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Cancer Genetics and Cytogenetics 170 (2006) 1–8

Cancer Genetics and Cytogenetics

Comparative genome hybridization reveals specific genomic imbalances during the genesis from benign through borderline to malignant ovarian tumors

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Received 23 February 2005; received in revised form 7 April 2006; accepted 11 April 2006

Abstract Ovarian cancer is one of the most common types of malignancy in women throughout the developed world. Despite recent therapeutic advances, long-term survival is poor because ovarian cancer is largely asymptomatic in its early stages. Comparative genomic hybridization (CGH) was applied to a series of 8 benign, 8 borderline, and 17 malignant ovarian to establish genomic imbalances associated with tumor progression. Benign and borderline tumors were characterized by losses at 1p32~p11, 2q14~q34, 4q13~q34, 5q11~q23, and 6q12~q24, as well as gains of 6p and chromosome 12. Similar chromosomal changes were also detected in malignant tumors but included additional chromosomal changes: gains at 1q21~q31, 2p, 3q, 5p, 7, 10p, 12p, 16p, 17, 19q, 20q, and 22q, as well as losses at X, 3p, 8p, 9, 11p, 13, 14, and 18. Some individual cases of benign and borderline tumors revealed no genetic alterations detectable by CGH, suggesting that these tumors may represent a subset of tumors that originate by an alternative mechanism of tumorigenesis. Furthermore, our findings reveal that borderline tumors are more similar to benign tumors than to malignant tumors with respect to their genetic profiles.

1. Introduction

Ovarian cancer has its highest incidence in Scandinavia, Israel, and the United States, and it is the fifth leading cause of cancer deaths among women in the United States (The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins; http://www.hopkinskimmelcancercenter.org/index.cfm). Ovarian tumors fall into three main categories — epithelial tumors (including adenocarcinomas), germ cell tumors, and stromal tumors. Ovarian tumors range from benign to carcinomas, where an intermediate group of tumors referred to as borderline can be distinguished.

The prognosis for ovarian cancer depends on tumor stage. The 5-year survival rates for stages III and IV are 15–20%, and for stages I and II, the survival rates are 70–90% (the National Ovarian Cancer Resource Center, http://www.ovarian.org/default.asp). Nonspecific biochemical markers such as CA 125 are currently being used for the diagnosis of ovarian cancer, but as of yet there are no reliable tests available for detection of early stage ovarian cancer because it is predominantly asymptomatic. Increasing our current knowledge of the genetic events associated with ovarian cancer progression will assist in the development of new prognostic markers and therapeutic agents. Cytogenetic studies have been performed on benign and primary ovarian tumors and reveal predominantly numerical aberrations (e.g., trisomy 12) [1,2]. Ovarian carcinomas, conversely, have highly complex karyotypes displaying numerous nonrandom chromosome changes (e.g., gains of chromosomes 1q, 2, 6p, 7, 8q, 12, 19p, and 20, and losses of chromosomes 1p, 4, 5, 8p, 9p, 11p, and 13 [3,4].

Comparative genomic hybridization (CGH) is a molecular cytogenetic method that detects global DNA sequence copy number changes in tumor genomes [5]. Using CGH, it is possible to measure quantitative changes, which are often difficult to identify and characterize using conventional

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0165-4608/06/$ – see front matter
doi:10.1016/j.cancergencyto.2006.04.010

cytogenetic banding methods that may be restricted by technical limitations such as low mitotic index or insufficient spreading of metaphase chromosomes. This makes CGH an ideal tool for analyzing chromosomal imbalances in solid tumors.

To identify specific DNA copy number changes in chromosomes and chromosome regions that occur during tumor development and progression from the benign through borderline to malignant ovarian tumors, CGH was applied to a series of patient samples consisting of 8 benign, 8 borderline, and 17 malignant ovarian tumors.

