Genetic alterations in gynecological malignancies

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The aim of the study was to estimate genetic alterations detected in ovarian and cervical cancer cells, in correlation with other available parameters of a histopathological and clinical character and to find the important associations and differences of both these tumor sites with diverse impacts on the cancer's prognosis. Sixty patients presenting with ovarian cancer and twenty patients manifesting cervical cancer were included in the study. The histological type and grade, MIB-1 and *p53* were estimated. For genetic testing, both conventional and molecular methods were applied. The results were subjected to statistical evaluation, using analysis of variances and χ^2 test. Ovarian cancer patients with extensive chromosomal rearrangements were assessed to be significantly younger . The typical findings, different in ovarian and cervical cancer cells have been found, including some less frequent findings (deletion of 22q in 36% of all ovarian cancer samples, as well as amplifications of chromosome 2 and deletions of chromosome 10, 11p and 21q in cervical cancer cells). The expression of proliferation marker MIB-1 was observed to be significantly higher in women with a high *p53* HSCORE . The significant importance of genetic alterations and the activity of proliferative markers, including common correlations with an unfavorable outcome with respect to ovarian tumors in younger women were found.

Keywords: chromosomal rearrangements, genetic alterations, ovarian cancer, cervical cancer, prognostic significance.

Ovarian cancer is the leading cause of death from gynecological malignancies. About 190,000 new cases and 114,000 deaths from ovarian cancer were estimated to occur worldwide, in 2003. The number of cases is not decreasing, according to the national cancer incidence and mortality statistics. The majority of ovarian cancers are sporadic tumors; more than 75% of cases are diagnosed at the advanced stages. In spite of the good response rate, more than 80% of patients experience recurrent disease. The mean five-year survival rate in Europe is 32%; however, in the advanced stage, it has been approximated to be less than 20%. This unfavorable outcome is largely ascribed to a lack of early warning symptoms and a lack of diagnostic tests that could allow early detection [1].

Cervical cancer is the second most common cancer among women worldwide – about 470,000 cases being diagnosed every year, with about 230,000 deaths annually. In spite of well-defined pre-malignant lesions, as well as both available and effective methods for their detection, the incidence of the tumor is still high. More than 80% of cases occur in the developing countries. The five-year survival rate is approximately 70%, and in the developing countries of the Third World, it has been estimated to be about 40%. The presence of HPV (human papilloma virus) seems to play a key role in cervical carcinogenesis of squamous cell carcinomas [1].

Over the last decades, the improved resolution of the cytogenetic techniques, which include the pathway from conventional cytogenetics to molecular karyotyping, has led to a significant increase in the detection rate of chromosomal aberrations in patients with cancer. Recent studies show that cytogenetic rearrangements can be an important adjunct to clinical data and, potentially, can be regarded as a new relevant marker for predicting tumor prognosis, drug sensitivity, or cancer risk assessment [2, 3].

The current limitations to the accurate use of genetic information relate to the multifactorial nature of cancer prognosis. It would be optimal to develop a prognostic "molecular ratio" for each patient, in order to lay a foundation for individualization of the therapeutic strategies.

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The aim of the study was to estimate the major genetic alterations in examined ovarian and cervical tumors and to predict their significance and prognostic value in correlation with other routinely examined clinical and laboratory markers.

Materials and Methods.

In tumor samples (60 of ovarian cancer and 20 of cervical cancer), for which histological type and grade were estimated, an immunohistochemical semi-quantitative method was applied to determine *p53* and the proliferative marker MIB-1. Another part of the tissue sample was used for cytogenetic processing, combining both conventional method and FISH (fluorescent *in situ* hybridization) method, using DNA specific probes and painting probes. The isolated DNA samples were preserved to be evaluated by the CGH (comparative genomic hybridization) method.

The samples of primary ovarian and cervical tumors, collected over a period of three years, were obtained during surgical procedure or from biopsy specimen, in cases of inoperable tumors. All clinical information, including CA125 tumor marker, were obtained and recorded. The patients studied had not received any cytotoxic therapy prior to cytogenetic study. Written informed consent was obtained from each patient.

A histopathologist examined each tumor and both histological type and tumor 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) [4].

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

For cytogenetic analysis, the disaggregated tumor tissue was processed for short-term culture in two media (BIOAMF-2 complete medium, and Amniomax C100 supplement with Amniomax C100 basal), in the ratio of 1:9 at 37°C in a carbon dioxide incubator. Chromosome slides were prepared in the conventional way. The culture was harvested by subjection to 0.1 ug/ml colcemid treatment (Life Technologies Inc., USA) for 3.5 h, hypotonic treatment with 0.075 M KCl, then fixed in the ratio 3:1 methanol acetic acid and finally, G-banding was performed using Wright's stain solution. The International System for Human Cytogenetic Nomenclature (1995) was used to describe the tumor karyotypes [5].

