

Accumulation of p53 protein is frequent in ovarian cancers associated with BRCA1 and BRCA2 germline mutations

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Abstract

Background—Mutations in the BRCA1 or BRCA2 genes are responsible for up to 95% of hereditary ovarian cancer cases. Both genes function as tumour suppressor genes, and development of a cancer is thought to require an accumulation of somatic genetic events in addition to the inherited germline predisposition. It is unknown whether these somatic events in BRCA associated ovarian cancer are similar to or distinct from those in sporadic cases. The most frequent somatic genetic event in ovarian cancer is a mutation of the p53 gene.

Aim—To study the role of p53 in hereditary ovarian cancer, by analysing accumulation of the p53 protein in ovarian cancers which occurred in BRCA1 or BRCA2 germline mutation carriers and comparing the results with a panel of ovarian cancers from patients who tested negative for both BRCA1 and BRCA2.

Methods—The study group consisted of 39 ovarian cancer patients in whom a BRCA mutation had been confirmed previously. p53 Immunohistochemistry was performed on archival tissue using a standard microwave antigen retrieval technique. The rate of p53 accumulation was compared with 40 ovarian cancer cases who tested negative for BRCA1 and BRCA2 germline mutations.

Results—p53 Accumulation was similar in BRCA related ovarian cancers and BRCA negative controls. Overall 27 of 39 BRCA1 or BRCA2 positive cases (69%) had evidence of p53 accumulation, compared with 24 of 40 invasive ovarian cancer cases (60%) which tested negative for BRCA1 and BRCA2 germline mutations. BRCA1 related ovarian cancers showed p53 accumulation in 22 of 30 cases (73%); p53 accumulation was present in five of nine BRCA2 related ovarian cancers.

Conclusions—In addition to germline BRCA1 and BRCA2 mutations, somatic p53 alterations leading to p53 accumulation are an important event in hereditary ovarian cancer and are as frequent as in non-BRCA-related ovarian cancer.

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Keywords: BRCA1 and BRCA2; p53; hereditary ovarian cancer; immunohistochemistry

Mutations in BRCA1 or BRCA2^{1,2} are responsible for approximately 95% of hereditary ovarian cancer cases that occur in families with an autosomal dominant inheritance pattern of ovarian cancer alone or in combination with breast cancer.³ Such germline mutations are associated with a life time risk for ovarian cancer of up to 60%.³⁻⁵ Both genes function as tumour suppressor genes, and development of a cancer is thought to require an accumulation of somatic genetic events in addition to the inherited germline predisposition. However, little is known about the nature or frequency of these events in ovarian cancers associated with germline BRCA mutations. It is possible that the somatic genetic events which occur in sporadic ovarian cancer are similar to those in BRCA associated cancers. Alternatively a germline BRCA mutation may represent a separate pathway of molecular carcinogenesis associated with distinct somatic genetic events.

The most frequent somatic molecular genetic event in ovarian cancer is a mutation of the p53 gene which acts as a tumour suppressor gene and controls entry into S phase of the cell cycle. Various studies have reported a high frequency of p53 accumulation or mutations in ovarian cancer, varying from 15% in early stages⁶ up to 81% in advanced stages.⁷ Missense mutations are the most common mutations in the p53 gene⁷ and a strong correlation between such mutations and immunohistochemical staining has been found in ovarian cancer as well as in other types of human cancers. p53 Mutations can be identified in 90% of ovarian cancers that accumulate p53 protein, whereas (missense) mutations are found in less than 10% of ovarian cancers that do not accumulate p53 protein.⁸

To study the role of p53 in hereditary ovarian cancer, we compared the frequency of p53 accumulation in ovarian cancers which occurred in BRCA1 or BRCA2 germline mutation carriers with a panel of ovarian cancers from patients who tested negative for mutations in both BRCA1 and BRCA2.

Methods

Ovarian cancer samples from patients known to harbour BRCA1 or BRCA2 mutations were identified from four sources: the University Hospital Vrije Universiteit, Amsterdam (n = 12), University of Toronto (n = 11), the UKCCCR Familial Ovarian Cancer Registry, Cambridge (n = 9), and Duke University, Durham, Connecticut (n = 7). These samples

