Distinction of Low Grade from High Grade Human Ovarian Carcinomas on the Basis of Losses of Heterozygosity on Chromosomes 3, 6, and 11 and HER-2/neu Gene Amplification

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ABSTRACT

We examined the frequencies of loss of heterozygosity at 13 different loci distributed on 9 chromosomes in 30 human ovarian carcinomas. The same tumors were also examined for the presence of amplification of the HER-2/neu and H-ras protooncogenes. The results confirmed earlier findings that losses of heterozygosity occurred at nonrandom frequencies on chromosomes 3, 6, and 11 in these tumors. None of the tumors examined showed amplification at the H-ras locus. The HER-2/neu gene, however, was amplified in approximately one-third of the tumors, in agreement with earlier studies from other laboratories. We subdivided our tumor specimens according to their histological grades, which can be regarded as representing different stages of tumor progression. Losses of heterozygosity on chromosomes 3 or 11 were not seen in low grade lesions, although they were present in most of the high grade tumors examined. Losses of heterozygosity on chromosome 6 as well as HER-2/neu amplification, in contrast, were present in several low grade tumors and were not more frequent in high grade lesions. We conclude that the latter two abnormalities are associated with cellular functions involved at earlier stages of ovarian tumor development, whereas inactivation of genes on chromosome 3 or 11 is associated with later steps that may be incompatible with the well differentiated phenotype.

INTRODUCTION

Ovarian carcinomas are the most lethal tumors of the female genital tract because most are detected late in the disease course, by which time the tumor has spread beyond the ovary and is difficult to eradicate. The genes controlling ovarian tumorigenesis and how they interact with each other in tumor development are still largely unknown. We (1) have recently shown that losses of heterozygosity occurred at nonrandom frequencies on chromosomal segments 3p, 6q, and 11p in these tumors, suggesting that inactivation of specific genes on these chromosomes is often associated with ovarian tumor development. Similar studies, reported by Lee et al. (2) also showed frequent losses of heterozygosity on chromosomes 6q and 11p, and expanded these observations to chromosome 17p. The latter chromosomal segment contains the p53 tumor suppressor gene, which is abnormal in a large number of different human tumor types. In addition to these studies addressing the importance of specific tumor suppressor genes, several laboratories have examined the frequencies of activation of specific oncogenes in ovarian tumors (3–10). Amplification of the HER-2/neu locus has been the most frequently observed oncogene abnormality, and such amplification has been associated with a poor clinical outcome (3).

In the present study, we examined further the consequences of the above-described genetic abnormalities on ovarian tumor phenotype. We determined whether specific abnormalities are associated with specific histological tumor grades because such grades may be regarded as representing different stages of tumor progression. The different histological grades are also a measure of aberrant cellular maturation, which is one of the most readily observed phenotypic alterations occurring in cancer. We used the International Federation of Gynecology and Obstetrics criteria for grading our ovarian tumors because such criteria reflect morphologically striking phenotypic changes associated with tumor progression: low grade tumors form differentiated structures such as glands and papillae, whereas high grade tumors have lost this ability and present as solid tumor nests with no architectural organization. We therefore examined the frequencies of losses of heterozygosity at 13 different loci in 30 ovarian tumors subdivided according to their histological grades. We determined the state of amplification of the HER-2/neu and H-ras protooncogenes in the same tumors. We conclude that genetic abnormalities on chromosomes 3 and 11 are associated with more poorly differentiated, anaplastic tumors, whereas abnormalities on chromosome 6, or HER-neu amplification, probably affect different sets of cellular functions and are compatible with the low grade (well differentiated) phenotype.

MATERIALS AND METHODS

Source of Blood and Tumor Specimens. All human tumor specimens were obtained in compliance with the rules and policies of the Human Subjects Committee at our Institution and after written approval by this Committee had been obtained. Source and handling of the specimens were described (1).

Histological Grading of Ovarian Carcinoma Specimens. The criteria of the International Federation of Gynecology and Obstetrics were used. Briefly, tumors that had a solid component accounting for 10% or less of the total tumor mass were assigned a grade I. The tumors were assigned a grade II if the solid component was more than 10% but less than 50%. Tumors that were more than 50% solid were assigned a grade III. Histological grading was done blindly, without knowledge of the molecular findings. Grading was done by one of us (L. D.), who is a practicing surgical pathologist.

DNA Extraction Procedures and Southern Transfer Experiments. All procedures, including digestion with restriction endonucleases, electrophoresis, transfer to nylon membranes, labeling of DNA probes, and hybridization conditions were described earlier (1). Precautions to ensure that tumor samples did not contain large amounts of admixed stroma were described (1). Densitometric analyses (Ultrascan XL 2222–020 laser densitometer; LKB, Bromma, Sweden) of autoradiographs were performed in rare cases in which accurate interpretation was complicated by stromal contamination.