Fluorescence *in situ* hybridization (FISH) 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 Spectrum Orange Probe (Vysis Inc., USA) were used, according to the manufacture's instructions. A whole chromosome-painting probe is made-up of sequences from the entire length of a given chromosome, labeled with a fluorochrome. Slides were analyzed on an Olympus BX 51 fluorescence microscope with a single band pass exciter filter for UV/DAPI (360 nm), Orange, and Green (Vysis Inc., USA).

DNAs from the tumors were analyzed by comparative genomic hybridization (CGH), to detect DNA sequence copy-number changes (loss or gain). Both tumor and normal reference DNA were labeled with different fluorochromes (green and red) and co-hybridized to normal metaphase chromosomes. The intensity of the two fluorochromes was compared and a corresponding profile was generated, showing gains and losses along each chromosome.

DNA from the samples was isolated by using the QIAamp® DNA Mini Kit (Qiagen Inc., USA). All CGH procedures were performed using reagents and kits produced by Vysis Inc., USA, following the manufacturer's instructions. Cell line DNA was labeled using the CGH nick translation kit. The hybridization mixture was prepared according to the CGH reagent kit, consisting of 200 ng of spectral-green labeled cell line DNA, 100 ng of spectrum-red labeled normal female reference DNA and 10 ug of human Cot-1 DNA. Hybridization was performed for 3 days at 37°C on normal female metaphase spreads (Vysis Inc., USA).

Ten to fifteen images were collected using LUCIA software (Laboratory Imaging Ltd, Czech Republic) with a LUCIA-CGH module, using a ≥ 1.2 ratio threshold for detecting DNA gains and ≤ 0.8 for losses, with a 95% confidence limit. Slides were analyzed on an Olympus BX 51 fluorescence microscope with a single band pass exciter filter for UV/DAPI (360 nm), Orange, and Green (Vysis Inc., USA).

Sex chromosomes and heterochromatic areas (centromeric and paracentromeric regions of chromosomes 1, 9, 16 p arms of acrocentric chromosomes) were excluded from the analyses.

In spite of the limitations of the conventional cytogenetic karyotyping method (still used at present time), in our study, two approaches were compared – the method of direct processing and the method of short-time culture, in order to estimate numerical and structural rearrangements of all chromosomes. The efficiency of these approaches was observed to be low. Both 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 (comparative genomic hybridization) proved to be the most reliable and suitable method for determining 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.

The statistical evaluation used the analysis of variances and χ^2 test and evaluated within the tested group of ovarian cancers, both the quantitative variables (age, CA125 before diagnosis, MIB-1 HSCORE, and *p53* HSCORE), as well as the qualitative parameters. The qualitative parameters in the

ovarian cancer group included: FIGO stage, histological type, grade, presence or absence of tumor residuum after 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 rearrangements. Response rates (RR) were the following: complete response (CR), partial response (PR) and progression of disease (PD) – all during the first year after diagnosis and treatment. Chromosomal rearrangements were divided into the following: none, small (1-7 aberrations), and large (more than 7 aberrations).

In the cervical cancer group, the quantitative variables included: age, MIB-1 HSCORE, *p53* HSCORE; among qualitative parameters the following were evaluated: stage, histological type, grade, response rate (RR), complete response (CR), partial response (PR), progression of disease (PD), chromosomal rearrangement: yes (big) or no (absent), *p53* HSCORE (positive, negative).

The statistical evaluation (ANOVA, chi-squared test) was provided using SPSS 13.0 software.

As many variables were compared, for post hoc pairwise mean comparisons in a one-way analysis of variances the Bonnferroni correlation of significance level was used.

The number of statistically evaluated cases in any particular parameter 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 problems (in some cases a small amount of tumor tissue and unsuccessful culture), chromosomal rearrangements were evaluated in 20 patients in the cervical cancer group and 47 patients in the ovarian cancer group.

Conventional cytogenetic karyotyping was successful in 17 patients (85%) in the cervical cancer group and in 35 patients from the 40 tested patients (87.5%) in the ovarian cancer group. In order to compare the results obtained by application of different methods and to evaluate the advantages and disadvantages of these different approaches, tumor samples were analyzed concurrently by the FISH method (6 patients (30%)), with previous successful conventional karyotyping, in the cervical cancer group and 6 patients (10% of the whole group), in the ovarian cancer group, with previous successful conventional karyotyping. The CGH method was applied in 4 patients (20%) from the cervical cancer group (three cases of unsuccessful conventional karyotyping, one case of successful conventional karyotyping) and in 12 patients (20%) of unsuccessful conventional karyotyping in the ovarian cancer samples. The concentration of isolated DNA was observed to be extremely low in 13 cases in the ovarian cancer group and these cases were excluded from genetic examination. All of these figures were both consistently and completely considered for statistical processing.

