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Interphase Molecular Cytogenetic Analysis of Epithelial Ovarian Carcinomas

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Karyotype information on ovarian carcinomas has been limited because the tumors are often difficult to culture and the resultant metaphases can have complex numerical and structural chromosomal anomalies. Fluorescent* in situ *hybridization is a rapid method of determining centromere copy number in metaphase cells and interphase nuclei. Fluorescent* in situ *hybridization was used to determine the numerical centromere complement of chromosomes X, 8, 12, and 17 and* HER-2/neu *gene amplification within interphase nuclei of 25 primary epithelial ovarian carcinomas. Touch preparations of the carcinomas were hybridized with two-color combinations of directly labeled α-satellite centromeric chromosome enumeration probes and a directly labeled* HER-2/neu *probe. Modal centromere copy numbers for each of the four chromosomes were used to determine numerical abnormalities relative to the flow cytometric DNA ploidy level for each tumor. Four cases were found to be normal with respect to the four chromosomes studied. In the remaining 21 cases a relative loss of chromosomes 17 (16 cases) and X (nine cases) and a relative gain of chromosomes 12 (10 cases) and 8 (nine cases) were the most common findings. In addition, the* HER-2/neu *gene was amplified in two of the 25 tumors. In conclusion, fluorescent* in situ *hybridization is an excellent method for rapid determination of numerical abnormalities and gene amplification in ovarian carcinomas. (Am J Pathol 1993, 142: 733–741)

Ovarian cancer is the most lethal of gynecological malignancies and the fourth leading cause of cancer death in women in the United States.[1] Ninety percent of all ovarian malignancies derive from the surface epithelium.[2] Unfortunately, the biology of this disease is poorly understood.

Cytogenetic analysis is an excellent tool to study the genetic changes associated with various malignancies. However, cytogenetic information on cultured primary epithelial ovarian carcinomas remains limited, because of difficulties in obtaining analyzable metaphases. Successful cytogenetic studies frequently reveal complex aneuploid karyotypes with multiple numerical and structural abnormalities.[3–11] Specific molecular genetic studies can also provide pathogenetic information. Evaluation of the *HER-2/neu* oncogene has shown it to be amplified and/or overexpressed in a subset of ovarian carcinomas.[12–17] Most studies have shown that *HER-2/neu* overexpression correlates with aggressive growth and poor clinical outcome.[13,14]

Fluorescent *in situ* hybridization (FISH) is a rapid method for determining the complement of specific chromosomes and chromosome regions within metaphase and interphase nuclei.[18] Centromere-specific probes were used to determine numerical abnormalities of chromosomes X, 8, 12, and 17 in 25 epithelial ovarian carcinomas. Ovarian tumors with numerical abnormalities of one or more of these chromosomes have been described previously by conventional cytogenetic analyses,[5–8,10] and directly labeled probes for these chromosomes are available. In this study, we show that FISH is an excellent method for examining numerical chromosomal abnormalities and *HER-2/neu* gene amplification within interphase nuclei of primary ovarian carcinomas.

Materials and Methods

Tissue Samples and Slide Preparations

Tissue samples were obtained from 25 primary ovarian carcinomas. None of the patients had received

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prior therapy. Histological subtypes and grades were determined by a single surgical pathologist (GLK). The histological subtypes of the carcinomas were as follows: serous, 16; endometrioid, 4; mixed epithelial, 2; mucinous, 1; clear cell, 1; and undifferentiated, 1. Five normal ovaries were randomized into the study as controls.

Touch preparations of fresh tissue or tissue previously frozen at −70 C were fixed in cold methanol for 20 minutes, followed by air drying. Slides were stored at −20 C until FISH hybridization was performed. Frozen histological sections were made from each specimen used for touch preparations, to verify the presence of tumor.

