RESEARCH NOTE

High frequency of *BRCA1/2* and *p53* somatic inactivation in sporadic ovarian cancer

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Introduction

Our knowledge about the role of *BRCA* genes in hereditary breast and ovarian cancer is rapidly expanding (Zikan *et al*. 2005). However, we know very little about their role in sporadic breast ovarian cancer. In epithelial ovarian cancer, allelic losses of the *BRCA1* gene are found in 23%-44% and of *BRCA2* in 25%–40% of samples, respectively (Gras *et al*. 2001; Geisler *et al*. 2002; Hilton *et al*. 2002; McCoy *et al*. 2003). Somatic mutations of *BRCA1/2* genes are typically extremely rare, but interestingly are found especially in clear cell carcinomas (Takahashi *et al*. 1996; Hilton *et al*. 2002). Other possible means of somatic inactivation of *BRCA* genes have been described: occasionally through gross genomic rearrangements, but most commonly by hypermethylation of promotor sequences or related (or independent) reduced expression of *BRCA* genes (Esteller *et al*. 2000; Miyamoto *et al*. 2002).

Here, we report on a study of the frequency and mechanisms of *BRCA1*, *BRCA2* and *p53* inactivation in 30 sporadic epithelial ovarian cancer samples using intragenic and flanking microsatellite markers. We examined loss of heterozygosity (LOH) for each gene, as well as the presence of somatic mutations and loss of expression, and attempted to relate the results to tumour histotype.

Materials and methods

Tumour samples

For analysis, we used blood and tissue of fresh-frozen tumour samples of ovarian epithelial cancer patients operated upon, at the Clinic of Obstetrics and Gynecology, First Faculty of Medicine, Charles University, Prague, and the General Teaching Hospital in Prague. All analysed tumours were sporadic i.e. no other tumours were referred to in the family history and all the patients were more than 40 years old at the time of diagnosis. All participants gave their written informed consent.

All freshly frozen samples were examined by a pathologist to evaluate the proportion of tumourous and nontumourous tissue. Only samples with over 50% tumourous tissue were used for our study. The histopathological characteristics of analysed tumours are given in table 1. Majority of patients (21/30 : 70%) were diagnosed in stage III International Federation of Gynocology and Obstetrics (FIGO). Stages I and IV were similarly represented (4/30 and 5/30: 13.3% and 16.7%, respectively), and none of the analysed tumours belong to stage II patient.

Table 1. Histopathological characterisation of examined tumours.

serous carcinoma	17/30 (56.7%)
G1	1
G2	5
G3–4	11
mucinous carcinoma	6/30 (20%)
G1	4
G2	1
G3–4	1
anaplastic	5/30 (16.6%)
G3–4	5
endometriod carcinoma	2/30 (6.7%)
G1	0
G2	1
G3–4	1

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Genetic material

DNA and RNA were isolated from freshly frozen tumour tissue and from peripheral venous blood by standard procedures, described in detail by Pohlreich *et al*. (2003). DNA from blood was used as a reference DNA for LOH experiments. In addition, mutation analysis of both *BRCA* genes was carried out on these blood samples to confirm the sporadic origin of analysed tumours. No hereditary mutations was found in the analysis of DNA from blood in these samples

LOH analysis

We used intragenic or flanking microsatellite markers. Three markers of each for BRCA1 (D17S855, D17S1322, D17S1323) and *BRCA2* (D13S1695, D13S1699, D13S1701) region and two markers for *p53* region (TP01, TP02) were used. LOH was evaluated visually by comparison of alleles from tumour and blood, or by area under curve (AUC) computation using the formula $(A_t \times B_n)/(B_t \times A_n)$, where A and B are alleles, and *t* and *n* indicate tumourous and normal tissue, respectively. LOH was considered to be found if intensity of one allele in tumour was lower then 50% of normal (blood) tissue allele intensity. Microsatellite fragments were amplified by PCR and then analysed by polyacrylamide Spreadex gel electrophoresis as described in Janatova *et al*. (2003).

Results and discussion

LOH analysis

We analysed LOH in three tumour-supressor genes — *BRCA1*, *BRCA2* and *p53* — using intragenic and flanking microsatellite markers. Markers heterozygous for appropriate patient were set as informative, homozygous ones as non-informative. Total heterozygosity (informativeness) ranged between 90.0% and 96.7% for the three gene regions studied.