The number of diploid tumors was considerably high in our study. It could be connected with the procedure of obtaining the tissue samples (a clear malignant tumor can contain connective tissue components). The results presented were obtained from both the methods – conventional and molecular ones. The FISH method was used to specify results achieved from the conventional method. The CGH method was used in the cases of unsuccessful culture. The number of studied metaphases varied from 3 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 the normal findings, we evaluated 30 metaphases.

Results

Ovarian cancer group. Patients in the ovarian cancer group were aged 39-81 years at the time of diagnosis (median 61).

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

Family history with the presence of oncological disease occurred in 7 patients (11.7%) (lung cancer, cancer of the pancreas, hematological malignancies, and brain tumors). In 6 patients (10%), another tumor occurred before the diagnosis of ovarian cancer – 3 patients suffered from breast cancer (ages 60, 77, 73 years), 1 patient from colorectal cancer (age 52 years) and 2 patients suffered from primary endometrioid cancer of the uterus (ages 43, 67 years).

Three patients from the ovarian cancer group were sent to a laboratory providing mutation analysis of *BRCA1* and *BRCA2* genes. The choice of patients was regulated following the criteria of the national consensus of indications for genetic examination in breast and ovarian cancer patients and their relatives [6].

We found the *BRCA1* mutation in one patient.

Conventional cytogenetic karyotyping was successful in 35 from the 40 tested patients (87.5%). In order to compare

Tab. 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 adenocarcinoma	51	85%
	G1	8	(15.7%)
	G2	7	(13.7%)
	G3	36	(70.6%)
	mucinous adenocarcinoma	3	5%
	G1	2	(66.7%)
	G2	1	(33.3%)
	G3	0	
	endometrioid adenocarcinoma	5	8.3%
	G1	1	(20%)
	G2	3	(60%)
	G3	1	(20%)
	undifferentiated	1	1.7%
	total	60	100%

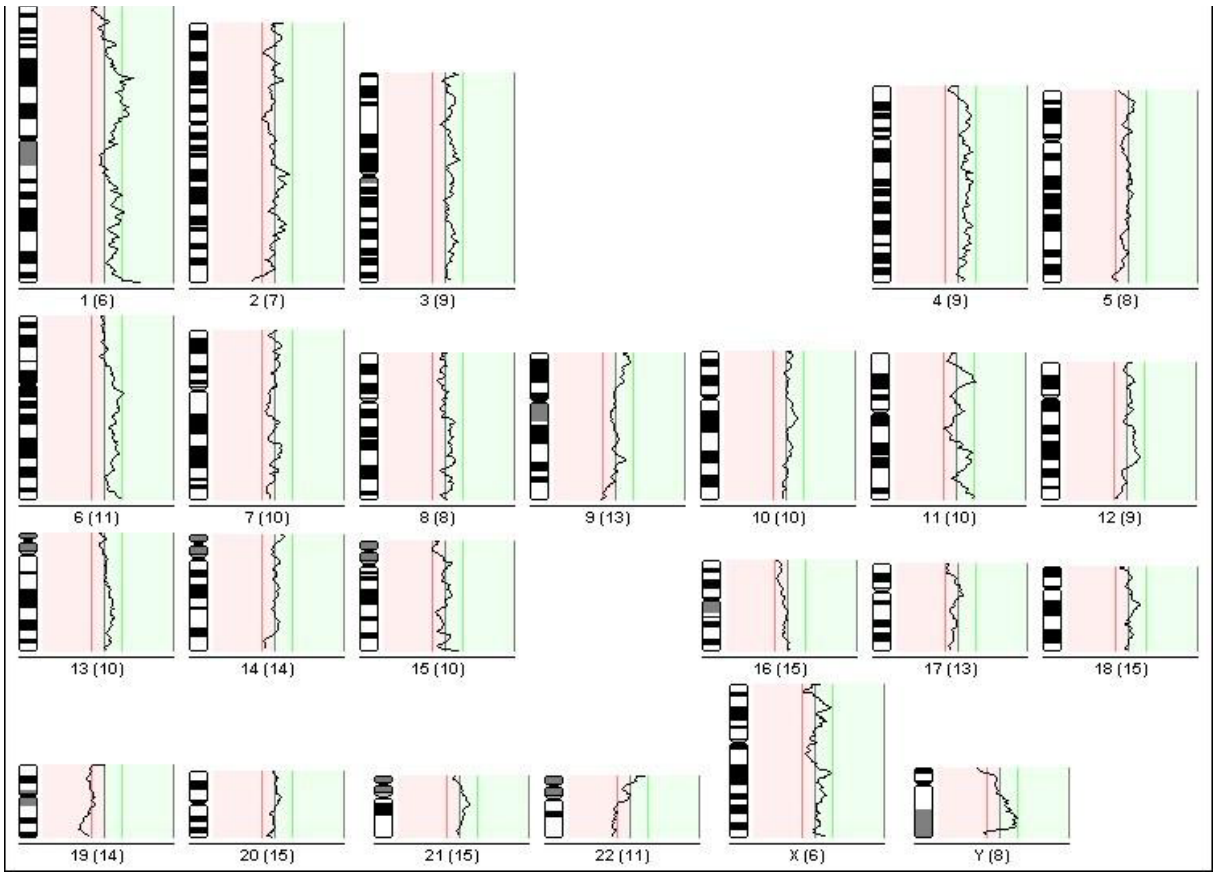

Fig. 1 Patient No 37, carcinoma of ovary, FIGO IIIA. A well-differentiated serous cystadenocarcinoma. A rare amplification 1p, considerable deletion 19q and deletion 22q (In the group of patients examined, detected in 36%; quite rare in terms of the available references).