In Situ *Hybridization with Centromere Enumeration Probes (CEPs)*

Frozen slides were thawed, fixed for 20 minutes in 3:1 methanol/acetic acid, dehydrated in 70%, 85%, and 100% ethanol, and air dried. Fluorescent, directly labeled, α-satellite DNA probes for chromosomes X, 8, 12, and 17 (probes available at the time of the study) and appropriate hybridization solutions were provided by Imagenetics Inc. (Framingham, MA). All probes and hybridization solutions are available from Imagenetics. Chromosome CEPs were used in two-color combinations. Ten microliters of the two probes and the hybridization mixture were placed on the appropriate area of the touch preparation, coverslipped, and sealed with rubber cement. The probe and target DNA were simultaneously denatured in a 90 C oven for 1 minute. Hybridization conditions and posthybridization washes were performed as recommended by Imagenetics. Briefly, slides were incubated overnight at 41 C in a moist chamber, followed by posthybridization washes (10 minutes each) including three 50% formamide washes in 2× standard saline citrate (SSC) (300 mmol/L sodium chloride, 30 mmol/L sodium citrate) at 45 C, 2× SSC at 45 C, 2× SSC/0.1% Nonidet P-40 at 45 C, and 2× SSC/0.1% Nonidet P-40 at room temperature. Nuclei were counterstained with 1 μg/ml 4′,6-diamidino-2-phenylindole dihydrochloride in the presence of the anti-fade compound 1,4-diazabicyclo(2,2,2)octane.

In Situ *Hybridization for* HER-*2/neu Amplification*

Slides were prepared for hybridization as described above. A mixture of directly labeled SpectrumOrange HER-2/*neu* (Imagenetics) and directly labeled SpectrumGreen CEP DNA probe 17 (Imagenetics) were mixed with the appropriate hybridization solution. The probe mixture was denatured for 5 minutes in a 75 C water bath and placed on ice for an additional 5 minutes. Target DNA was denatured by incubation of the slides in 70% formamide/2× SSC at 75 C for 5 minutes, followed by dehydration through an ethanol series and air drying. The probe mixture was then placed on the slide, coverslipped, sealed, and incubated overnight at 41 C (optimal hybridization temperature recommended by Imagenetics) in a moist chamber. Posthybridization washes and counterstaining were as described above for centromere probes, except that the duration of all washes was reduced to 5 minutes.

Analysis of Interphase in Situ *Hybridization*

Green and orange signals for each two-color probe combination were enumerated in each of 500 interphase nuclei per specimen. Photomicrographs were taken with a Zeiss Axioplan microscope equipped with a triple-pass filter (IO2-I04-IO1O; ImagenOptics, Framingham, MA) for simultaneous detection of SpectrumOrange, SpectrumGreen, and 4′,6-diamidino-2-phenylindole dihydrochloride. Exposure times were approximately 2 minutes, with Kodak Ektachrome 400 film (Eastman Kodak, Rochester, NY). Overlapping nuclei were not used for evaluation. To reduce sampling error because of heterogeneity of chromosomal abnormalities within the touch preparations, representative areas in two quadrants on each hybridized area were evaluated by one observer and the remaining two quadrants were evaluated by another observer (DLP, JFH, or TJB). Although the proportion of nuclei containing different chromosomal abnormalities varied slightly from quadrant to quadrant and from observer to observer, in each case the specific abnormalities were consistently identified (data not shown). In addition to heterogeneity of chromosomal abnormalities within tumor nuclei, differences in proportions of normal to abnormal nuclei existed in various areas on each slide. This variability resulted from regional differences in the infiltrative pattern of the tumor. To reduce the probability of randomly excluding tumor nuclei from evaluation, each quadrant was scanned for areas containing representative proportions of nuclei containing normal and abnormal centromere copy numbers. All normal and abnormal nuclei within that area were then evaluated.

Flow Cytometry

Paraffin-embedded specimens were processed by the technique described by Hedley et al,[19] by using a 2.5-hour 37 C incubation in 0.5% pepsin (0.9% NaCl, pH 1.5) of the dewaxed rehydrated sections. The extracted nuclei were stained for DNA content by using a modification of the method of Vindelov[20] requiring a 30-minute 37 C incubation in RNase. The propidium iodide-stained nuclei were stored at 4 C overnight and were analyzed on a Becton Dickinson FACScan (Becton Dickinson, San Jose, CA) after a 2-minute sonication step to remove doublets.[21] Ten thousand stained nuclei were analyzed for each sample, and cell cycle analyses were performed on a Consort 30 computer (Becton Dickinson). DNA ploidy levels were defined as follows: diploid, DNA index (DI) = 1.0; near-diploid, DI = 1.01 to 1.25; near-triploid, DI = 1.26 to 1.75; tetraploid, DI = 1.90 to 2.10; near-tetraploid, DI = 1.76 to 1.89 or 2.11 to 2.25.

Results

Evaluation of Interphase in Situ *Hybridization for CEPs*

The average centromere copy numbers for the five normal ovary specimens are summarized in Table 1. A very low percentage of tetrasomic nuclei were found in these specimens. Other aneusomy, whether because of technical or biological variation, was quite low. The largest variation from the normal two copies of any of the four chromosomes was a loss of an X centromere signal in 6% of the cells in a single normal ovary from a 54-year-old woman (data not shown).