2. Materials and methods

2.1. Tumor samples

Frozen material from 33 ovarian tumors was collected at the Karolinska Hospital in Stockholm, Sweden. The tumors were subjected to histopathologic characterization using hematoxylin and eosin–stained sections of formalin-fixed, paraffin-embedded specimens. Tumors were classified using the WHO system [6]. The clinical data for each tumor sample is summarized in Table 1: 17 tumors were classified as malignant, 8 as borderline, and 8 as benign.

2.2. DNA extraction and metaphase chromosome preparations

DNA was successfully extracted from the 33 tumors according to standard procedures using phenol/chloroform extraction (http://www.riedlab.nci.nih.gov/protocols.asp). Normal reference DNA was prepared from human female lymphocytes using the QIAamp DNA Blood Kit (Qiagen, Valencia, CA). Normal metaphase slides for CGH were prepared from healthy female peripheral blood cultures. The entire CGH experiment was carried out using the same batch of slides.

2.3. CGH

CGH was carried out as described previously (http://www.riedlab.nci.nih.gov/protocols). Tumor and control DNA were labeled by nick translation with biotin dUTP and digoxygenin-11-dUTP (DIG); 1–2 μg of nick-translated test and control DNA were coprecipitated in 3 mol/L

Table 1
Clinical and histopathologic characteristics of samples

Sample	True type (A/B/C)	Pathologic diagnosis	Tumor size (cm)
OC14	A	Serous cystadenoma IA	14
OC19	A	Serous cystadenoma IA	8
OC34	A	Serous cystadenoma IA	15
OC38	A	Serous cystadenoma IA	9
OC82	A	Cystadenofibroma	
OC95	A	Serous cystadenoma IA	
OC66	A	Serous cystadenoma IA	
OC90	A	Serous cystadenofibroma	
OC39	B	Borderline seropapillary IB	13
OC46	B	Borderline seropapillary IB	
OC50	B	Borderline seropapillary IB	10
OC21	B	Borderline mucinous IIB	19
OC59	B	Borderline mucinous IIB	19
OC72	B	Borderline serous IB	
OC77	B	Borderline serous IB	
OC96	B	Borderline serous IB	
OC07	C	Serous papillary adenocarcinoma IC	8
OC08	C	Serous papillary adenocarcinoma IC	9
OC09	C	Serous papillary adenocarcinoma IC	8
OC20	C	Serous papillary adenocarcinoma IC	11
OC30	C	Serous papillary adenocarcinoma IC	14
OC40	C	Serous papillary adenocarcinoma IC	
OC43	C	Serous papillary adenocarcinoma IC	12
OC04	C	Mixed tumor	25
OC06	C	Clear cell tumor IVC	10
OC27	C	Clear cell tumor IVC	16
OC33	C	Endometrioid carcinoma IIIC	16
OC48	C	Serous papillary adenocarcinoma IC	12
OC49	C	Endometrioid carcinoma IIIC	16
OC84	C	Clear cell tumor IVC	7
OC74	C	Endometrioid carcinoma IIIC	17
OC73	C	Serous papillary adenocarcinoma IC	16
OC89	C	Serous papillary adenocarcinoma IC	7

A, benign; B, borderline; C, malignant.