the results obtained from applying different methods and to evaluate the advantages and disadvantages of these different approaches, tumor samples were analyzed concurrently by the FISH method in 6 patients -10% of the whole group, with previous successful conventional karyotyping. The CGH method was applied in 12 patients (20%) of unsuccessful conventional karyotyping. The concentration of isolated DNA was extremely low in 13 cases in the ovarian cancer group and these cases were excluded from genetic examination.

Tab. 2 The most frequent genetic alterations in ovarian cancer group, and methods of their detection.

GENETIC ALTERATIONS		N (total 47)	%	Methods	
				Conv.*	CGH
Amplifications	1q	17	36.2	yes	yes
	3q	8	17	yes	yes
	20q	8	17	no	yes
Deletions	4p	8	17	yes	yes
	4q	8	17	yes	yes
	18p	4	8.5	no	yes
	18q	4	8.5	yes	yes
	19q	4	8.5	no	yes
	22q	17	36.2	yes	yes
Translocations	t(10;15)	2	4.3	yes	no

* conventional karyotyping

Genetic findings. Numerical and structural aberrations were detected in more than 63% of ovarian cancer cases. The most frequent structural aberrations were unbalanced translocations and deletions in both ovarian and cervical cancer groups. Highly complex and abnormal karyotypes were discovered in the analyses accomplished. The number of chromosomes ranged from 63 to 85. Using CGH analysis, deletions were a more common finding than amplifications. This may be connected with the fact that deletions of the particular chromosomes reflected the role of the tumor suppressor genes, which are located in special regions and play an important role in the pathogenesis of ovarian cancer.

Amplifications, typical in ovarian cancer, were found on chromosomes 1q in 17 (36.2%) examined cases, 3q in 8 (17%) cases and 20q in 8 (17%) cases, deletions on chromosomes 4p, 4q in 8 (17%) cases each, 18p, 18q in 4 (8.5%) cases each and 19q in 4 (8.5%) cases (Tab. 2).

