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Immunohistochemical analyses of sporadic and familial (185delAG carriers) ovarian cancer in Israel

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Abstract

A single germ line mutation in *BRCA1*, (185delAG) is detected in a substantial portion of Jewish Israeli patients with ovarian cancer. Whether disease phenotypes differ in *BRCA1* mutation carriers and sporadic cases is presently a subject for debate. To gain insight into this issue, we analysed tumours from 65 Jewish women with ovarian cancer, 29 (45%) were 185delAG *BRCA1* mutation carriers, and 36 (55%) were non-carriers of any of the predominant Jewish mutations in *BRCA1* or *BRCA2* (sporadic). In 19/29 mutation carriers (66%) diagnosis was made prior to age 60 years, compared with 14/36 (39%) of the non-carriers ($P=0.03$; Yates corrected $P=0.06$). Low malignant potential ('borderline') tumours were detected less frequently among carriers (2/29; 7%) than non-carriers (9/36; 25%) ($P=0.03$; one tail $P=0.05$). Immunohistochemical analysis in invasive carcinoma ($n=54$) showed that 17/27 carriers (63%) and 18/27 non-carriers (67%) had positive nuclear staining with a p53 antibody. In 4/27 carriers (15%) and 3/25 non-carriers (12%), 25% or more of the tumour cells stained positive for Ki-67, an insignificant difference. Results were not altered by including borderline tumours ($n=11$) in these analyses. We conclude that the rate of *TP53* inactivation and proliferative index in ovarian cancer, are similar for 185delAG *BRCA1* mutation carriers and sporadic cases.

Keywords: Ovarian cancer; *BRCA1*; *BRCA2*; p53; Prognostic factors; Inherited predisposition

1. Introduction

Ovarian cancer is the fourth leading cancer-related cause of death in women in Western society [1,2]. In 1994 in Israel, 357 Jewish women developed the disease [3], and only 35–40% of them are predicted to be alive 5 years after diagnosis, based on data from industrialised countries [4]. The genetic factors that modify disease outcome and influence therapeutic response are not clear, and remain an intense area of research. The inherited form of ovarian cancer occurs in approximately 10% of incidence cases, and germ line mutations within *BRCA1*, apparently account for the majority of these familial cases [5,6]. Uniquely in Israel, up to one third of unselected Jewish ovarian cancer patients harbour a single germ line mutation in *BRCA1* (185delAG), irrespective of their ethnic origin, family history of cancer or age at diagnosis [7,8]. The same mutation occurs in approximately 1% of the general Ashkenazi population [9] and also in non-Ashkenazi women [10,11]. An unresolved issue with conflicting data is whether disease outcome and/or therapeutic response in sporadic ovarian cancer differ from the

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inherited form of the disease. Some studies show a more favourable prognosis [12,13] while others have found that a germ line mutation is associated with an equal or less favourable outcome [14]. Germ line mutations in *BRCA1* are also associated with inherited breast cancer [6], so legitimate analogies may be drawn from similar studies on patients with inherited and sporadic forms of breast cancer. Analyses of histopathological features and proliferative indices in breast cancer, showed that *BRCA1* mutation carriers differ from non-carriers. Tumours tend to be more often of the ductal type, have a more pleomorphic nuclei, be non-diploid with high S phase fraction values, a higher mitotic index, be more often grade III with massive lymphocyte infiltration, be oestrogen and progesterone receptor negative, and at times, have a high rate of p53 positive staining presumably indicative of a mutant *TP53* gene [15–19]. Despite these somatic features suggestive of a more aggressive course, and a less favourable response to therapy, the overall survival in *BRCA1* mutation carriers with breast cancer is reportedly similar to sporadic cases [20]. The precise implication of these differences in histopathological features on long-term survival and therapeutic response in breast cancer is presently unclear. Rhei and coworkers analysed 40 *BRCA*-linked ovarian tumours and detected *TP53* mutations (by full mutational analysis) in 80% of tumours [21].

The somatic features in ovarian cancer that are suggestive of a less favourable prognosis have not yet been fully elucidated. Inactivation of the *TP53* gene has been suggested as an indicator of poor prognosis. Mutant p53 is detected in up to 81% of epithelial ovarian cancer (range: 50–81%) [22–24]. Inactivation of the gene results in prolongation of the half-life of the mutant allele. Thus, positive immunohistochemical staining for p53, is generally interpreted as representing mutant protein. In studies comparing immunohistochemistry and DNA-based mutation analyses, the correlation between the two methods in estimating the rate of p53 inactivation was found to be satisfactory [25–28]. The prognostic significance of mutant p53 in epithelial ovarian cancer is still debated: some investigators showed that mutant p53 is associated with poor prognosis in ovarian cancer [29–32] while others could not demonstrate this trend [22,23,33]. Notably, in these studies no distinction between sporadic and familial cases based on *BRCA1* genotyping was made.

To gain more insight into the potential differences in disease phenotype in ovarian cancer between inherited and sporadic disease, we evaluated tumours from Jewish ovarian cancer patients. Somatic analysis of the p53 expression pattern and the proliferative indices in 185delAG *BRCA1* mutation carriers were compared with sporadic, non-mutation carrier Jewish ovarian cancer patients.