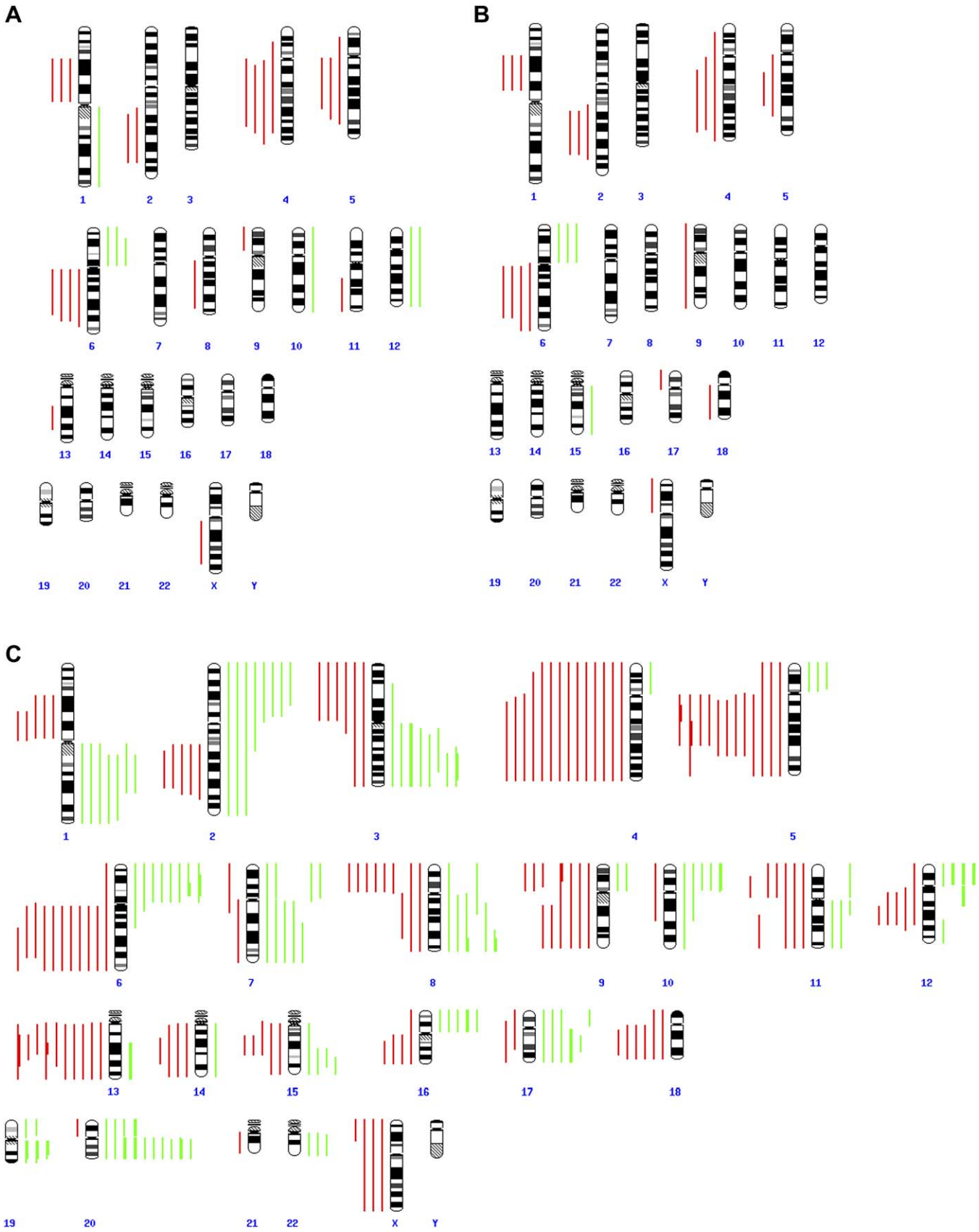
A
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y
B
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y
C
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X Y

Table 2
Comparison of frequent CGH alterations of different subtypes in this study and previous CGH studies

Ovarian subtype	CGH results:current study	CGH results:other studies	Reference nos.
Serous	Loss: 1p32~p11, 4q13~q34, 5q11~q23, 6q12~q23 Gain: 1q, 3q, 6p	Similar to our studies except loss of 1p32~p11 and gain of 5q, 8q	[17,20,35–38]
Endometrioid	Loss: 2q14~q34, 3p, 4, 5q11~q23, 6q, 9p, 12q, 13q Gain: 15q, 17, 20q	Loss: 4, 18 Gain: 1q, 3q, 7q, 10q, 20	[17,35,36]
Clear cell	Loss: 6q, 8p, 9p, 13q Gain: 2q, 8q, 16p, 17q, 20q	Loss: 13, 15, 18 Gain: 2, 8	[17,38]
Mucinous	Loss: 6q, 9, 17p, 18q, Xp Gain: 15q	Loss: 9, 20q Gain: 2q, 3q, 5, 13q	[38]