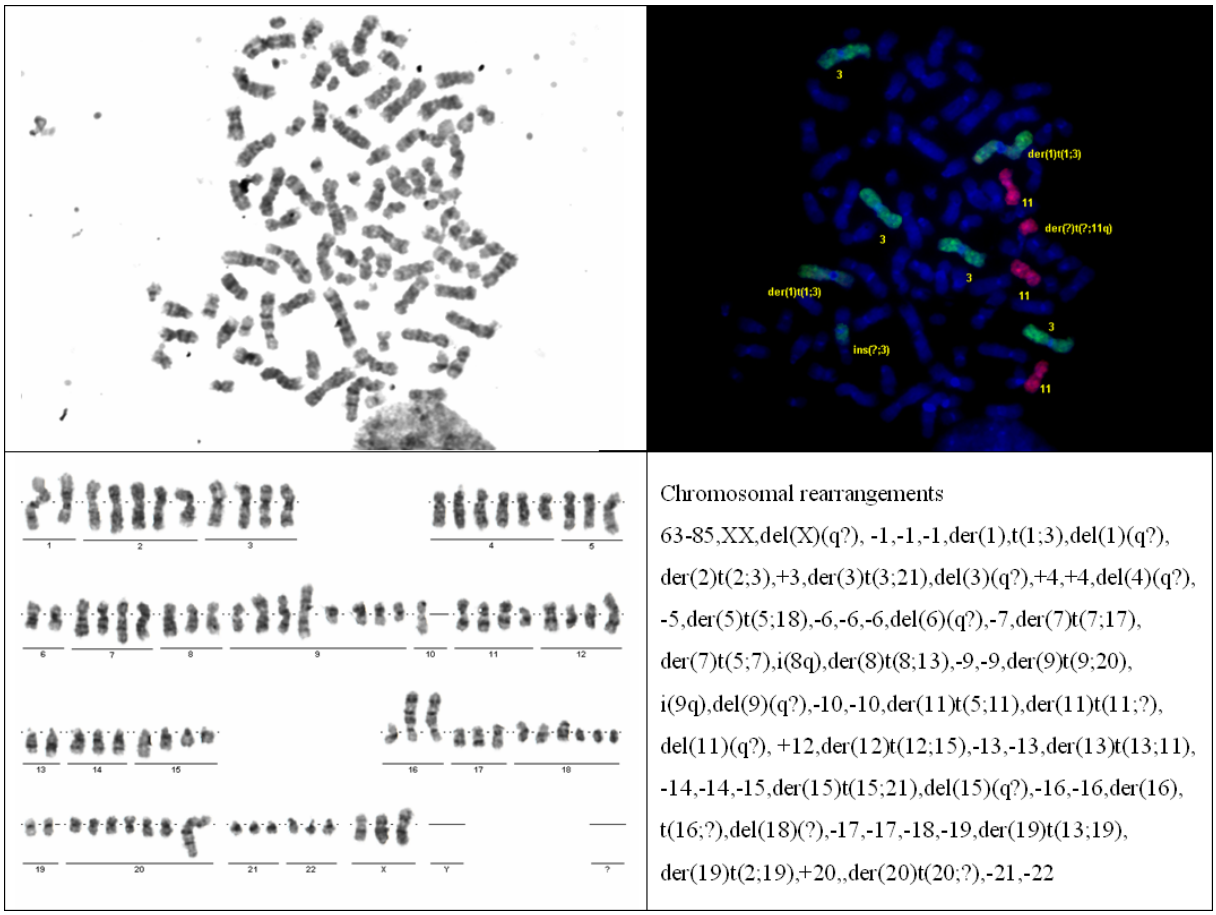

Fig. 2 Patient No 01 – carcinoma of ovary, FIGO IVB. The chromosomal finding corresponds with the advanced stage. Considerable chromosomal rearrangements – numerical and structural – were detected. The number of chromosomes is 63-85, the number of aberrant chromosomes is higher than seven, e.g. the number of aberrations with a suggested favorable prognosis. Histological finding: serous papillary cystadenocarcinoma, grade 2. The patient is a BRCA1 mutation carrier (five-year survival, partial treatment response).

Deletion 22q was found in 17 (36.2%) cases; such finding however was quite rare describing in the available references (Fig. 1).

The isolated balanced translocation t(10;15) was found in 2 of the examined cases (4.3%) (Tab. 2). This finding was found in all the examined cells. A constitutional translocation was excluded, by examination of the peripheral blood lymphocytes cultures. An examination of chromosomal breakpoints was not performed.

All other findings occurred in less than 5% (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 the examined tumors, extensive changes were found. The number of aberrant chromosomes was greater than seven (Fig. 2). In 37%, a diploid karyotype was found.

The relations of clinical, histopathological and molecular paramaters. The statistically significant associations of *quantitative* variables in the *ovarian* cancer group were the following:

1. Women with FIGO stage I are significantly ($p < 0.01$) older (median age 74) than women with advanced stages II-IV (median ages 59, 61, 60). A lower aggressiveness, including slow tumor growth, is suggested. No other correlation of quantitative parameters (CA125 before diagnosis, MIB-1 HSCORE, *p53* HSCORE) and stage were found.
2. Women with tumor grade 1 have significantly ($p < 0.05$) lower MIB-1 HSCORE (141), in comparison with women that have tumor grade 2 (209) or grade 3 (195).
3. Women with tumor grade 1 have significantly ($p < 0.05$) lower *p53* HSCORE (126), in comparison with women who have grade 2 (236) and grade 3 (173).
4. Women with a high p53 HSCORE were found to have a high MIB-1 HSCORE also ($p < 0.001$), in comparison with women with a low *p53* HSCORE.
5. Women with large number of chromosomal rearrangements (more than 7) are younger (median age 54) than women with small number of rearrangements, i.e. 1-7 (median age 60) or no rearrangements (median age 66) ($p < 0.1$).

Tab. 3 The structure of cervical cancer group (FIGO stage, histology, grade)

PARAMETER		N	%
FIGO stage	I	6	30%
	II	6	30%
	III	6	30%
	IV	2	10%
	total	20	100%
HISTOLOGY, GRADE:	squamous cell carcinoma	20	100%
	G1	0	
	G2	8	40%
	G3	12	60%
	total	20	100%

Tab. 4 The most frequent genetic alterations in cervical cancer group, and methods of their detection

GENETIC ALTERATIONS		N (total 20)	%	Methods	
				conv.*	CGH
Amplifications	3q	12	60	yes	yes
	3p	8	40	yes	yes
	5p	4	20	no	yes
	20p	4	20	no	yes
	22q	4	20	no	yes
Deletions	13q	4	20	yes	no
	2q	4	20	yes	no
	6q	4	20	yes	no
Isochromosome	5p	8	40	yes	no

*conventional karyotyping

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

1. The stage of the disease and differentiation of tumor (grade) are dependent on the $p < 0.001$ significance level (from χ^2 statistic).
2. The stage of the disease and presence of tumor residuum after surgical procedure are dependent on the $p < 0.001$ significance level.
3. The histological type of tumor and grade are dependent on the $p < 0.05$ significance level.
4. The tumor differentiation (grade) and the presence of tumor residuum after surgical procedure are dependent on the $p < 0.001$ significance level.
5. The tumor differentiation (grade) and response rate (RR: CR complete response, PR partial response, PD progression of disease) are dependent on the $p < 0.01$ significance level.
6. The tumor residuum after surgical procedure and RR are dependent on the $p < 0.05$ significance level.
7. The tumor residuum after surgical procedure and level of tumor marker CA125 after treatment are dependent on the $p < 0.05$ significance level.

