelial derived ovarian tumors, when malignant, constitute one of the most lethal forms of cancer. These tumors are themselves a heterogeneous group of neoplasms. Of most importance are the specific genetic alterations encountered in each separate morphological subgroup of EOC. As proposed by Shih and Kurman [7], the model of ovarian carcinogenesis encompasses type I tumors, characterized by mutations of BRAF and KRAS for low-grade serous tumors, KRAS mutations for mucinous tumors and β-catenin and PTEN mutations for endometrioid tumors while the only distinct molecular event for the more aggressive type II tumors, high-grade serous carcinomas and undifferentiated carcinomas, is the accumulation of p53 mutations. Nevertheless, one should also consider the crucial role of the responsiveness of each tumor to cisplatin-based chemotherapy. Nucleotide excision repair (NER), has a central role in DNA repair and is associated with resistance to cisplatin-based chemotherapy. Likewise, apoptosis, when inefficient, may be an important cause of chemoresistance.

Table 2. — *Correlations between the IHC score of the molecules under study and Stage/Grade. Significant observations are underlined*.

	IHC Score	Gamma co-efficient	*p* level
Stage	p53	0.080	0.542
	bcl-2	0.348	0.004
	ERCC-1	-0.343	0.004
	bax	-0.025	0.834
Grade	p53	0.103	0.438
	bcl-2	0.050	0.706
	ERCC-1	-0.046	0.709
	bax	-0.171	0.167

In the present study, the immunohistochemical expression of ERCC-1 has been evaluated in patients with epithelial ovarian cancer treated with platinum-based chemotherapy. Since the initial work of Olaussen *et. al.* [8] on the predictive/prognostic role of ERCC1 on the clinical course of patients with small cell lung cancer treated with platinum-based therapy, many authors have tried to investigate its role on other tumors [9].

In the present study, patients with ERCC-1 positive carcinomas, irrespective of histological type, had a significant higher disease-free interval in comparison to those with ERCC-1 negative tumors, the latter being statistically correlated with high-stage disease. Moreover, ERCC-1 positivity was found to be significantly reduced in serous and endometrioid carcinomas in comparison to clear cell carcinomas. It has been emphasized by some investigators that ERCC1 does not only act by removing the platinum adducts from DNA but it is also involved - as a repair mechanism - in the prevention of mutagenesis and cancer development [10]. This complex role may lead to an inconsistent effect of ERCC-1 expression on survival, providing a satisfactory explanation for the contradictory results of our study.

Recently, the existence of ERCC1 exon VIII alternative splicing in ovarian cancer cells has been demonstrated [11]. It was found that its overexpression did not change the protein level of ERCC1 in cancer cells but decreased the excision repair function of ERCC1 and enhanced the sensitivity of cancer cells to cisplatin in a dose-dependent manner. Taking under consideration the above observation, evaluating the protein expression of ERCC-1 might prove to be of limited value. Our results also question the validity of immunohistochemistry in the evalua-