sodium acetate and ethanol using an excess of human Cot-1 DNA (Invitrogen, Carlsbad, CA) and salmon sperm DNA (Sigma, St. Louis, MO). The precipitate was resuspended in 5 μL deionized formamide (pH 7.5) at 37°C for 1 hour, and then an equal volume of master mix (20% dextran sulfate and 2× standard saline citrate, pH 7.0) was added to the probes, followed by incubation at 37°C for 30 minutes. Probes were denatured at 80°C for 5 minutes, followed by pre-annealing at 37°C for 1–2 hours. Probes were hybridized onto metaphase slides for 72 hours at 37°C and detected with avidin-fluorescein (FITC) antibody (Vector Laboratories, Burlingame, CA) for the test DNA and mouse monoclonal anti-digoxigenin (Sigma), tetramethylrhodamine isothiocyanate (TRITC)-conjugated rabbit anti-mouse antibody for the control DNA. Chromosomes were stained with 4',6-diamidino-2-phenylindole (DAPI). Ten to 15 metaphases per case were imaged using a Leica (Cambridge, UK) DM RXA microscope with a cooled CCD camera (Sensys; Roper Scientific, Tucson, AZ). The ratio between the FITC and TRITC intensities was analyzed with CW 4000 software from Leica. FITC/TRITC ratios above 1.2 were defined as gains and those above 1.4 were defined as amplifications. Ratios below 0.8 defined a loss of genomic material.

3. Results

Recurrent losses of chromosomal material in 1p32~p11, 2q14~q34, 4q13~q34, 5q11~q23, and 6q12~q23, as well as gain of 6p and chromosome 12, were detected in benign and borderline tumors (Fig. 1, A and B). In three cases of benign and four cases of borderline tumors, no copy number changes were detected. We noted that losses were more frequent than gains in both benign and borderline tumors, and that the average numbers of chromosomal changes for benign tumors were 5.25 losses and 1.75 gains, and for borderline tumors, 6.33 losses and 1.33 gains.

The chromosomal changes detected in benign and borderline tumors were also recurrent in malignant ovarian tumors with additional chromosomal changes present, such as gains at 1q21~q31, 2p, 3q, 5p, 7, 8q, 10p, 12p, 16p, 17, 19, 20, and 22q, and losses at X, 2q22~q34, 3p, 4, 5q11~q31, 6q, 8p, 9, 11p, 13, 14, and 18. All chromosomes were involved in copy number variation in the malignant tumors (Fig. 1C). The average numbers of chromosomal aberrations detected in the malignant tumors were 7.17 losses and 5.58 gains. Fig. 1 summarizes the DNA copy number changes for all tumor types, and individual CGH profiles are displayed at http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi [7].

4. Discussion

Several reports suggest that the occurrence and progression of neoplastic disease require multiple genetic events, ranging from DNA mutations to large chromosomal rearrangements, occurring sequentially in a cell lineage [8]. A number of theories supporting different models for ovarian cancer have been proposed. One theory suggests that benign, borderline, and malignant tumors are unrelated entities, each arising de novo from the ovarian epithelium [9,10]. A second theory suggesting tumor progression of a portion of ovarian tumors commences with benign, evolving through borderline to malignant [11].

In the present investigation, CGH was used to analyze chromosomal aberrations that occur during the transition from benign through borderline to malignant ovarian tumors. Invasive ovarian carcinoma has been extensively investigated using karyotypic and CGH techniques [10,12–18]. The few analyzed cases of benign and borderline tumors have suggested that gains of 8q and loss of 9p represent early changes in ovarian cancer progression [19,20]. No gains of 8q were seen in our benign or borderline tumors, and only one benign tumor in our study had loss of 9p.