An association for the genetic findings (*chromosomal rearrangements*), in the ovarian cancer group, was found to be just within the group of quantitative variables (age). No statistically significant association was found between the genetic findings and the qualitative variables.

Cervical cancer group. Patients in the *cervical* cancer group were aged 39-84 at the time of diagnosis (median 52 years).

The Tab. 3 presents the structure of the ovarian cancer group (FIGO stage, histology, grade).

All women from this small group had their last gynecological examination 5-20 years prior to cervical cancer diagnosis was made. All women were cigarette smokers.

Conventional cytogenetic karyotyping was successful in 17 patients (85%). The tumor samples were analyzed concurrently by the FISH method (6 patients (30%)), with previous successful conventional karyotyping; The CGH method was applied in 4 patients (20%) – three cases of unsuccessful conventional karyotyping and one case of successful conventional karyotyping.

Numerical and structural aberrations were detected in 29% of *cervical* cancer cases. The number chromosomes of cancer cells ranged from 44 to 82.

Genetic findings. The most frequent findings in the *cervical* cancer cells were amplifications 3q, found in 12 (60%) examined cases and isochromosome 5p in 8 (40%) examined cases. Less frequently was found the amplification – in 4 (20%) cases; and deletions 13q and 2q were found in 4 (20%) cases. In advanced cervical tumors, great numerical and structural rearrangements were found on the following chromosomes: amplifications 3p in 8 (40%) cases, 5p in 4 (20%) cases, 20p in 4 (20%) cases, 22q in 4 (20%) cases and deletions 13q in 4 (20%) cases, 6q in 4 (20%) cases, and isochromosome 5p in 8 (40%) cases – as typical findings for cervical tumors (Tab. 4). The unique amplification of chromosome 2p, and 2q (in one case), and the rare deletions of chromosomes 10p, 10q, 11p, and 21q were found in our study. In 71% of the cases, diploid karyotypes were found.

The relations of clinical, histopathological and molecular paramaters

In the cervical cancer group, just two significant associations were found between the evaluated *quantitative* parameters:

1. Women with FIGO stage IV are significantly ($p < 0.05$) older (median age 80) than women at stages I-III (median age 55, 51, 54).
2. Women with tumor grade 2 have significantly ($p < 0.1$) higher *p53* HSCORE in comparison with women with grade 3.

No statistically significant associations among all possible couples of *qualitative* variables in cervical cancer group were found.

Genetic alterations and their relations to selected parameters in ovarian and cervical cancer groups. As the study was partly focused on genetic changes in tumor cells, our attention was concentrated on all hypothetic relations of chromosomal rearrangement with the above-mentioned parameters of quantitative and qualitative character.

We divided the *ovarian* cancer patients into the groups: no aberrations , a small number of aberrations (1-7) and a large number of aberrations (more than 7). These three groups of chromosomal rearrangements were correlated to age, FIGO stage, histological type and grade, RR (response rate), CA125 level, MIB-1 HSCORE and *p53* HSCORE, remaining surgical residuum, and nodal metastases. Despite some promising relationships, no statistically significant conclusions were found, except as mentioned above. The cases with minimal or no chromosomal rearrangements will be submitted to further follow-ups in order to evaluate significance of small numbers of aberrations.

Among the *cervical* cancer patients, divided into two groups (with or without chromosomal rearrangements), no significant relationships of genetic changes or of other variables, of either a quantitative or qualitative character were found.

We neither evaluate correlations between the severity of chromosomal rearrangement nor the overall survival, because of the short follow- up time of all patients participating in the study (one year).

The mean values of MIB1-HSCORE were similar in both the *ovarian* and *cervical* cancer groups (187.8, 209.3). The mean values of *p53* HSCORE were significantly higher in ovarian cancer cells, than in cervical cancer cells (180.5, 119.2). It appeared that the *p53* HSCORE is a more important marker than the MIB-1 HSCORE; its importance is obvious in the light of the significant associations with other quantitative and qualitative variables, as described above.

The genetic changes seem to be less extensive in the cervical cancer group, in comparison with the ovarian cancer group. It may be related to the different conditions in the obtaining of the tumor tissues, small numbers of patients in the cervical cancer group, as well as the different pathways of carcinogenesis.

