Original Article

Chromosomal Rearrangements and Their Relations to Histopathological and Clinical Parameters in Epithelial Ovarian Cancer

(chromosomal rearrangements / ovarian cancer / prognostic significance)

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Abstract. The aim of the study was to estimate genetic alterations detected in ovarian cancer cells in correlation with other available parameters of histopathological and clinical character and to find important relations with impacts on the cancer prognosis. Additionally, we wanted to compare methods for evaluating genetic changes. Sixty patients with ovarian cancer were included in the study. The histological type and grade were defined, MIB-1 and p53 were estimated using an immunohistochemical method. For genetic testing, both conventional and molecular methods were applied – direct culture and G-banding technique, FISH method with whole chromosome painting probes, and CGH method. The results were submitted to statistical evaluation, using analysis of variances and χ^2 test, with Bonnferroni correlations of the significance level. Numerical and structural aberrations have been detected in more than 63 % examined ovarian cancer cases. Patients with extensive chromosomal rearrangements were significantly younger. Specific genetic alterations, including some rare findings such as deletion 22q in 36 % of all ovarian cancer samples, have been found, together with associations between particular prognostic factors.

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Abbreviations: CGH – comparative genomic hybridization, CR – complete response, FISH – fluorescence *in situ* hybridization, FIGO – International Federation of Gynaecology and Obstetrics, PD – progression of disease, PR – partial response, RR – response rate.

Introduction

Ovarian cancer is the most lethal malignant tumour of the female reproductive system and represents about 30 % of all gynaecological malignancies. It is the fourth leading cause of cancer deaths in women. About 190,000 new cases and 114,000 deaths from ovarian cancer are estimated to occur annually. Women with ovarian cancer have a poor prognosis. The mean five-year survival rate in Europe is 32 %; in advanced stage, it is less than 20 %. This unfavourable outcome is largely ascribed to a lack of early warning symptoms and a lack of diagnostic tests that allow early detection. As a result, approximately 75 % patients present when this cancer is in advanced stage. In spite of the good treatment response, more than 80 % patients get recurrent disease. Most of the ovarian cancers are sporadic tumours. About 5–10 % of ovarian cancers have a hereditary background; the majority of them seem to be due to mutation in the *BRCA1* and/or *BRCA2* genes (Stewart and Kleihues, 2003).

The outcome in a cancer patient depends on a variety of variables referred to as prognostic factors. These factors can concern the tumour (tumour-related prognostic factors), patient (host-related prognostic factors), or patient environment (environment-related prognostic factors) (Gospodarovicz et al., 2001).

Recent studies show that certain genetic alterations can be an important adjunct to clinical data and, potentially, can be regarded as a new relevant marker for predicting tumour prognosis, drug sensitivity, or cancer risk assessment (Huang et al., 2002; Teixeira, 2002). The aim of the study was to estimate genetic alterations detected in ovarian cancer cells, in correlation with other available parameters of a histopathological and clinical character, and to find the associations with impact on the cancer prognosis.

Material and Methods

The tumour samples, collected over a period of three years, were obtained during a surgical procedure (laparoto-

my) in 60 patients with primary ovarian tumours. The patients were from Prague and Central-Bohemian region. All clinical information, including tumour marker CA125 results, was recorded. The patients studied had not received any chemotherapy prior to cytogenetic study. Platinum-based chemotherapy in combination with taxanes or cyclophosphamide followed the surgical procedure. Written informed consent was obtained from each subject. The study was approved by the Local Ethical Committee.

The tumour was examined by a histopathologist – histological type and tumour differentiation were determined. An immunohistochemical method was applied to evaluate p53 and the proliferative marker MIB-1 (semi-quantitative method with quantification of HSCORE, according to McCarty, using computer-assisted image analysis) (Mc Carty et al., 1985).

Conventional cytogenetic karyotyping, fluorescence *in situ* hybridization (FISH) with whole chromosome painting probes and comparative genomic hybridization (CGH) were used to screen for losses and gains of DNA sequences.

For cytogenetic analysis, the disaggregated tumour tissue was processed for short-term culture in two media (BIOAMF-2 complete medium, and Amniomax C100 supplement with Amniomax C100 basal) (Life Technologies Inc., Gaithersburg, MD). Chromosome slides were prepared in the conventional way. G-banding was performed using Wright's stain solution. The International System for Human Cytogenetic Nomenclature (1995) was used to describe the tumour karyotypes (Mitelman, 1995).