Fig. 1. Summary of all chromosomal changes detected by the CGH analysis. (A) The analysis of 8 benign ovary tumors. (B) The analysis of 8 borderline tumors. (C) The analysis of 17 malignant ovary tumors. Bars to the right of each chromosomal ideogram represent chromosomal gain, and bars to the left represent chromosomal loss.

Trisomy 12 has been reported in benign ovarian tumors [19,21–25]. The authors have concluded that this aberration may predict a higher risk for developing malignant tumors. This numerical aberration was detected in two out of eight benign tumors in this study.

When results from the analysis of the 17 malignant tumors were combined with those from the 8 borderline and 8 benign tumors, a very clear pattern emerged, as shown in Fig. 1. A subset of chromosomal imbalances (regional losses of 1p, 4q, 5q, 6q, as well as gains of 6p) were maintained throughout progression, whereas the malignant group increased in overall numbers and complexity of genomic imbalances, suggesting that borderline tumors can be classified as being closer to benign than to malignant tumors. The malignant group had recurrent gains of 2p, 5p, 7, 10p, 15q, 16p, and 17, as well as amplifications detected at 3q21~q28, 6p21.1~p11, 8q23~q24, 12p13~p11, and 19q11~q13. Similar regional chromosomal amplifications were detected in a previous study but occurred at higher frequencies [26] (http://www.helsinki.fi/cmg/cgh_data.html). In the four analyzed subtypes, substantial differences and similarities in regional chromosomal alterations were detected. A comparison of our results with those reported by other investigators is shown in Table 2. The

Table 3
Comparison of CGH alterations in this study and the Progenetix database

	Present CGH study (% abnormal)			
Chromosome region	Benign (8)	Borderline (8)	Malignant (17)	Progenetix CGH (% abnormal) (320)
Loss				
1p	38	38	38	11
2q	25	38	29	4
3p	–	–	35	5
4	–	–	35	12
4q	50	25	65	20
5q	25	25	53	18
5q low loss	13	–	24	NA
6q	50	50	65	11
8p	–	–	47	9
9	13	13	47	11
11p	–	–	35	6
12q	–	–	29	3
13	13	–	59	13
14	–	–	24	4
15	–	–	29	9
16	–	–	24	13
18	–	13	35	14
22	–	–	24	16
X	13	13	24	9
Gain				
1q	13	–	41	19
2	–	–	18	14
2p	–	–	47	12
3q	–	–	47 (amp 12)	27 (amp 4)
5p	–	–	18	12
6p	38	38	47	14
7p	–	–	24	7
7q	–	–	29	14
8q	–	–	35 (amp 12)	35 (amp 5)
10p	13	–	29	9
11q	–	–	18	6
12	25	–	–	10
12p	25	–	24 (amp 18)	16 (amp 2)
15	–	13	24	6
16p	–	–	29	8
17	–	–	18	4
17q	–	–	12 (amp 6)	10 (amp 1)
19p	–	–	12	6
19q	–	–	18	6
20	–	–	24	14
20q	–	–	59 (amp 18)	22 (amp 2)
22q	–	–	18	5

The numbers in parentheses indicate the number of cases.
All figures in the table are percentages of cases involved, including the cases demonstrating amplification or low loss.