Figure 1A illustrates a low-grade mixed epithelial cystadenocarcinoma (case 2) hybridized with a mixture of centromere probes for chromosomes 12 and 8. The distribution of the number of nuclei containing different ratios of green (centromere 12) to orange (centromere 8) signals (2:2, 1:1, 2:3, 3:3, 4:4, etc.) for the same tumor is graphically illustrated in Figure 1*B*. The majority of nuclei in the low-grade tumor contained two centromere 12 and two centromere 8 signals. Figure 2*A* is an example of a high-grade serous cystadenocarcinoma (case 23) hybridized with the same combination of centromere probes. The distribution of centromere signals within nuclei from this tumor is illustrated in Figure 2*B*. This high-grade tumor had two major populations. One contained eight centromere 12 and five centromere 8 signals; the second major population had two copies each of chromosome centromeres 12 and 8. The two-probe combination illustrates that the gain of centromere 12 and centromere 8 signals occurred within the same tumor nuclei. This method, however, cannot establish whether the diploid population represents normal cells or a diploid tumor population.

Table 1. *Normal Ovary Centromere Copy Number*

	Average centromere copy number (%)*			
	1	2	3	4
Chromosome X	1.9	97.1	0.3	0.7
Chromosome 8	1.6	96.8	0.7	0.9
Chromosome 12	1.6	97.0	0.3	1.1
Chromosome 17	2.7	96.3	0.2	0.8

* Average percentage of nuclei with stated centromere copy number for five normal ovaries (500 nuclei/case).

Ploidy Analysis and Determination of Partial Numerical Karyotypes

Centromere copy numbers for each of the four chromosomes studied (X, 8, 12, and 17) were determined for each tumor. An example, the centromere copy numbers for tumor 23, is illustrated in Table 2. Because the distribution of the number of specific centromere signals was often broad within individual tumors, the modal centromere copy numbers were used for further data analysis. Modes were defined as those copy numbers comprising >20% of the 500 cells evaluated. Twenty percent was chosen as a conservative cut-off value to ensure identification of major cell populations while at the same time avoiding minor populations that might reflect variables such as base-line copy number abnormalities, hybridization failure, or observer variation.

After modal centromere copy numbers were identified for the four chromosomes studied, gains or losses of each chromosome, relative to the ploidy level established by flow cytometry, were expressed as partial numerical karyotypes. Table 3 summarizes the DNA ploidy level by flow cytometry, the modal centromere copy numbers by FISH, and the derived partial numerical karyotypes for the 25 carcinomas.

Ten of the 25 tumors were DNA diploid by flow cytometry. A tumor was defined as DNA diploid when a normal histogram was observed and the G_2-M peak/tetraploid peak comprised <20% of the total cell population. Notably, of the 10 DNA-diploid tumors observed by flow cytometry, seven were aneuploid by FISH. The numerical abnormalities detected by FISH (among the chromosomes studied) ranged from the gain of a total of four additional chromosome centromeres in two cases to the loss of a single chromosome 17 centromere in three cases. Thus, the greater sensitivity of FISH, compared with