Table 1 Histology, grade, stage, and p53 accumulation in BRCA1 and BRCA2 related ovarian cancers and BRCA1 and BRCA2 negative controls

	p53 Accumulation in BRCA1 mutation carriers (%)	p53 Accumulation in BRCA2 mutation carriers (%)	p53 Accumulation in all mutation carriers (%)	p53 Accumulation in BRCA1 and BRCA2 negative controls (%)
<i>Histology</i>				
Serous	22/30 (73)	5/9	27/39 (69)	20/27 (74)
Mucinous	–	–	–	2/3
Other epithelial*	–	–	–	2/10
<i>Grade</i>				
1	–	–	–	0/4
2	12/17 (71)	3/5	15/22 (68)	13/20 (65)
3	9/13 (69)	2/4	11/17 (65)	11/16 (69)
<i>FIGO stage</i>				
I	1/1	–	1/1	3/9
II	1/1	–	1/1	3/4
III	12/18 (67)	4/7	16/25 (64)	14/27 (52)
IV	2/3	–	2/3	–
Unknown	6/7	1/2	7/9	–

*Clear cell (3), endometrioid (2), undifferentiated (3), and mixed (2) epithelial ovarian cancers.

included 30 cases of ovarian cancer with 19 different germline mutations in BRCA1 and nine cases with seven different mutations in BRCA2.

The control group consisted of 43 randomly selected epithelial ovarian cancer samples from a total of 300 patients who participated in an Ontario-wide study based at the University of Toronto and who tested negative for germline mutations in both BRCA1 and BRCA2. Three cases were borderline tumours and were excluded from further analysis. Of the remaining 40 invasive epithelial ovarian cancers, 26 had a negative family history of breast and ovarian cancer, two had one first degree relative with breast cancer (> 60 years), and 12 ovarian cancer patients had one or more first degree relatives with early onset breast cancer (< 60 years) or ovarian cancer.

The study group consisted of 39 patients in whom a BRCA1 or BRCA2 germline mutation had been confirmed previously.

Several techniques were used to detect such mutations including the protein truncation test (PTT), direct sequencing, and heteroduplex analysis. In the control group, consisting of 40 cases in whom no mutation could be found, a standard protocol from the study based at the University of Toronto was used on lymphocyte DNA. Exons 11 of BRCA1 and 10-11 of BRCA2 were screened by PTT, and the Ashkenazi Jewish founder mutations in exons 2 and 20 of BRCA1 were screened using a PCR based heteroduplex analysis. For the detection of known founder mutations in patients of French-Canadian ancestry, direct sequencing for these founder mutations was performed.

p53 Immunohistochemistry was performed in one laboratory and interpreted by one gynaecological pathologist (PS).

Sections were dewaxed and taken to water, after which endogenous peroxidase activity was blocked using 3% hydrogen peroxide in Tris buffer for 10 minutes. A standard microwave antigen retrieval method was performed using a microwavable pressure cooker at high power for 2.5 minutes in 10 mM citrate buffer, after full pressure was reached. Slides were cooled before transferring to 0.05 M Tris-buffer (pH 7.5) for five minutes, incubated in 10% normal goat serum (Zymed) for 10 minutes, and subsequently incubated with the primary antibody

(DO7, Novacastra Laboratories) for one hour, followed by washing in Tris buffer. A secondary biotinylated antibody was applied (goat anti-mouse, anti-rabbit, anti-guinea pig, anti-rat IgG, Zymed) for 10 minutes and after washing in Tris buffer streptavidin-horseradish peroxidase was applied, washed again, and followed by the chromagen (DAB, Sigma) activated with 0.6% hydrogen peroxide. The reaction time was microscopically controlled. Counterstaining was performed with Mayer's haematoxylin.

p53 Accumulation was considered present if more than 10% of tumour nuclei stained positively.⁹ For histopathological review and immunohistochemistry, the pathologist was blinded to the BRCA carrier status. Tumours were graded according to the GOG (Gynecological Oncology Group) grading system, considering both architectural and cytological features. Grade 1 carcinomas have mild cytological atypia and less than 5% solid areas, while grade 3 carcinomas have marked cytological atypia and more than 50% of the area involved by solid growth.¹⁰

p53 Immunostaining was compared between the study group and controls using the two tailed Fisher exact test.

Results

Overall 27 of 39 BRCA1 or BRCA2 positive cases (69%; 95% confidence interval (CI) 54.1% to 84.4%) had evidence of p53 accumulation, compared with 24 of 40 ovarian cancer cases which tested negative for BRCA1 and BRCA2 germline mutations (60%; 95% CI 44.1% to 75.9%) (p = 0.48).

p53 Accumulation occurred in 22 of 30 BRCA1 related ovarian cancers, and in five of nine BRCA2 related ovarian cancers (p = 0.42).

In the control group, p53 accumulation was detected in 15 of 26 cases with a negative family history and in nine of 14 cases with a positive family history.

The distribution of histopathological subtype, grade, FIGO stage, and p53 status for the study group and control group are shown in table 1. All BRCA related ovarian cancer cases were serous cystadenocarcinomas, whereas only 68% of ovarian cancers in the control group had this histopathological diagnosis.