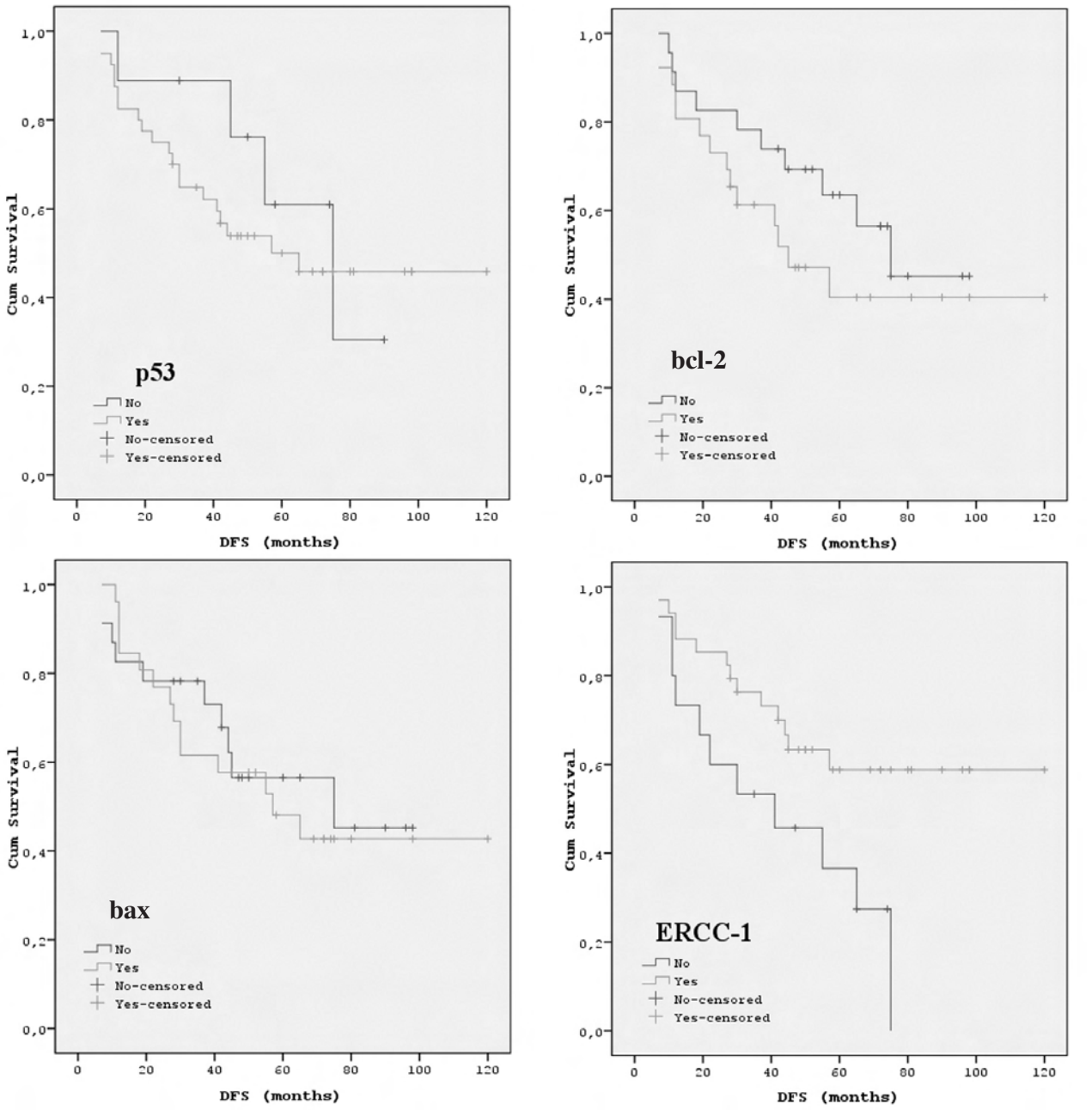

Figure 2. — Kaplan-Meier curves of the disease free survival among EOC cases with positive or negative expression of the molecules of the current study.

tion of the biological behavior of a tumor. The expression of the cell growth regulators p53 and bcl-2 was found to be more inversely related than expected. The crucial point is the relation between a gene's mutation and its protein immunohistochemical expression. In ovarian carcinogenesis, many of the common miss-sense mutations that occur in TP53 are associated with post-translational stabilization and relative overexpression of p53, allowing for its immunohistochemical detection [12]. Most authors speculate over a positive relation between p53 positive tumors, the immunohistochemical expression of bcl-2 and bax, their prognostic significance and their predictive value in patients treated with platinum-based therapy [13, 14]. Our results from this relatively limited series are more in agreement with those reported by Beale *et al*. [15]. Most probably, single-gene expressions are unreliable to be used as potential predictive markers. It appears that the most crucial feature of any ovarian tumor is the existence of genetic alterations that determine its development and its progression. It has been shown that tumor biomarkers have significantly different expression patterns in each tumor subtype and that more than half of those biomarkers lose their predictive value when survival analyses are made subtype specific [16]. Therefore,

despite the fact that - as shown in the present study - platinum-based chemotherapy had different effects in women with ERCC1 (+) and ERCC1 (-) EOC, significantly longer series probably with histological-type matched cell regulator genes should be evaluated to draw more distinct conclusions.