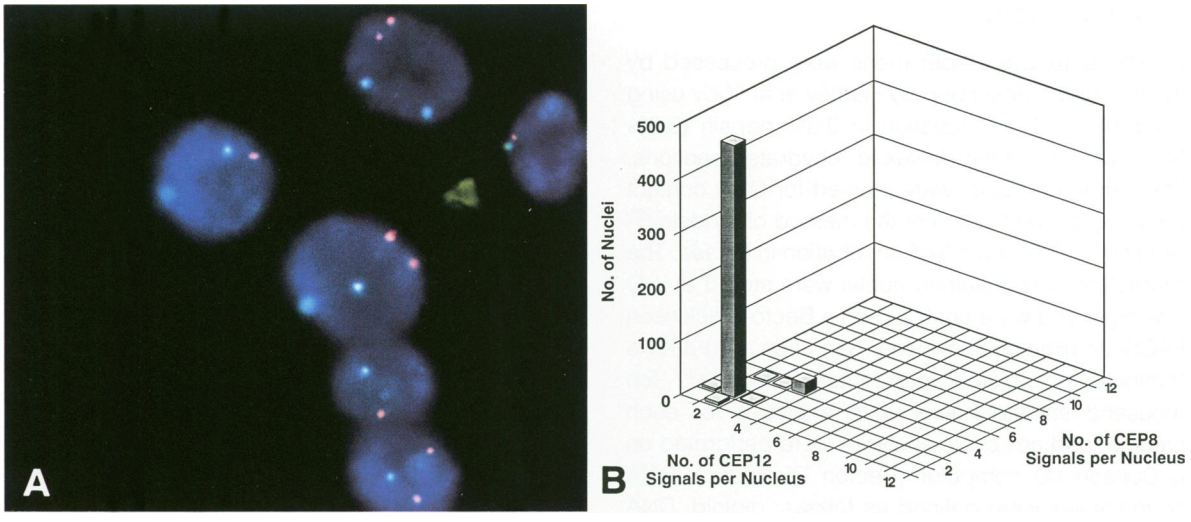

Figure 1. **A:** *Representative photomicrograph from a touch preparation of case 2 hybridized with SpectrumGreen CEP DNA probe 12 and SpectrumOrange CEP DNA probe 8 (× 780).* **B:** *Distribution of the number of nuclei with the indicated number of CEP DNA probe 12 and CEP DNA probe 8 signals in case 2 (modal peak, 2:2).*

flow cytometry, in aneuploidy detection is the result of its ability to identify lesser degrees of aneuploidy.

The remaining 15 cases were not DNA diploid by flow cytometry; two were near-diploid, seven near-triploid, three tetraploid, and three near-tetraploid. These ploidy levels corresponded closely to the modal copy numbers of the four chromosome centromeres in 13 of the cases (exceptions were cases 5 and 19). Case 5 had a small (19%) near-tetraploid (DI = 2.18) population of cells. However, the proportion of the abnormal population detected by FISH approached the proportion of the DNA-diploid population and not the near-tetraploid population. Thus, the partial numerical karyotype for this tumor was based on diploidy. Likewise, in case 19 the partial numerical karyotype was based on diploidy. This tumor was classified as tetraploid by flow cytometry because 24% of the cells were observed in the G_2-M/tetraploid peak. However, by FISH only 7% of the cells were found to be tetrasomic for both centromeres of chromosomes 8 and 12. The most likely reason for these discrepant flow cytometry and FISH results is that the G_2-M/tetraploid peak detected by flow cytometry is composed of an admixture of true tetraploid cells in G_0-G_1 and diploid cells in G_2-M (both contain a 4N DNA content). FISH can distinguish these two populations. In general, tetraploid nuclei have four FISH signals and diploid nuclei in

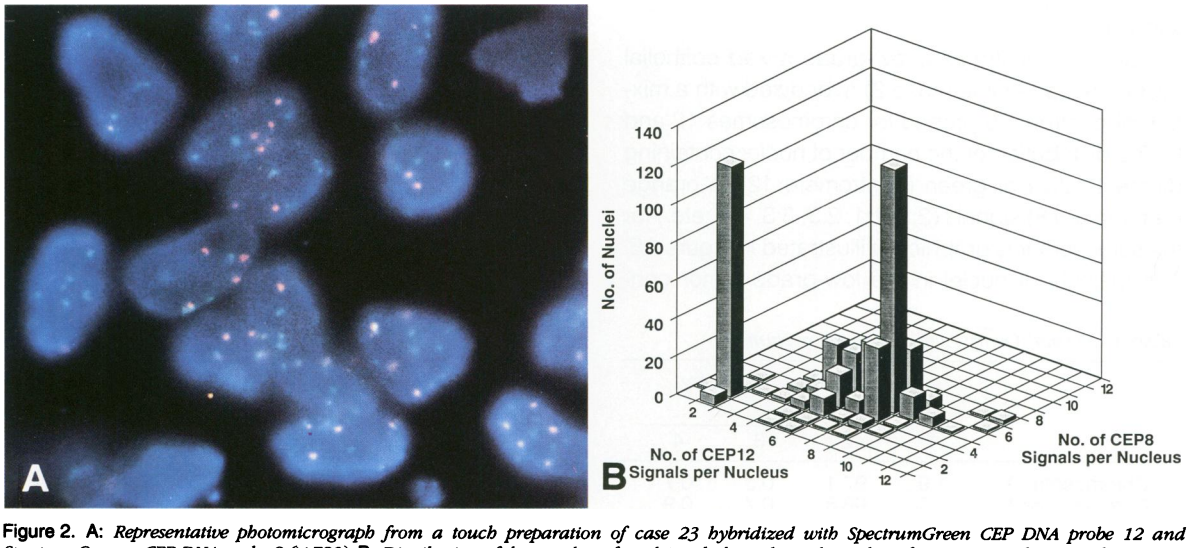

Figure 2. **A:** *Representative photomicrograph from a touch preparation of case 23 hybridized with SpectrumGreen CEP DNA probe 12 and SpectrumOrange CEP DNA probe 8 (× 780).* **B:** *Distribution of the number of nuclei with the indicated number of CEP DNA probe 12 and CEP DNA probe 8 signals in case 23 (modal peaks, 8:5 and 2:2).*