Source of DNA Probes. The source of pH3H2, pBH302, p2–2, J0209E-B, pMCT15, phins 214, pT24-C3, pOR8, and pYNZ132 was mentioned earlier (1). The following probes were obtained from the American Type Culture Collection: pEFD1263, pPOS-2, pTHI62,
RESULTS

Frequencies of Losses of Heterozygosity at Selected Loci in Human Ovarian Carcinomas. We examined the frequencies of losses of heterozygosity at 13 different loci from 9 different chromosomes in a population of 30 ovarian tumors to determine whether any of these loci were deleted at nonrandom frequencies in these tumors. Fig. 1 shows representative results for chromosomes 3, 6, and 11. DNA from blood and tumor specimens (Fig. 1, b and t) for each patient was analyzed by Southern blotting using probes complementary to polymorphic DNA sequences on the 3 chromosomes. Each blood DNA

Fig. 1. Detection of losses of heterozygosity on chromosomes 3, 6, and 11 in human ovarian carcinomas. DNA was extracted from blood (b) and tumor (t) specimens from each individual patient, digested with either HindIII (for pBH302 and pH3H2), MspI (for pYNZ132 and pT24-C3), PvuII (for p2-2 and pOR8), or RsaI (for pHINS214) restriction endonucleases, and analyzed by Southern blotting using the indicated probes. The higher molecular weight bands seen in tumor no. 1 with pBH302 and p2-2 are constant bands. These bands are not apparent in the corresponding blood DNAs because of unequal loading and do not represent allelic rearrangements. kb, kilobases.
GENETIC ABNORMALITIES AND OVARIAN TUMOR DEVELOPMENT

Table 1  Frequencies of losses of heterozygosity at selected loci in 30 different human ovarian carcinomas

<table>
<thead>
<tr>
<th>Probe</th>
<th>Chromosomal assignment</th>
<th>No. of cases with loss of heterozygosity over total no. of informative cases</th>
<th>% loss</th>
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<tbody>
<tr>
<td>H3H2</td>
<td>3p21</td>
<td>2/16</td>
<td>13%</td>
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<tr>
<td>BH302</td>
<td>3p24.1-2p22</td>
<td>6/15</td>
<td>40%</td>
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<td>JO209 E-B</td>
<td>5p15.2-p51.1</td>
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<td>p2-2</td>
<td>6p21-qter</td>
<td>5/9</td>
<td>56%</td>
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<td>YNZ128</td>
<td>6p21-qter</td>
<td>6/16</td>
<td>38%</td>
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<tr>
<td>OR8</td>
<td>6q24-q27</td>
<td>3/15</td>
<td>20%</td>
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<tr>
<td>EFD126.3</td>
<td>9q34-qter</td>
<td>1/10</td>
<td>10%</td>
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<tr>
<td>OS-2</td>
<td>10q21-q26</td>
<td>2/16</td>
<td>13%</td>
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<td>T2-C3</td>
<td>11p15.5</td>
<td>8/23</td>
<td>35%</td>
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<td>HINS214</td>
<td>11p15.5</td>
<td>4/14</td>
<td>29%</td>
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<tr>
<td>THI62</td>
<td>13q12-q21</td>
<td>1/17</td>
<td>7%</td>
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<tr>
<td>YNZ22</td>
<td>17p13</td>
<td>5/19</td>
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<tr>
<td>MCT15</td>
<td>21q22</td>
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</table>

The sample shown in Fig. 1 contains 2 alleles. All of the tumors except tumor 1 at the pH3H2 locus contain only one allele, implying that the other was lost. Table 1 summarizes the results for all 13 loci studied and shows that those most commonly affected by such losses were on chromosomes 3, 6, and 11. In addition, chromosome 17, which contains the p53 tumor suppressor gene (12) as well as perhaps an additional and presently unidentified tumor suppressor (13), was also affected, although at slightly lower frequencies. These findings are consistent with our earlier studies (1) on chromosomes 3, 6, and 11 as well as with those of Lee et al. (2), who recently reported similar findings for chromosomes 6, 11, and 17. Although the frequencies of losses of heterozygosity on 17p shown in Table 1 are lower than those reported by Lee et al. (2), those differences may be explained by the fact that these authors have studied only high grade ovarian tumors, whereas many of our tumors are grade 1 or II (see below).