Grade distribution was similar between BRCA1 or BRCA2 positive cases and those who tested negative for BRCA1 and BRCA2 germline mutations. There was, however, a statistically significant difference in FIGO stages between the two groups: 28 of 30 BRCA1 or BRCA2 positive ovarian cancer cases (93%) compared with 27 of 40 BRCA1 and BRCA2 negative ovarian cancers (68%) were diagnosed in advanced stage (FIGO III and IV) ($p = 0.017$). When correcting for stage, p53 overexpression was similar in both groups: 16 of 25 advanced stage BRCA1 or BRCA2 positive cases and 14 of 27 advanced stage BRCA1 and BRCA2 negative ovarian cancers stained positive for p53 ($p = 0.41$). Correcting for differences in histological subtype or grade resulted in very similar rates of p53 accumulation in BRCA related cases and controls (table 1).

Discussion

To our knowledge this is the first study comparing p53 accumulation in ovarian cancers from BRCA1 and BRCA2 germline mutation carriers with p53 accumulation in ovarian cancers of non-carriers. Crook *et al* (11) found p53 mutations in all breast and ovarian cancers in four families with BRCA1 mutations and postulated that a somatic p53 mutation may be required in the development of breast cancer in BRCA1 mutation carriers.¹¹ Auranen *et al* found no difference in p53 overexpression between ovarian cancer cases occurring in families and patients with no family history but they did not test for BRCA mutations.¹²

We detected a similar rate of p53 accumulation in BRCA1 and BRCA2 related ovarian cancers compared with BRCA1 and BRCA2 negative controls, regardless of family history. Our results indicate that as for sporadic ovarian cancer, the pathway of carcinogenesis in BRCA1 and BRCA2 related ovarian cancer involves alterations in p53 leading to accumulation of the protein. It is possible that the accumulation of p53, noted in BRCA related cases, involves a mechanism other than a mutation. This is unlikely since there is a high concordance between p53 missense mutations and immunohistochemical staining, with a false positive rate of 2% and false negative rate of 3%.¹³ However, not all p53 mutations result in protein accumulation that can be detected immunohistochemically. Insertion, deletion, nonsense, splice site, and missense mutations outside exons 5–9 may result in weak or negative immunohistochemical staining.^{13 14} Casey *et al* found mainly deletions, insertions, or nonsense mutations in 22 of 94 cases (23%) that did not overexpress p53.¹³ Therefore possible differences in the distribution of these types of mutation may be missed by studying immunohistochemical staining alone.

Our control group consisted of patients who tested negative for BRCA1 and BRCA2 germline mutations and of these 26 of 40 cases were known to have a negative family history for both breast and ovarian cancer. Since the prevalence of BRCA1 mutations in women unselected for family history has been esti-

mated at less than 3%,¹⁵ the prevalence of mutations in women known to have a negative family history will be even lower. It remains possible that patients in the control group harboured a mutation that was not detected by our analysis. In the 12 patients with a positive family history the prevalence of BRCA1 germline mutations can be estimated to be approximately 20%.¹⁶ The method of mutation detection used in the control group can be expected to have detected approximately 60% of mutations in BRCA1 and 50% of mutations in BRCA2. It is therefore unlikely that undetected mutation carriers are significantly confounding our control group. Moreover, the rate of p53 expression in BRCA negative familial and BRCA negative sporadic ovarian cancer cases was similar and compared well with the rate of protein accumulation in BRCA1 and BRCA2 mutation carriers, thus providing further evidence that p53 accumulation is not related to BRCA status or family history.

There is some evidence of differences between BRCA related and sporadic ovarian cancer. First, the clinical characteristics of hereditary ovarian cancer appear to differ from sporadic cancer. Ovarian cancers in BRCA1 or BRCA2 germline mutation carriers are more likely to be of the serous type, to occur at a younger age, and to present at a more advanced stage compared with sporadic cancer.¹⁷ Second, the biological behaviour of hereditary ovarian tumours may be different and a better survival in hereditary ovarian cancer patients has been suggested.¹⁸ Third, mutations in BRCA1 or BRCA2 are rarely found as a somatic event in sporadic ovarian cancer cases. This may be explained by the fact that these genes are switched off by a different molecular mechanism than the mutations occurring in hereditary cases. Alternatively, the cellular control pathways involving BRCA1 and BRCA2 may not be of importance in sporadic ovarian cancer.

In order to explore these questions further, studies of other molecular events known to occur in sporadic ovarian cancer are in progress in our research group. Our present study suggests that molecular genetic differences between BRCA related and sporadic ovarian cancer are unlikely to include differences in the role of mutations in p53 as a somatic genetic event.

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