2. Patients and methods

2.1. Study population

The study group encompassed 65 epithelial ovarian cancer cases who were selected from an ongoing Israeli nationwide study of ovarian cancer. All patients reported herein had pathologically proven ovarian cancer, diagnosed in one of 22 medical centres around Israel, after March 1993. All were tested for being carriers of one of the three predominant Jewish mutations in *BRCA1* (185delAG, 5382InsC) or *BRCA2* (6174delT). The study was approved by the Institutional Review Board and all participants signed a written informed consent.

2.2. Analysis for the three predominant Jewish BRCA1 and BRCA2 germ line mutations

Detection of the 185delAG, 5382InsC and 6174delT mutations was performed using DNA extracted by standard protocols from peripheral blood samples. 100 to 250 ng of DNA was used as template and mutations were detected by a modified PCR restriction digest as previously described [8].

2.3. Histopathological evaluation

Formalin-fixed paraffin-embedded tissues from all patients, were cut to 4 micron thick sections, and placed on polylysine-coated slides. One slide of each case was stained with haematoxylin and eosin, and was re-evaluated and ascertained by two experienced pathologists at the Department of Pathology. Classification of surface epithelial ovarian tumours was done according to the WHO histological classification of ovarian tumours [34,35]. Staging of these tumours was done according to the International Federation of Gynecology and Obstetrics (FIGO staging).

2.4. Antibodies

Primary antibodies used in immunohistochemical staining were: monoclonal anti-p53 clone 1801 (Zymed Laboratories Inc, USA) and monoclonal anti-Ki67 clone 7B11 (Zymed Laboratories Inc). For p53, the technique and the reagents applied are described in detail elsewhere [36], and was previously applied for the analysis of ovarian cancer [37].

2.5. Immunohistochemical analysis

Paraffin-embedded sections were thaw-mounted onto Fisherbrand super frost plus slides. After air drying at 37°C for 16 h and incubation for 30 min at 60°C, slides were deparaffinised and rehydrated. For antigen expo-

sure, slides for p53 and Ki-67 were placed in 10 mM citrate buffer (pH 6.0) and heated twice for 5 min in a 750 W microwave oven, with the buffer being replenished between each heating interval. Slides were then allowed to cool down to room temperature for 10 min, then rinsed for 5 min in 0.1% bovine serum albumin (BSA)-tris-buffered saline (TBS (0.05 M Tris/HCl 0.1 M NaCl, pH 7.6)) and 0.05% Tween 20 solution. To reduce background signals, all slides were incubated at room temperature with 10% non-immune goat serum for 15 min, followed by a CAS block (Zymed Laboratories Inc) for 30 min. Staining was performed for both antibodies at 4°C for 16 h, with labelled avidin–biotin [38,39]. Control sections consisted of serial sections from the same blocks that were similarly processed and analysed during the experiment, except that no antibodies for p53 and Ki-67 were used. Immunostaining was scored as positive for p53 in any case of even focal nuclear staining [40]. Ki-67 positive nuclei were counted per 1000 cells. The sections were examined at high power (×40), 10 fields were chosen in the area showing most proliferation, with 100 cells assessed in each field. Proliferation score of more than 250 cells per 1000 cells was considered a high proliferation index.

2.6. Statistical analyses

For each patient, data on age at diagnosis, ethnic origin, type and stage of tumours were analysed. Comparisons of the percentage of tumours with a positive staining for p53 and Ki-67 in *BRCA1* 185delAG mutation carriers with the non-carriers was performed using Chi square test or Fisher's exact test, according to the numbers in each category. A Yates corrected P value of <0.05 was considered significant.

3. Results

3.1. Patients' and histological characteristics

The study group comprised of 65 epithelial ovarian cancer cases, 29 (45%) 185delAG mutation carriers, and 36 (55%) non-carriers of any of the three predominant Jewish mutations in *BRCA1*/*BRCA2*. Of the entire study group, 54 (83%) had invasive ovarian cancer (27 mutation carriers and 27 non-carriers), and 11 (17%) had low malignant potential ('borderline') tumours: 2 carriers and 9 non-carriers. In 19/29 (66%) mutation carriers, diagnosis was made prior to 60 years of age, compared with 14/36 (39%) in non-carriers ($P=0.03$; Yates corrected $P=0.06$). Low malignant potential tumours were detected less frequently among carriers (2/29; 7%) than in non-carriers (9/36; 25%) ($P=0.03$; one tail $P=0.05$). There were no differences in the proportions of stage III–IV disease between carriers (22/29; 76%) and non-carriers (25/36; 69%). There were 41 (63%) patients with serous ovarian cancer (21 carriers and 20 non-carriers), 5 (8%) with endometroid ovarian cancer (2 carriers and 3 non-carriers) and only one non-carrier with mucinous cancer (Table 1).