Discussion

The genetic profile of the tumor and knowledge of particular molecular factors (which we are able to detect due to the last years' progress in biotechnology), are believed to be able to help predict prognosis for the course of the disease. They are suggested to be predictive of response to treatment modality, to present a target for therapy, as well as to monitor activity of the tumor disease.

Cytogenetic analyses of the cervical carcinomas (using different methods, as referred from different laboratories) have revealed numerous structural and numerical aberrations. However, primary aberrations have not been found yet.

Amplifications are predominantly found on chromosomes 1q, 3q, 5p, 8q, 17q and 20q. Deletions occur predominantly on chromosomes 2q, 3p, 4p, 6q, 11q, 13q, 17q, 18q and Xq. The aberrations have been found in tumors of different stages, including pre-malignant lesions – CIN (cervical intraepithelial neoplasia). The findings in pre-malignant lesions may refer to the malignant potential of these lesions and to the high probability of cancer development [7, 8, 9, 10].

Amplification 3q (3q24-3q28) is the most frequent alteration that has been found in cervical cancer cells, as well as in high grade pre-malignant lesions and initial carcinomas. It is in the region of the *TERC* gene (telomerase gene), with a role in the immortalization of cells that is a genetic marker for risk prediction. The gain of 3q was a typical finding in women with a pre-malignant lesion developing into invasive carcinoma [11, 12, 13, 14]. It is suggested that the loss of tumor suppressor genes on chromosomes 3p, 9p, 11p and 18q is associated with a worse prognosis [15, 16]. Deletion of 3p is another typical finding in invasive and metastatic cervical carcinomas [17]. The importance and associations of the inactivated tumor suppressor gene *p53* (17p13.3) to the development of cervical cancer are suggested in the co-existence of HPV infections. HPV (human papilloma virus) infection is considered the most important factor in cervical carcinogenesis, although its presence alone is not sufficient for tumor development. The association of an HPV infection with the specific alterations has not been found [18]. A high number of chromosomal aberrations are associated with the advanced stage of the tumor and a worse prognosis for the disease, including those tumors with metastatic spread. Deletions 11p and 18q are also associated with an outcome being worse [19]. Another typical finding for cervical tumors, detected by conventional cytogenetic studies as well as by the molecular cytogenetic techniques, is the presence of isochromosome 5p [20].

The most frequent finding in our study was the amplification on chromosomes 3q and 5p, and deletion 13q. These findings correspond with those described in other studies. Advanced stages were associated with the higher number of chromosomal aberrations; in our study, this finding was not statistically significant. We observed the rare finding in our study of the deletion of chromosome 10 and another unique finding of the amplification of chromosome 2 at an early stage (IB).

A high and increasing number of molecular biological studies on cancer of the ovary have been published in the last few years. That the genetic loss of the *BRCA1/2* genes is important in the pathogenesis of both hereditary breast and ovarian cancer is well established. However, the role of *BRCA1/2* genes in non-familial cancers, which represent the majority of these diseases, is not yet clear. Women who carry mutations in the *BRCA1* gene develop ovarian cancer at a younger median age, compared to non-carriers. A series of studies has implicated *BRCA1* mutations as both a favorable and an unfavorable prognostic factor; a U.S. case control study showed a highly significant survival advantage in *BRCA1*-carriers patients affected by advanced ovarian cancer [21].

The only patient in our study with the *BRCA1* mutation, FIGO stage IVB, survived for over five years; it corresponds with the opinions of a survival advantage of *BRCA1*-carriers. However, in this case it is just a case-report.

Amplifications, typical for epithelial ovarian cancer cells, have been found on chromosomes 1q, 3q, 8q and 20q; deletions occur predominantly on chromosomes 4q, 13q, 16q, and 18q. Complex aberrations have been found in mucinous and endometrioid tumors; in benign and low malignant potential tumors, aberrations do not occur. Moderate aberrations are found in well-differentiated tumors, while complex rearrangements are typical for undifferentiated tumors. Serous tumors of advanced stages contain twice as many aberrations than do tumors of early-stage carcinomas. Amplifications have been found on chromosomes 3q, 6p, 7, 8q and 20; with deletions on 4q, 6q, 12q, 13q and 16q. Common aberrations for different histological types are amplifications 3q, 6p and deletion 4q. Aberrations related to a worse prognosis were amplifications 6p, 7q, 13q and deletions 15q, 17p, 18q and 21q [22, 23, 24]. Relationships between the number of aberrations, stage and overall survival rate have been found [25]. Inactivation of the tumor suppressor genes is detected more often than the activation of oncogenes. It is not clear whether this is due to the method applied or to the more common pathway in carcinogenesis.

Comparisons of both early-stage and advanced tumors have revealed some differences between both groups: deletions were more frequent than amplifications in early-staged tumors. Typical deletions were the following: 2q, 4q, 5q, 6q, 13q and 18q. Amplifications have been found particularly at advanced stages – on chromosomes 3q, 8q, 11q, 12p, 17q and 20q [26].