The fluorescence *in situ* hybridization method using whole chromosome painting probes WCP 1 SpectrumGreen Probe, WCP 3 SpectrumGreen Probe, WCP 4 SpectrumOrange Probe, WCP 7 SpectrumGreen Probe and WCP 11 SpectrumOrange Probe (Vysis Inc., Downers Grove, IL) were used according to the manufacturer's instructions. Slides were analysed in an Olympus BX 51 fluorescence microscope (Olympus Co., Ltd., Tokyo, Japan) with single band pass exciter filter for UV/DAPI (360 nm), Orange and Green (Vysis).

Total DNA from the tumours was analysed by comparative genomic hybridization (CGH). DNA from the samples was isolated by using the QIAamp® DNA Mini Kit (Qiagen Inc., Valencia, CA). All CGH procedures were performed using reagents and kits produced by Vysis Inc., following the manufacturer's instructions. Ten to fifteen images were collected using LUCIA software (Laboratory Imaging Ltd., Prague, Czech Republic) with a LUCIA-CGH (Laboratory Imaging) module. Slides were analysed in the Olympus BX 51 fluorescence microscope with single band pass exciter filter for UV/DAPI (360 nm), Orange and Green (Vysis).

The statistical evaluation used analysis of variances (ANOVA) and χ^2 test. As many variables had been compared, the Bonnferroni correlation of significance level was used for post hoc pairwise mean comparison in a one-way analysis of variances.

The evaluation in the tested group of ovarian cancers concerned both the *quantitative* variables (age, CA125 before diagnosis, MIB-1 HSCORE, p53 HSCORE), and the *qualitative* parameters. The qualitative parameters included: FIGO stage, histological type, grade, presence or absence of tumour residuum after the surgical procedure, operation with or without lymphadenectomy (with presence of nodal metastases), response rate (RR), half decline of CA125 after treatment (yes or no), and chromosomal rearrangement. Response rates (RR) were the following: complete response (CR), partial response (PR), progression of disease (PD) – during the first year after diagnosis and treatment. Chromosomal rearrangements were divided into the following: none, small – 1–7 aberrations, large – more than 7 aberrations. The number of statistically evaluated cases in particular parameters differed – clinical parameters, histology including grade were available in all cases; immunohistochemical examination (MIB-1 HSCORE, p53 HSCORE) was missing in several cases because of incorrect tissue fixation of the sample earmarked for this processing. As a consequence of some technical troubles (in some cases an insufficient amount of tumour tissue and unsuccessful culture), chromosomal rearrangements were evaluated in 47 patients in the tested ovarian cancer group.

Conventional cytogenetic karyotyping was successful in 35 patients out of the 40 tested (87.5 %) in the ovarian cancer group. In order to compare results obtained from applying different methods, and to evaluate advantages and disadvantages of these different approaches, tumour samples were analysed concurrently by the FISH method (six patients, i.e. 10 % of the entire group), with previous successful conventional karyotyping. The CGH method was applied in 12 patients (20 %) of unsuccessful conventional karyotyping in the ovarian cancer samples. The concentration of isolated DNA was extremely low in 13 cases in the ovarian cancer group; these cases were excluded from genetic examination. All these figures were both consistently and completely considered in the statistical processing.

The number of diploid tumours was quite high in our study. This may be connected with the way of obtaining the tissue sample (a clear malignant tumour may contain connective tissue components). The presented results come from both methods – conventional and molecular. The FISH method was used to specify results coming from the conventional method. The CGH method was used in the cases of unsuccessful culture. The number of studied metaphases varied from three to 28. Our aim was to evaluate all of the metaphases found – in the case of pathological findings, we evaluated all metaphases; in the case of normal findings we evaluated 30 metaphases.

Results

Patients in the tested ovarian cancer group were aged 39–81 years at the time of diagnosis (median 61). Table 1 presents the structure of the ovarian cancer group (FIGO stage, histology, grade).

Three patients from the ovarian cancer group were sent to a laboratory providing mutation analysis of

BRCA1 and *BRCA2* genes (according to criteria of the national consensus of indications for genetic examination in breast and ovarian cancer patients and their relatives (Bartoňková et al., 2003)). The *BRCA1* mutation was found in one patient.

Genetic findings

Numerical and structural aberrations were detected in more than 63 % ovarian cancer cells. The number of chromosomes ranged from 63 to 85. Using CGH analysis, deletions were more common findings than amplifications.

Table 2 presents the most frequent genetic alterations found in our study. The most frequent amplification 1q was found in 17 patients (36.2 %). Deletion 22q was also found in 17 (36.2 %) cases; this finding is quite rare according to the literature.