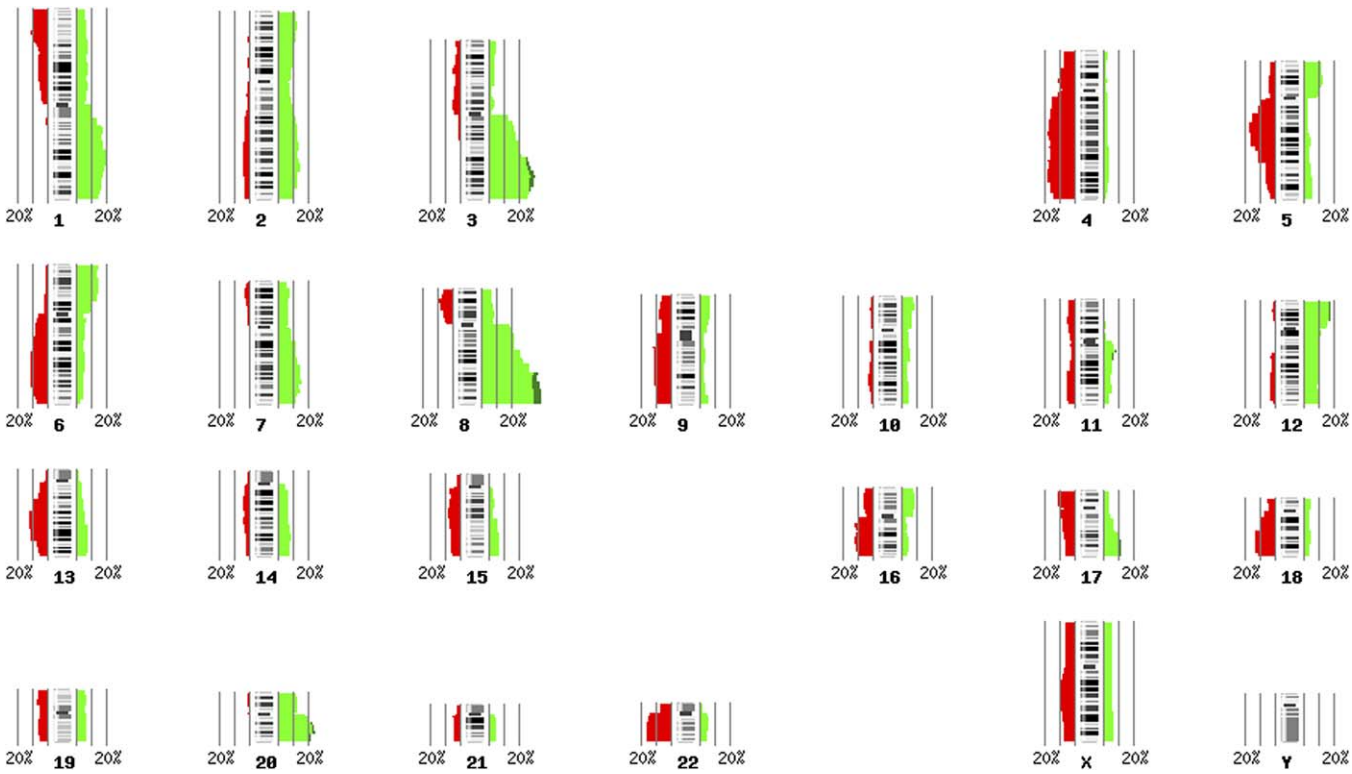

Fig. 2. CGH profile generated from 320 cases of ovarian cancer included in the Progenetix online database [27]. The ideogram shows the summary of imbalances in all cases analyzed by CGH. Green represents gains, and red shows losses (percent per band). See Table 4 for a list of all ovarian tumor diagnoses included in this figure.

discrepancies between our results and those of others may be due to the small number of endometrioid ovarian carcinomas (threee cases), clear-cell carcinomas (three cases), and mucinous tumors (two cases) included in our study.

Table 3 shows the comparison among the most frequent copy number gains and losses detected in our study with CGH profiles from 320 other ovarian tumor cases summarized in http://www.progenetix.net/ [27] and shown in Fig. 2; the morphology and International Classification of Diseases codes for the 320 cases are listed in Table 3. All the copy number changes identified in previous studies were also detected in a previous study but occurred here

Table 4
Morphology and ICD codes for 320 ovarian tumors included in the Progenetix database