Loss of genetic material on chromosome 4 was a typical finding at the advanced stage [27]. Different histological types do not differ in specific aberrations; complex aberrations are rare in mucinous and endometrioid tumors. Moderate aberrations are found in well-differentiated tumors; complex rearrangements are typical of poorly differentiated tumors [28, 29, 30].

Patients with tumors containing less than 7 aberrations have a better survival time, these patients are suggested to have better treatment response. Tumors with amplifications 1p, 10p, 20q and deletion 5q are at a greater risk of a recurrence. The results indicate that the number of the chromosomal aberrations may be associated with the survival time of the patients [31]. The data indicate that tumors of low malignant potential and invasive carcinomas include different aberrations and so they may be considered two different groups of ovarian tumors [32, 33, 34]. The borderline tumors seem to be more similar to benign tumors than to malignant tumors with respect to their genetic profiles. The issue of whether borderline tumors are precursors of invasive carcinoma or distinct clinical entities, however, is still the subject of discussion. The distinct cytogenetic alterations could be early events of serous ovarian tumors and could also characterize a subgroup of borderline ovarian tumors that may have potential to progress and develop malignancy [35, 36]. The comparison of primary and metastatic tumors has revealed more aberrations in the group of primary tumors [37]. More recently, an i(5p) was described as a novel recurrent abnormality in ovarian cancer [38].

Cytogenetic analyses of ovarian carcinomas in our study proved the presence of complex karyotypes with a wide range of numerical and structural rearrangements such as fragmented chromosomes, telomeric fusions and complex rearrangements. The number of genetic rearrangements was significantly higher in the group of younger women. Because of a small number of evaluated cases, we hoped that statistical significance on $p < 0.1$ had its special importance too and could show the direction of the future concentrations on these associations. In the groups of higher number of evaluated cases, the significance of $p < 0.01$ or $p < 0.05$ level could be suggested. No other interactions were found between the severity of chromosomal rearrangements and *p53* HSCORE, stage of disease, histological type, grade, response rate, or decline of CA125 after surgical procedure. Significant correlations were found among several other particular parameters of histopathological and clinical characteristics.

On the other hand, some findings were less frequently found in both groups of patients – rare deletions on chromosome 11p and 21p, amplification of chromosome 2 in cervical cancer, as well as the chromosomal finding of an isolated balanced translocation t(10;15) and amplification 1p, deletion 19q, and deletion 22q in ovarian cancer. The interpretation of these particular findings is in conflict; they seem to contribute toward future directions in genetic research – on the molecular basis and with a concentration on special aberrations.

Conclusion

The results presented have to do with the project, which is concentrated upon systematic research of chromosomal aberrations of the gynecological malignant tumors and their correlations with available parameters of both molecular biological and clinical characteristics.

Different methods and approaches were used studying and evaluating genetic alterations in gynecological malignant tumors – from classical cytogenetic procedures to molecular-cytogenetic methods, in order to determine the most suitable approach for the elected research. We compared the methods from various points of view – requirements upon time and financial resources, failure and exploitability. The CGH (comparative genomic hybridization) has been found to be the most suitable contemporary method, supplemented by FISH after a modified short-time culture.

Specific genetic alterations, including some rare findings, have been found both in ovarian and cervical cancer cells: rare deletions on chromosome 11p and 21p; and amplification of chromosome 2 in cervical cancer. There was also found a chromosomal expression of an isolated balanced translocation t(10;15) and amplification 1p, deletion 19q and deletion 22q in ovarian cancer (in the group of patients examined, it was detected in 36%; being quite rare in terms of available references).

The results arising from the statistical evaluations of the appointed parameters informed us about the significant im-

portance of the genetic findings and the activity of proliferative markers, including the common correlation with an unfavorable outcome in ovarian tumors of younger women. Future research ought to be concentrated on just this group of patients and the group of patients with borderline tumors, in order to explain the special process of carcinogenesis with the goal of implementation to both an individual and effective clinical approach.

The study has found a whole series of associations between particular prognostic factors in the examined patients. The role of chromosomal rearrangements and genetic changes, as found in the cancer cells, is not quite so clear and straightforward.

The number of aberrations in ovarian cancer cells seems to be an important prognostic marker, especially when associated with younger age. A better prognosis is associated with less than 7 aberrations found in the cancer cells.

Comparisons of the genetic findings in ovarian and cervical cancer cells point to differences in both tumors; different pathways of carcinogenesis are suggested. The presence of chromosomal rearrangements in pre-malignant lesions – CIN (cervical intraepithelial neoplasia) or early stages revealed the group of women with a high risk of an unfavorable prognosis.

Now, in the light of the results presented, future genetic examinations could be useful in the early stages of cancer disease in younger women, where genetic changes could contribute to an improved prediction of outcome and a greater individualization of therapeutic interventions.

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