Table 2. *Centromere Copy Numbers for Case 23*

	Centromere copy number (%)*								
	1	2	3	4	5	6	7	8	>8
Chromosome 17	2	38†	55†	3		2			
Chromosome 12		26†		2	8	13	9	37†	5
Chromosome 8	2	26†	5	13	45†	9			
Chromosome X	5	91†	2	1		1			

* Percentage of 500 nuclei with indicated centromere copy number.
† Modal centromere copy number (>20%).

G_2-M have two signals. Thus, in case 19 we concluded that the major tumor cell population was diploid.

Several tumors had multiple modes for a single chromosome, likely reflecting tumor heterogeneity, the presence of a normal cell population, and/or the presence of a disomic tumor cell population. When multiple modes were present for a given chromosome the most frequent abnormal mode (different from the flow cytometry ploidy level) was used to determine the partial numerical karyotype. In three tumors (cases 11, 22, and 23) the use of the two-probe combination identified disomic populations ranging from 17% to 29% of the cells for all four centromere probes. These populations most likely represent normal cells or diploid tumor cells and were, therefore, not interpreted as chromosomal losses relative to the DNA triploidy or tetraploidy observed with flow cytometry. In case 22, in addition to the normal cell/disomic tumor cell population for chromosomes X and 17 at least two abnormal tumor populations were identified by using the two-color probe combination. A gain of both a chromosome X and a chromosome 17 centromere was observed in one cell population, whereas a gain of a chromosome X centromere and a loss of a chromosome 17 centromere were observed in a separate cell population. Thus, this tumor had two abnormal cell populations, with partial numerical karyotypes of +X,+8,+12,+17 and +X,+8,+12,−17. A similiar situation was present in case 18, in which two abnormal populations had partial numerical karyotypes of −X,−8,−17 and −X,−8,+17.

Numerical Chromosome Abnormalities

As shown in Table 3, four carcinomas had diploid centromere counts for each of the four chromosomes studied. The remaining 21 cases had at least one numerical abnormality. No correlation was found between the presence of numerical chromosome abnormalities and different histological subtypes.

The presence or absence of numerical chromosome abnormalities within different tumor grades is shown in Table 4. All three cases of low-grade tumors (grade 2) were diploid, whereas all but one of the

Table 3. *Ploidy, Modal Centromere Copy Number, and Partial Numerical Karyotype*

Case	Diagnosis (adenocarcinoma)	Grade	Ploidy by flow cytometry (DI)	Modal chromosome centromere copy number				Partial numerical karyotype*
				X	8	12	17	
1	Mucinous	2	Diploid (1.0)	2	2	2	2	Normal
2	Mixed epithelial	2	Diploid (1.0)	2	2	2	2	Normal
3	Endometrioid	2	Diploid (1.0)	2	2	2	2	Normal
4	Serous	3	Near-triploid (1.60)	2, 3	4	4	2	−X, +8, +12, −17
5	Serous	3	Near-tetraploid (2.18)	1	3	2	1	−X, +8, −17†
6	Serous	3	Tetraploid (1.92)	4	4	6, 8	2, 3, 4	+12, +12, +12, +12, −17, −17
7	Serous	3	Near-tetraploid (2.20)	2	5	5, 6	4	−X, −X, +8, +12
8	Serous	3	Near-diploid (1.08)	2	2	2, 3	2	+12
9	Serous	3	Tetraploid (2.00)	3	3	4, 5	2, 3	−X, −8, +12, −17
10	Serous	3	Diploid (1.0)	2	2	2	1	−17
11	Serous	3	Near-triploid (1.38)	2, 3	2, 3	3	1, 2	−17, −17†
12	Endometrioid	3	Near-triploid (1.30)	1	2, 3	3, 4	3	−X, −X, −8, +12
13	Endometrioid	3	Diploid (1.0)	1, 2	2	2	1, 2	−X, −17
14	Clear cell	3	Diploid (1.0)	2	2, 3	2	1	+8, −17
15	Mixed epithelial	3	Near-triploid (1.61)	3	3	3	2	−17
16	Serous	4	Near-diploid (1.06)	2	2	2	2	Normal
17	Serous	4	Diploid (1.0)	2	2	2	1, 2	−17
18	Serous	4	Near-triploid (1.61)	2	2	3	2, 4	−X, −8, +17/−X, −8, −17†
19	Serous	4	Tetraploid (2.04)	2	2	2	1, 2	−17†
20	Serous	4	Diploid (1.0)	2	2, 5	2, 3, 4	2	+8, +8, +8, +12
21	Serous	4	Near-triploid (1.73)	1	5	2, 3	2, 3	−X, −X, +8, +8, −12, −17
22	Serous	4	Near-triploid (1.33)	2, 4	2, 4	2, 4	2, 4	+X, +8, +12, +17/+X, +8, +12, −17†
23	Serous	4	Near-tetraploid (2.23)	2	2, 5	2, 8	2, 3	−X, −X, +8, +12, +12, +12, +12, −17†
24	Endometrioid	4	Diploid (1.0)	2	2	2	1	−17
25	Undifferentiated	4	Diploid (1.0)	2	2, 4	2, 4	2	+8, +8, +12, +12