References

[1] Jemal A., Siegel R., Ward E., Hao Y., Xu J., Thun M.J.: "Cancer statistics, 2009". *CA Cancer J. Clin.,* 2009, *59*, 225.

[2] Leitao M.M. Jr., Chi D.S.: "Operative management of primary epithelial ovarian cancer". *Curr. Oncol. Rep.,* 2007, *9*, 478.

[3] Gonzalez-Martin A., Toledo G., Chiva L.: "Epithelial ovarian carcinoma: current evidences and future perspectives in the first-line setting". *Clin. Trans. Oncol.,* 2010, *12,* 418.

[4] Li Q., Yu J.J., Mu C., Yunmbam M.K., Slavsky D., Cross C.L. *et al*.: "Association between the level of ERCC-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells". *Anticancer Res*., 2000, *20,* 645.

[5] Niedernhofer L.J., Odijk H., Budzowska M., van Drunen E., Maas A., Theil A.F. *et al*.: "The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks". *Mol. Cell. Biol*., 2004, *24*, 5776.

[6] Skirnisdottir I., Seidal T., Gerdin E., Sorbe B.: "The prognostic importance of p53, bcl-2, and bax in early stage epithelial ovarian carcinoma treated with adjuvant chemotherapy". *Int. J. Gynecol. Cancer,* 2002, *12*, 265.

[7] Kurman R.J., Shih IeM.: "The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory". *Am. J. Surg. Pathol*., 2010, *34*, 433.

[8] Olaussen K.A., Dunant A., Fouret P., Brambilla E., Andre F., Haddad V. *et al*.: "DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy". *N. Engl. J. Med*., 2006, *355,* 983.

[9] Kim D.Y., Cho M.S., J.Y. C., Lee E., Mun Y.C., Im S. *et al*.: "Significance of ercc1 protein expression in predicting response for advanced gastric cancer patients receiving cisplatin containing chemotherapy". *Proc. Am. Soc. Clin. Oncol.,* 2003, *22* (abstr. 1079).

[10] Kim M.K., Cho K.J., Kwon G.Y., Park S.I., Kim Y.H., Kim J.H. *et al*.: "Patients with ERCC1-negative locally advanced esophageal cancers may benefit from preoperative chemoradiotherapy". *Clin. Cancer Res*., 2008, 14, 4225.

[11] Sun Y., Li T., Ma K., Tian Z., Zhu Y., Chen F., Hu G.: "The impacts of ERCC1 gene exon VIII alternative splicing on cisplatin-resistance in ovarian cancer cells". *Cancer Invest.,* 2009, 27, 891.

[12] Leitao M.M., Soslow R.A., Baergen R.N., Olvera N., Arroyo C., Boyd J.: "Mutation and expression of the TP53 gene in early stage epithelial ovarian carcinoma". *Gynecol. Oncol.,* 2004, *93,* 301.

[13] Geisler J.P., Geisler H.E., Miller G.A., Wiemann M.C., Zhou Z., Crabtree W.: "p53 and bcl-2 in epithelial ovarian carcinoma: their value as prognostic indicators at a median follow-up of 60 months". *Gynecol. Oncol.,* 2000, *77,* 278.

[14] Ziolkowska-Seta I., Madry R., Kraszewska E., Szymanska T., Timorek A., Rembiszewska A., Kupryjanczyk J.: "TP53, BCL-2 and BAX analysis in 199 ovarian cancer patients treated with taxane-platinum regimens". *Gynecol. Oncol*., 2009, *112*, 179.

[15] Beale P.J., Rogers P., Boxall F., Sharp S.Y., Kelland L.R.: "BCL-2 family protein expression and platinum drug resistance in ovarian carcinoma". *Br. J. Cancer*, 2000, 82, 436.

[16] Kobel M., Kalloger S.E., Boyd N., McKinney S., Mehl E., Palmer C. *et al*.: "Ovarian carcinoma subtypes are different diseases: implications for biomarker studies". *PLoS Med.,* 2008, *5*, e232.

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