LOH in *BRCA1* region was found in 11 out of 29 informative tumours (37.9%). Histological types were represented more or less equally in this subgroup: serous carcinoma: 4 (36.4%); mucinous carcinoma: 3 (27.2%); and anaplastic carcinoma: 4 (36.4%).

In *BRCA2* region, LOH was found in 10 out of 27 informative tumours (37.0%): 2 serous (20.0%); 4 mucinous (40.0%); 3 anaplastic (30.0%); and 1 endometroid (10.0%).

In more then half of the informative tumours (16/29: 55.1%), LOH in *p53* locus was detected, and the proportion of three major carcinoma histotypes was rougly equal: 6 serous (37.4%); 5 mucinous (31.3%); and 5 anaplastic (31.3%). There was no endometroid carcinoma expressing LOH in *p53* (table 2).

Table 2. Allelic losse in informative tumours with respect to tumour histotype.

	LOH		
Tumour histotype	*BRCA1*	*BRCA2*	*p53*
serous	4/11(36.4%)	2/10 (20.0%)	6/16 (37.4%)
mucinous	3/11(27.2%)	4/10 (40.0%)	5/16 (31.3%)
anaplastic	4/11 (36.4%)	3/10 (30.0%)	5/16 (31.3%)
endometroid	0	1/10 (10.0%)	0

In sum, out of 30 tumours at least one region of LOH was found in 18 tumours (60.0%). LOH in a sole region was present in 5 out of 18 tumours (27.8%): thrice in *p53* and twice in *BRCA*. LOH of *BRCA2* and *p53* were observed in conjunction in 2 out of 18 tumours (11.1%). Interestingly, LOH in *BRCA1* region was never present solely, but only in conjunction with either *p53* LOH (5/18 tumours: 27.8%) or *p53* and *BRCA2* LOH (6/18 tumours: 33.3%). And, in contrast, no tumours with only *BRCA1* LOH along with *BRCA2* LOH were found (table 3). These results suggest that it might be worthwhile to study the inactivation succession of such genes in sporadic ovarian epithelial tumours.

Somatic mutations analysis

Standard mutation analysis of coding and exon-flanking sequences of *BRCA1* and *BRCA2* genes were done in tumour samples with LOH in at least one of these gene regions. Neither somatic nor hereditary mutations were found.

Considering the rarity of high frequency of repetitive sequences in both *BRCA1* and *BRCA2* regions, it is thought that gross genomic rearrangements due to inadequate homologous recombination repair are most often the events leading to somatic inactivation of these genes (Szabo and King 1997). However, such events would require complex techniques based on RNA or on MLPA methods for their detection.

Table 3. Conjunction of allelic losses and tumours divided to subgroups due to LOH pattern.

LOH regions	Number of tumours	Percentage of tumours with LOH	Percentage of all analysed tumours
BRCA1 + BRCA2 + p53	6	33.3%	20.0%
BRCA1 + p53	5	27.8%	16.7%
BRCA2 + p53	2	11.1%	6.7%
BRCA2	2	11.1%	6.7%
p53	3	16.7%	10.0%

Loss of expression analysis

We examined expression of *BRCA1* and *BRCA2* genes by RT-PCR (reverse transcription PCR). Two tumours (6.7%) lacked expression of *BRCA1* gene. In one of them, no LOH was found in *BRCA1* gene region. However, in the other tumour, allelic loss was present in all the three examined microsatellite markers for *BRCA1*, suggesting that alleles are inactivated in different ways in these two cases: by allelic loss (expressed as LOH), and lack of expression, without change in the genomic sequence.

One tumour (3.3%) lacked expression of *BRCA2* gene this tumour was heterozygous for all three *BRCA2* microsatellite markers, but no LOH was detected in *BRCA2* region. Promoter hypermethylation is thought to be a cause of lack of expression (Hilton *et al*. 2002), and such epigenetic changes are extremely important for modulation of expression of many genes during cell differentiation as well as during tumour transformation (Chan *et al*. 2002; Verma and Srivastava 2002).

Conclusion

Research of *BRCA1/2* genes role in sporadic ovarian cancer is still beginning. As our data and those from literature cited here suggest, these genes (or their defects) appears to be important in approximately one-third of sporadic ovarian epithelial cancer cases. Consequently, subsequent studies need to focus on how these tumours differ in biological features or in response to therapy.

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