* Partial numerical karyotypes were determined from the most frequent modal copy numbers in reference to the flow cytometry ploidy level. Diploid, DI = 1.0; near-diploid, DI = 1.01 to 1.25; near-triploid, DI = 1.26 to 1.75; tetraploid, DI = 1.9 to 2.1; near-tetraploid, DI = 1.76 to 1.89 or 2.11 to 2.25.
† Refer to text (Results) for additional discussion on determination of partial numerical karyotype.

Table 4. *Histological Grade versus FISH Chromosomal Numerical Abnormalities*

Histological grade	Normal cases	Abnormal cases
Grade 2	3	0
Grade 3	0	11
Grade 4	1	10

high-grade tumors (grades 3 and 4) had numerical chromosome abnormalities. The one high-grade tumor (case 16) that was diploid by FISH had an abnormal near-diploid DNA cell population by flow cytometry. This was the only case in which flow cytometry results were abnormal and FISH results were normal. The use of additional probes for FISH would most likely have identified chromosomal abnormalities in this case. In contrast, with the use of only four centromere probes, FISH detected aneusomy in seven of the 10 tumors that were diploid by flow cytometry.

Table 5 summarizes the numerical abnormalities found for the four chromosomes studied. The most common abnormality was loss of chromosome 17 (16 cases), followed by gain of chromosome 12 (10 cases), gain of chromosome 8 (nine cases), and loss of chromosome X (nine cases). The most common single abnormality was the loss of chromosome 17 (six cases). One case had a gain of chromosome 12 as the sole abnormality.

HER-*2/neu* Gene Amplification

Amplification of the HER-2/*neu* gene was demonstrated in two of the 25 tumors (cases 12 and 22). Figure 3 illustrates the amplification in case 12. Two small orange signals were present in nonamplified cases (Figure 3, *inset*). In contrast, multiple signals, clustering around the chromosome 17 centromere probe, were seen in the amplified cases.

The majority of cells in case 12 had amplification associated with one chromosome 17. Occasionally, two clusters of amplified signals were present, both of which were located around the chromosome 17 centromere signal. These nuclei also had four centromere 17 hybridization signals.

Table 5. *Summary of Numerical Chromosome Abnormalities in 25 Epithelial Ovarian Carcinomas*

	Number of cases
Normal	4
Relative loss of chromosome centromeres*	
Chromosome 17	16
Chromosome X	9
Chromosome 8	3
Chromosome 12	1
Relative gain of chromosome centromeres*	
Chromosome 12	10
Chromosome 8	9
Chromosome 17	2
Chromosome X	1

* Loss and gain of chromosomes were determined relative to the ploidy level of each tumor.

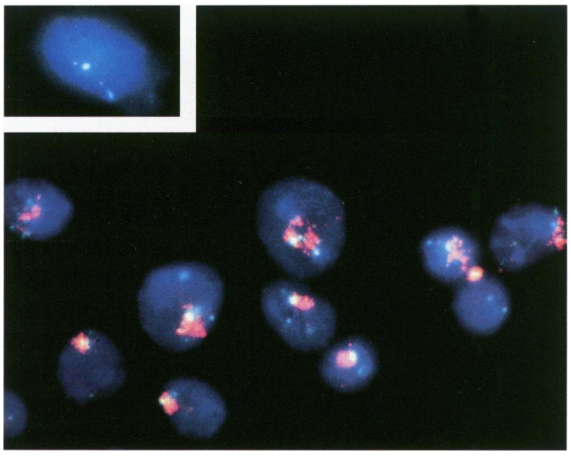

Figure 3. *HER-2/*neu *gene amplification is demonstrated in case 12 with the use of SpectrumOrange-labeled* HER-2/neu *and SpectrumGreen CEP DNA probe 17 (×780). Inset, example of a tumor without* HER-2/neu *amplification (case 15).*

In case 22, two clusters of amplified *HER-2/neu* gene were present in the majority of cells. Both clusters were consistently associated with chromosome 17 centromere signals. In this case, however, the total number of chromosome 17 centromere signals was four, corresponding to the apparent tetraploid level of the tumor.