Another rare finding, isolated balanced traslocation t(10;15) was found in two examined cases (4.3 %) This finding was observed in all examined cells. A constitutional translocation was excluded by examination of the peripheral blood lymphocyte cultures. Examination of chromosomal breakpoints has not been performed.

All other findings, except for those given in the Table, occurred in less than 5 % of cases (amplifications: 8q, 11q, 17q, 19q, 12p, 12q, 5p,5q, 6p, 6q, 21q, deletions: 16q, 17q, 11p, 11q, 13q, 12p, 5q, 9q, 2p, 2q, Xp, 1q, 15q).

In 32 % of examined tumours, extensive changes were found; the number of aberrant chromosomes was greater than seven. In 37 % of cases, a diploid karyotype was found.

The efficiency of the conventional cytogenetic karyotyping (the method of direct processing and the method of short-time culture) was low. Both the FISH method and painting probes specified the structural rearrangements. Mitoses of good quality from previous short-time culture were necessary for the application of this method. The CGH seems to be the most reliable and suitable method for the ability to determine the loss or gain of DNA sequences. The CGH method also has some disadvantages as compared to the conventional karyotyping (e.g. it fails to identify balanced translocations and ploidy variations). The advantage of the method is the minimal amount of isolated DNA necessary, without particular previous culture.

Table 1. The structure of the ovarian cancer group (FIGO stage, histology, grade)

Parameter		N	%
FIGO stage:	I	10	16.7
	II	4	6.7
	III	40	66.7
	IV	6	10
	total	60	100
HISTOLOGY, GRADE:	serous adenoca	51	83
	G1	8	(15.7)
	G2	7	(13.7)
	G3	36	(70.6)
	mucinous adenoca	3	5
	G1	2	(66.7)
	G2	1	(33.3)
	G3	0	
	endometrioid adenoca	5	8.3
	G1	1	(20)
	G2	3	(60)
	G3	1	(20)
	undifferentiated	1	1.7
	total	60	100

adenoca – adenocarcinoma

The relations of clinical, histopathological and molecular parameters

The statistically significant associations of *quantitative* variables in the group of patients with ovarian cancer were the following:

1. Women with FIGO stage I were significantly ($P < 0.01$) older (median age 74) than women with advanced stages II–IV (median age 59, 61, 60). Lower aggressiveness including slow tumour growth is suggested. No other correlation of quantitative parameters and stage were found.
2. Women with tumour grade 1 had significantly ($P < 0.05$) lower MIB-1 HSCORE (141) in comparison with women who had tumour grade 2 (209) or 3 (195).
3. Women with tumour grade 1 had significantly ($P < 0.05$) lower p53 HSCORE (126) in comparison with women who had grade 2 (236) and 3 (173).
4. Women with high p53 HSCORE were also found to have high MIB-1 HSCORE ($P < 0.001$) in comparison with women with low p53 HSCORE.
5. Women with extensive chromosomal rearrangements (more than 7) were younger (median age 54) than women with small number of rearrangements, i.e. 1–7 (median age 60) or no rearrangements (median age 66) ($P < 0.1$).

The statistically significant associations between *qualitative* variables in the ovarian cancer group were the following:

1. Stage of disease and differentiation of tumour (grade) were dependent on $P < 0.001$ (from χ^2 statistics).
2. Stage of disease and presence of tumour residuum after surgical procedure were dependent on $P < 0.001$.

Table 2. The most frequent genetic alterations in the ovarian cancer group

GENETIC ALTERATIONS		N (total 47)	%
Amplifications	1q	17	36.2
	3q	8	17.0
	20q	8	17.0
Deletions	4p	8	17.0
	4q	8	17.0
	18p	4	8.5
	18q	4	8.5
	19q	4	8.5
	22q	17	36.2
Translocations	t(10;15)	2	4.3

3. Histological type of tumour and grade were dependent on $P < 0.05$.
4. Tumour differentiation (grade) and presence of tumour residuum after surgical procedure were dependent on $P < 0.001$.
5. Tumour differentiation (grade) and response rate (complete response, partial response, progression of disease) were dependent on $P < 0.01$.
6. Tumour residuum after surgical procedure and RR were dependent on $P < 0.05$.
7. Tumour residuum after surgical procedure and level of tumour marker CA125 after treatment were dependent on $P < 0.05$.

No other statistically significant associations have been found among the evaluated parameters.