3.2. Immunohistochemical studies

p53 Analysis was completed for 64/65 samples, for technical reasons. No differences were detected in the rates of positive nuclear staining with p53 antibody in the invasive cancer cases ($n=54$): in 17/27 of carriers (63%) and in 18/27 of non-carriers (67%). Extending the analysis to include borderline tumours ($n=64$), did not alter the results: 17/29 (59%) of carriers and 22/35 (63%) of non-carriers had positive p53 nuclear staining.

Table 1
Patient and tumour characteristics

(a) Clinical and pathological by *BRCA1* 185delAG status

	Total ($n=65$) n (%)	Carriers ($n=29$) n (%)	Non-carriers ($n=36$) n (%)	Significance (P value)
Age (years)				
<60	33 (51)	19 (66)	14 (39)	0.06[a]
⩾60	32 (49)	10 (34)	22 (61)	NS[c]
Stage				
I–II	15 (23)	6 (21)	9 (25)	NS
III–IV	47 (72)	22 (76)	25 (69)	NS
Unknown	3 (5)	1 (3)	2 (6)	
Morphology				
Borderline	11 (17)	2 (7)	9 (25)	0.05[b]
Invasive	54 (83)	27 (93)	27 (75)	
Serous	41 (63)	21 (72)	20 (56)	NS
Endometroid	5 (8)	2 (7)	3 (8)	ND[d]
Mucinous	1 (2)	0	1 (3)	ND
Anaplastic	5 (8)	2 (7)	3 (8)	ND
Other epithelial	2 (3)	2 (7)	0	ND

(b) p53 and Ki-67 staining patterns by *BRCA1* 185delAG status (for invasive ovarian cancer only)

	Total n (%)	Carriers n (%)	Non-carriers n (%)	Significance n (%)
p53				
Total	54 (100)	27 (100)	27 (100)	
Positive[e]	35 (65)	17 (63)	18 (67)	NS
Negative	19 (35)	10 (37)	9 (33)	NS
Ki67				
Total	52 (100)	27 (100)	25 (100)	
Positive[f]	7 (13)	4 (15)	3 (12)	NS
Negative	45 (87)	23 (85)	22 (88)	NS

[a] Calculated by the Yates correction.
[b] Calculated by the Fisher's exact test.
[c] NS denotes non significant.
[d] ND not done.
[e] Positive means that more than 1% of the cells were staining positive.
[f] Positive indicates that 25% or more of the cells were positively stained with that antibody.

Ki-67 was technically successful in 62/65 tumours, and the rates of tumours in whom more than 25% of the tumour cells stained positive for Ki-67 were similar within the invasive ovarian cancer group: 4 of 27 mutation carriers (15%) and 3/25 (12%) of non-carriers. Results were not altered by analysis that included borderline tumours ($n=62$): 4/31 carriers (13%) and 3/33 non-carriers (9%) exhibited this staining pattern. This difference did not reach statistical significance.

4. Discussion

In this study, no differences in histopathological features suggestive of a higher malignant potential, p53 inactivation and proliferation, as assessed by Ki-67, were noted in Jewish patients with inherited ovarian cancer compared with their sporadic counterparts. Our data admittedly are based on a relatively small number of tumours but indicate that, unlike breast cancer, in ovarian cancer the 185delAG *BRCA1* mutation, may not have a bearing on disease outcome and/or therapeutic response. Thus, it is plausible that in Jewish individuals the molecular mechanisms of tumorigenesis of sporadic and inherited ovarian cancer are similar, and unaffected by the presence of specific germ line mutations in *BRCA1*. This study analysed a single mutation in *BRCA1*, 185delAG. While this offers an advantage in terms of stratification of the analysed group, specific genotype-phenotype correlations have been reported in *BRCA1* [41], thus, limiting the generalibility of our conclusions to other mutations. It is possible, and plausible, that mutations in other regions of *BRCA1* are associated with statistically significant differences between mutation carriers and non-carriers. Indeed, Ramus and colleagues showed that ovarian tumours from *BRCA1* and *BRCA2* mutation carriers displayed a significantly higher p53 mutation rate and were less differentiated than sporadic tumours [42]. The difference between our results and those reported by Ramus and colleagues [42], may be related to the exact location of the mutations.

Based on the present study, no statement can be made regarding disease outcome in mutation carriers versus sporadic cases. However, mutant p53 is considered a marker indicative of poor prognosis [30,31] and advanced stage disease [27,43] in ovarian cancer by some investigators. The similar rates of mutant p53 in both patient groups, suggests that the biological behaviour of the ovarian tumour may not be affected by the status of *BRCA1* germ line mutation. Indeed, preliminary data in Jewish ovarian cancer patients, indicate that mutation carriers and non-carriers have similar survival rates (data not shown).

The study was performed on a relatively small number of tumours, raising questions as to the statistical power, validity and the significance of our conclusions. However, the fact that the overall rate of p53 positivity (39/64; 61%) is similar to that reported in the literature [22–24], might indicate that the sample analysed was not grossly skewed and highly selected.

In conclusion, we could not demonstrate any significant differences in the rate of p53 positive staining and a proliferative marker, between Jewish ovarian cancer patients with and without the 185delAG *BRCA1* germ line mutation. The prognostic implication of our finding and its generalisability to all *BRCA1* mutation carriers is presently unclear.

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