Although accurate quantitation of the gene amplification detected by FISH was not attempted, the majority of cells in cases 12 and 22 contained >10 specific *HER-2/neu* signals. The majority of cells in the remaining cases contained two copies of *HER-2/neu*. Three tumors (cases 7, 8, and 21) contained occasional nuclei with more than 2 but less than 10 signals. Additional studies using Southern analysis for detection of *HER-2/neu* gene amplification confirmed that only cases 12 and 22 contained significant amplification of the *HER-2/neu* gene (data not shown).

Discussion

In the present study, we used FISH to evaluate four specific numerical chromosomal abnormalities and *HER-2/neu* gene amplification in epithelial ovarian carcinomas. The classical cytogenetic approach of culturing solid tumor cells to evaluate chromosomal abnormalities has several limitations. The low mitotic index and poor quality metaphases seen with cultured solid tumor cells often make it difficult to obtain the desired 20 to 30 analyzable metaphases. In addition, there is the potential for normal stromal cells to

overgrow tumor cells in culture. In contrast, by using FISH in interphase nuclei significantly more cells (500 or more) can be evaluated, the results can be obtained rapidly (24 hours, compared with several days to weeks for classical cytogenetic analysis), and cell culture is not required.

The use of CEPs for detection of chromosome gains and losses can rapidly target specific chromosomes that may be involved in tumorigenesis. A decrease in the number of interphase signals for a specific α-satellite probe can be interpreted as a loss of the centromere of that chromosome in a given cell population. However, the use of CEPs gives no information on structural abnormalities that may be present. Thus, translocated portions of an apparently absent chromosome may be present on other chromosomes. An increase in the number of specific signals can represent the gain of an intact normal chromosome, a gain of that chromosome with additional structural abnormalities, or the identification of a chromosome that, in classical cytogenetic analysis, would be considered a marker (unidentifiable) chromosome. In very rare cases, the presence of two signals may represent an isodicentric chromosome containing two distinct α-satellite regions.

The use of touch preparations made directly from the tumor specimens ensures analysis of abnormalities that are present *in vivo* and not those selected for in tissue culture. Other *in vitro* artifacts are also avoided. When evaluating nuclei on touch preparations, however, heterogeneity of the sample must be considered. Clonal heterogeneity within ovarian carcinomas has been described previously[8,22] and was noted in several of the tumors in this study. In addition, different areas within the touch preparation have varying proportions of normal cells and tumor cells, reflecting the different infiltrating patterns of the tumor within normal tissues. We found that evaluating representative areas within each quadrant and including a large number of cells (500) in the evaluation ensured the identification of the major abnormalities. The use of the two-probe combination was also found to be very useful in selected cases in differentiating abnormal cells from normal cells. When a population of nuclei had two copies of one chromosome and an abnormal copy number for the second chromosome, the population could easily be defined as abnormal. If only single probes had been used these tumor cell populations could not have been differentiated from normal cells. It must be kept in mind that the FISH procedure, even when using two-probe combinations, cannot differentiate between normal cells and disomic tumor cells.

In this study, FISH was found to be more sensitive than flow cytometry in detecting aneuploid tumors. With the use of only four centromere probes, tumors were found to be aneuploid in seven of the 10 cases in which flow cytometry showed DNA diploidy. In only one case flow cytometry identified an aneuploid population (near-diploid) that was not detected using the four centromere probes. FISH, therefore, is an excellent method for detection of aneuploidy in solid tumor touch preparations.

With the use of the four CEPs in this study, we found loss of chromosomes 17 and X and gain of chromosomes 12 and 8 to be common abnormalities in epithelial ovarian carcinomas. All chromosomes have been shown to be aneuploid in at least a few ovarian carcinoma cases reported in the limited classical cytogenetic analysis literature for this disease.[3–11] We selected chromosomes X, 8, 12, and 17 for study because numerical alterations of these chromosomes have been previously reported to be associated with ovarian epithelial neoplasia. For example, in several studies of these tumors by Pejovic et al[5–7,23] the most common numerical abnormalities were losses of chromosomes X, 8, 13, 14, 17, and 22 and gain of chromosome 12. Our findings of loss of chromosome X and 17 and gain of chromosome 12 correlate well with and confirm these previous reports.