The relations of genetic alterations and selected parameters

As the study was partly focused on genetic changes in tumour cells, our attention was concentrated on all hypothetic relations of chromosomal rearrangements with the above-mentioned parameters. Patients with chromosomal rearrangements were divided to the groups of no aberrations, a small number of aberrrations (1–7) and a large number of aberrations (7 and more). These three groups of chromosomal rearrangements were correlated to age, FIGO stage, histological type and grade, RR, CA125 level, MIB-1 HSCORE and p53 HSCORE, surgical residuum, nodal metastases. The only association in genetic findings was found within the group of *quantitative* variables (age). Patients with extensive chromosomal rearrangements were significantly younger (see above). No statistically significant associations have been found between the genetic findings and the qualitative variables.

The particular findings point to further direction in genetic research at the molecular level, with focus on special aberrations and cases with low numbers of aberrations.

Associations between the severity of chromosomal rearrangement and overall survival were not evaluated because of a short follow-up period for all patients participating in the study (one year).

Discussion

Typically, most investigators regard prognostic factors as those directly related to the particular tumour. Commonly used tumour-related prognostic factors are tumour pathology, anatomic disease extent, biochemical markers, expression of proliferation-related factors and, increasingly, molecular tumour characteristics including genetic alterations. Host-related prognostic factors include inherent demographic characteristics such as age, performance status, co-morbid conditions – all these factors may have a profound impact on the outcome. Environment-related factors comprise those that operate outside the patient – such as socioeconomic status, choice and quality of treatment, healthcare policy of the region – the impact of some of these factors may also be profound.

To consider the relevance of prognostic factors in clinical practice, we distinguish three groups of factors: essential (stage, histological type and grade, anatomic extent of disease), additional (proliferative parameters – MIB-1, patterns of invasion, host-related factors such as performance status, co-morbid conditions, function of vital organs), and the new and promising ones (molecular biological and genetic characteristics).

The present study included a number of well-known factors and was focused on their significance and relation with the found genetic alterations, considered to become the new and promising prognostic factors.

The stage of the disease is the most important and independent factor deciding about survival of patients with ovarian cancer. Risk of death is almost doubled when tumours are moderately or poorly differentiated, compared with those that are well differentiated. The prognostic impact of histological grade in advanced stage of the disease is not as strong as in early stages (Villa et al., 1998).

Our ovarian cancer group consisted mostly of patients in advanced stages. Early-stage cancer patients were significantly older. Advanced stages significantly correlated with poor differentiation ($P < 0.001$).

From the histopathological point of view, a typical histological finding in our group was serous adenocarcinoma. The histological type of tumours has not been found to be a prognostic factor in ovarian carcinoma (Pieretti et al., 2002).

Both absolute levels and half-life of CA125 have been demonstrated to be good prognostic indicators. CA125 half-life of less than 20 days correlates significantly with survival and with tumour regression (Geisler and Geisler, 2001; Baron et al. 2005).

A highly significant survival advantage in mutations *BRCA1*-carrier patients affected by advanced ovarian cancer was reported in several studies (Tapper et al., 1998; Patael-Karasik et al., 2000). The course of disease of the only patient – *BRCA1*-carrier corresponds with this finding; in spite of the advanced stage of disease she has been surviving for seven years.

In patients diagnosed with advanced-stage tumours, the overall survival is not only related to the initial burden, but trends to decrease with the post-surgical tumour mass. A significant correlation between tumour surgical residuum and response rate, as well as between surgical residuum and decline of CA125 level, has also been found in our ovarian cancer group.

The studies of cellular proliferative activity in tumour cells – MIB-1 (Ki-67), AgNOR (silver-stained nucleolar organizer region-associated protein) have produced conflicting results and failed to establish such counts as an independent prognostic factor. An increased proliferation activity measured by monoclonal antibody MIB-1 significantly correlates with histological grade, no significant correlation with histological type or stage of the disease has been found. A correlation has been

found between Ki-67 expression and the response rate on second-line chemoterapy (Sah et al., 2004; Wang et al., 2004).

Mutations of p53 (a „molecular guard“) and overexpression of mutant p53 products are more common in advanced stages (40–60 %) than in early-stage disease (15 %). Some studies have suggested that p53 overexpression in stage III–IV disease is associated with a 10–20% decrease in 5-year-survival (Nielsen et al., 2004).

A significant correlation between proliferative activity and grade has been found in our ovarian cancer group. These results are conclusively in agreement with other studies.