ICD Code	Morphology	No of cases
8010/3	Carcinoma, NOS	2
8041/3	Small cell carcinoma, NOS	1
8070/3	Squamous cell carcinoma, NOS	46
8120/3	Transitional cell carcinoma, NOS	1
8140/0	Adenoma, NOS	9
8140/2	Adenocarcinoma in situ	11
8140/3	Adenocarcinoma, NOS	66
8260/3	Papillary adenocarcinoma, NOS	4
8310/3	Clear cell adenocarcinoma, NOS	6
8441/3	Serous carcinoma, NOS	56
8442/1	Serous cystadenoma, borderline malignancy	21
8450/3	Papillary cystadenocarcinoma, NOS	2
8460/3	Micropapillary serous carcinoma (C56.9)	3
8461/3	Serous surface papillary carcinoma	11
8470/3	Mucinous cystadenocarcinoma, NOS	2
8472/1	Mucinous cystic tumor of borderline malignancy	13
8480/3	Mucinous adenocarcinoma	8
8620/3	Granulosa cell tumor, malignant	56
8460/3	Sertoli cell carcinoma	1
8951/3	Mesodermal mixed tumor	1

Abbreviation: ICD, International Classification of Diseases.

at higher frequency (Table 3). It is well known that different population groups have different propensities for specific cancers and many factors have to be considered, such as tumor subtype and patient's age, when making comparisons among different genetic studies. The fact that the tumors in this study come from a relatively homogeneous population in Sweden may explain some of the differences in the frequencies of alterations observed.

The recurrent chromosomal alterations in the malignant tumors analyzed in this study involved chromosomes 1, 2, 3, 4, 8, 13, 18, and 20, and have also been reported by other investigators [1,16,18,28,29]. The most frequent chromosomal abnormality observed was loss of chromosome 4 in 21/33 tumors. Previous studies on ovarian carcinoma using CGH [1,30] have also demonstrated loss of chromosome 4. The PBXW7 tumor-suppressor gene, which encodes a protein regulating the degradation of cyclin E, is located at 4q31.3 and has been reported to be mutated in ovarian cancer cell lines [31].

Twelve of the 17 malignant tumors contained loss of 5q. The *APC* gene, located at 5q21~q22 (which is in the region of loss for tumors of this study), has been reported to be inactivated through methylation in ovarian cancers [32], suggesting that inactivation of this gene may provide a proliferative growth advantage for ovarian tumor cells.

Eleven out of 17 malignant tumors contained loss of 6q. Loss of heterozygosity analysis for genes contained in 6q has been reported in ovarian tumors and, specifically, the candidate tumor suppressor gene *PARK2*, which is located at 6q25~q27, has been reported to be inactivated by deletions in 3/20 ovarian tumors [33]. In addition, the Mitelman Database (http://www.cgap.nci.nih.gov/Chromosomes/Mitelman) has identified recurrent deletions and unbalanced translocations involving 6q in ovarian carcinomas.

In the present study, benign and borderline tumors have a significantly lower average frequency of chromosomal alterations than carcinomas, while other reported tumors do not show any aberrations [9,20], which is in concordance with our results. The tumors that showed no alterations, all of which were either benign or borderline, might contain changes smaller than 5−10 megabases not readily detected by conventional CGH, or they may involve an alternative mechanism of tumorigenesis, such as mutations. Because the numbers of tumors investigated in our study is low, further studies using larger sets of ovarian tumors are needed to verify the significance of the tumor-specific genomic imbalances we observed. Additional molecular techniques, such as microarray analysis, can be recruited to perform a detailed analysis of the regions affected by copy number changes detected by CGH to identify the genes involved.

Ovarian cancer involves a complex set of common, recurrent, and less frequent genomic abnormalities. The aim is to develop a model for the progression of ovarian cancer similar to that proposed for the malignant progression of colorectal cancer [34]. The identification of chromosomal aberrations detected in this study, together with findings from previous studies, may highlight the potential sites for new oncogenes and tumor suppressor genes involved in ovarian tumor initiation and progression.

Acknowledgments

The authors are most grateful to Michael Baudis for permission to use the CGH data from his Progenetix Database and for his generous assistance in producing the profile. This work was supported by the King Gustav Jubilee Clinic Cancer Research Foundation.

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