The loss of chromosome 17 found in this study also correlates well with loss of heterozygosity results reported by several investigators.[24–26] Loss of heterozygosity for chromosome 17 has been observed in up to 77% of epithelial ovarian carcinomas. Allelic loss of the region surrounding the *TP53* gene, located on the chromosome 17 p-arm, was detected in 16 of 20 cases (80%) of ovarian cancers by Okamoto et al.[27] Loss of heterozygosity studies are currently in progress in our laboratory on the 25 carcinomas used in this study. Preliminary results indicate loss of heterozygosity on some portion of chromosome 17 in all cases (WA Cliby, S Ritland, L Hartmann, KC Halling, G Keeney, K Podratz, RB Jenkins, unpublished results), suggesting that loss of genetic material from chromosome 17 may be an important event in ovarian carcinogenesis.

Gain of chromosome 12 has occasionally been described in ovarian carcinomas.[7,28–30] Trisomy 12 has been seen more consistently, however, in benign and borderline ovarian tumors, including cystadenomas,[23,31] fibromas,[23,32] thecomas,[33] and granulosa cell tumors.[32] This particular trisomy has also been associated with other female genitourinary tract tumors,[34] including leiomyomas[35–37] and endometrial adenocarcinoma.[38] In the present study, a gain of chromosome 12 was seen in 10 of the 21 high-grade tumors but was not detected in any of the three low-grade tumors.

Gain of chromosome 8 has been reported infrequently in the classical cytogenetic studies performed on ovarian carcinomas. In fact, as mentioned previously, the loss of chromosome 8 has been observed in some studies.[5,7] The discrepancy between these findings and our FISH results (gain of chromosome 8 in nine cases) is most likely the result of the fact that FISH can identify chromosomes that cytogenetically are classified as markers. We have shown this to be true in case 23, in which FISH identified five copies of the chromosome 8 centromere. Cytogenetic studies of the same tumor revealed two major clones with complex karyotypes, including loss of chromosome 8 and multiple markers (RB Jenkins, D Bartelt Jr., P Stalboerger, D Persons, RJ Dahl, K Podratz, G Keeney, L Hartmann, unpublished results). FISH analysis of the cultured tumor verified the presence of five chromosomes containing chromosome 8-specific centromeres within multiple metaphases (RB Jenkins et al, unpublished results).

Although only four chromosomes were evaluated in this study, the consistency of numerical chromosomal abnormalities among the different histological subtypes of epithelial ovarian carcinomas suggests that common pathogenetic mechanisms may underlie the various histological categories of this disease. The examination of additional chromosomes will be necessary to verify this hypothesis. The demonstration of abnormalities in all but one of the high-grade tumors, but in none of the low-grade tumors, suggests that multiple genomic anomalies accompany the more malignant appearance and behavior of high-grade ovarian carcinomas.

Amplification of the *HER-2/neu* gene has been associated with a poor prognosis in ovarian cancer.[13,14] Berchuck et al,[14] using an immunohistochemical technique on frozen tissue sections, demonstrated a high level of *HER-2/neu* expression in 23 of 73 cases (32%) of ovarian cancer. Slamon et al[13] reported that 31 of 120 cases (26%) of primary ovarian carcinomas had *HER-2/neu* gene amplification by Southern analysis. However, only eight of the 31 amplified cases had a >5-fold amplification. Other investigators[16] have reported much lower incidences (4%) of *HER-2/neu* gene amplification in epithelial ovarian carcinomas. These discrepancies may reflect variations in the methods and controls used in the various studies. The two cases of *HER-2/neu* amplification identified by FISH in our study appeared to have >5-fold amplification of the gene. Southern analysis, performed on the 25 carcinomas, confirmed the FISH results.

In conclusion, FISH can be used to rapidly determine the complement of specific chromosomes and chromosome regions within interphase nuclei of solid tumors. The use of FISH, in this study, demonstrated that loss of chromosomes 17 and X and gain of chromosomes 12 and 8 are common abnormalities in epithelial ovarian carcinomas. *HER-2/neu* gene amplification was seen in two of the 25 cases studied. The use of additional CEPs will help identify other numerical chromosomal abnormalities in ovarian carcinomas as well as other solid tumors.

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