From the point of view of genetic alterations, ovarian cancer presents a wide variety of findings, and interpretation of these findings is still subject of continuing research. Comparison of early-stage and advanced tumours revealed some differences between both groups: deletions were more frequent than amplifications in early-stage tumours. Typical deletions were the following: 2q, 4q, 5q, 6q, 13q, 16q, 18q. Amplifications were found particularly in advanced stages – on chromosomes 1q, 3q, 8q, 11q, 12p, 17q, 20q (Aunoble et al., 2000; Shridhar et al., 2001).

Loss of genetic material on chromosome 4 was a typical finding in advanced stage (Mark et al., 1999). Different histological types do not differ in specific aberrations, complex aberrations are rare in mucinous and endometrioid tumours. Moderate aberrations are found in well-differentiated tumours, complex rearrangements are typical for poorly differentiated tumours (Kiechle et al., 2001; Kim et al., 2003).

Patients with tumours containing less than seven aberrations presented better survival time; these patients are suggested to have better treatment response. Tumours with amplifications 1p, 10p, 20q and deletion 5q are at greater risk of recurrence (Sham et al. 2002).

Serous tumours of advanced stages contain twice more aberrations than tumors of early-stage carcinomas. Amplifications have been found on chromosomes 3q, 6p, 7, 8q and 20, deletions on 4q, 6q, 12q, 13q and 16q. Common aberrations for different histological types are amplifications 3q, 6p and deletions 4q. Aberrations related to worse prognosis were amplifications 6p, 7q, 13q and deletions 15q, 17p, 18q and 21q (Hu et al., 2003).

Data indicate that tumours of low malignant potential and invasive carcinomas include different aberrations and so they may be considered to be two different groups of ovarian tumours (Hauptmann et al., 2002; Tibiletti et al., 2003). The issue of whether borderline tumours are precursors of invasive carcinoma or distinct clinical entities, however, is still subject of discussion. The distinct cytogenetic alterations could be early events of serous ovarian tumours and could also characterize a subgroup of borderline ovarian tumors that may have potential to progress and develop malignancy (Helou et al., 2006; Ősterberg et al., 2006). Comparison of primary and metastatic tumors has revealed more aberrations in the first explored group of tumours (Staebler et al., 2002; Fishman et al., 2005). More recently, an i(5p) was described as a novel recurrent abnormality in ovarian cancer (Panani and Roussos, 2006).

Cytogenetic analyses of ovarian carcinomas in our study proved the presence of complex karyotypes with a wide range of numerical and structural rearrangements. Deletions were more common findings than amplifications, in spite of the fact that most of the tumours were in advanced stages in our cancer group, while in the literature they are more typical findings for early-stage carcinomas.

The number of genetic rearrangements was significantly higher in the group of younger women. No other interactions were found between severity of chromosomal rearrangements and the selected parameters. The correlation between the severity of chromosomal rearrangements and the overall survival were not evaluated, either, due to the short follow-up period (one year).

On the other hand, some unique findings described in the Results have been found. The interpretation of these findings is conflicting and their further research will be necessary. Deletion 22q, quite rare according to the literature, was found in 17 (36.2 %) cases in our study.

The 22q11.2 deletion syndrome is a common chromosomal disorder with highly variable phenotypic expression and immunologic defects that affect more than 70 % of individuals regardless of their clinical presentation. The genetic basis of the syndrome is complex and it is still not fully understood. The associations between recurrent, interstitial deletions in 22q11.2 and predisposition to cancer were reported recently – in malignant rhabdoid tumours, pancreatic adenocarcinoma, and squamous cell cervical carcinoma (Wieser et al., 2005; Choi et al., 2007; Jackson et al., 2007; Loukopoulos et al., 2007).

Conclusions

Different methods and approaches were used to study and evaluate the genetic alterations in gynaecological malignant tumours – from the classical cytogenetic procedure to molecular-cytogenetic methods, in order to determine the most suitable approach for the selected research. The methods were compared from various points of view – time and financial requirements, failure, and exploitability.

The CGH has been found to be the most suitable contemporary method, supplemented by FISH after a modified short-time culture.

The study has found associations between particular prognostic factors, as well as between prognosis and number of aberrations in tumour cells. The number of aberrations in ovarian cancer cells seems to be an important prognostic marker, especially when associated with younger age.

Specific genetic alterations, including some rare and unique findings in ovarian tumour cells, have been found – isolated balanced translocation t(10;15), amplification 1p, deletion 19q, and deletion 22q (in the group of ex-

amined patients detected in 36 % of cases, quite rare in terms of available literature).

Further research ought to be concentrated on the group of ovarian tumours in younger women and the group of patients with bordeline tumours, in order to explain the special process of carcinogenesis, with implementation to an individual and effective clinical approach.

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