Several of the loci in Table 1 show either absence or low frequencies of losses of heterozygosity in the tumors examined. This implies that our findings for chromosomes 3, 6, 11, and 17 did not result from random chromosomal aberrations associated with the malignant phenotype in general. It is significant that some of the loci that show low frequencies of losses of heterozygosity in ovarian tumors show considerably higher frequencies in other tumor types. For example, the long arm of chromosome 10 is one of the loci most frequently affected by losses of heterozygosity in glioblastoma multiforme (14, 15), and the long arm of chromosome 9 shows similar losses in 67% of transitional cell carcinomas (16). Probe THI62 is complementary to a locus close to the retinoblastoma susceptibility locus on chromosome 13, which is thought to be involved in a large number of different tumor types (17–20). The results support the notion that inactivations of genes on chromosomes 3, 6, 11, and 17 are common in human ovarian carcinomas and probably play an important role in their development, whereas a number of other genes with suspected tumor suppressor activities important for the control of other tumor types are probably not commonly associated with ovarian cancers.

Only 13% of the informative tumors studied showed losses of heterozygosity at the H3H2 locus assigned to 3p21, whereas losses of heterozygosity were found in 40% of cases at the BH302 locus assigned to 3p24.1–p22. Although not statistically significant (P = 0.19), these differences suggest that the latter locus may be closer to a putative tumor suppressor gene important in the control of ovarian tumor development than H3H2. The finding of 3 different tumors with losses of heterozygosity on the chromosome 10p short arm has also been described (21). However, no significant losses of heterozygosity were observed at this locus in our study.

Table 2: Allelic changes in human ovarian carcinomas of varying histological grades

<table>
<thead>
<tr>
<th>Tumor FIGO grade</th>
<th>H3H2</th>
<th>BH302</th>
<th>JO2597</th>
<th>2-2</th>
<th>YNZ132</th>
<th>OR8</th>
<th>EFD126.3</th>
<th>OS-2</th>
<th>T2-C3</th>
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a FIGO, International Federation of Gynecology and Obstetrics.

b x, homozygous (non-informative) individuals; ●, heterozygous individual with loss of heterozygosity in her tumor; ○, heterozygous individual with no loss of heterozygosity in her tumor.
at the BH302 locus but not at H3H2 (Fig. 1; Table 2) further substantiates this conclusion and indicates that the chromosomal breakpoint resulting in genetic losses occurred between the 2 loci in these tumors.

Relationship between Frequencies of Losses of Heterozygosity at Selected Loci and Ovarian Tumor Grades. We analyzed our ovarian tumors according to grades of malignancy (Table 2) to determine whether specific losses of heterozygosity would be associated with specific grades. Fig. 2 shows a statistically significant relationship between increasing tumor grade and the total number of losses of heterozygosity at all loci examined (P < 0.00005). This is consistent with the concept that more anaplastic tumors contain larger numbers of chromosomal aberrations. Some specific molecular abnormalities, however, do not show a similar relationship. Chromosome 6, one of the chromosomes associated with high frequencies of losses of heterozygosity in the present study as well as in that of Lee et al. (2), contained losses in several of our grade I tumors. There was no statistically significant (P = 0.70) increase in the frequency of losses of heterozygosity observed on this chromosome in tumors with higher histological grades (Table 2; Fig. 2). In contrast, chromosomes 3 and 11, which contain the other most commonly deleted loci in our tumor population, showed no losses of heterozygosity in grade I tumors and significant increases (P = 0.02 and 0.01, respectively) in such losses in higher grade lesions. These results suggest that inactivation of a gene on chromosome 6 may be associated with early stages of malignant transformation and is compatible with the low grade phenotype, whereas inactivations of genes on chromosome 3 or 11 probably result in more aggressive tumor behavior.

Relationship between Tumor Grade and Oncogene Amplification. Dominantly acting oncogenes constitute another class of genes involved in the control of tumor development in addition to tumor suppressor genes. Recent data suggest that the HER-2/neu oncogene, in particular, is frequently amplified in ovarian as well as breast tumors and that such amplification may be associated with poorer clinical outcome (3). We therefore determined the extent of amplification of this gene in our ovarian tumors and asked if such amplification is associated with specific losses of heterozygosity or tumor grades. We also looked at the extent of amplification of the H-ras gene in the same tumors because this locus was frequently affected by losses of heterozygosity in our tumor population (see above). High copy numbers of this gene have been reported in some ovarian cancers (5), although H-ras point mutations are rare in ovarian carcinomas (7, 8). However, there is no demonstrable amplification of the H-ras locus in any of the tumors that we examined using the insulin locus as our control nonamplified gene (results not shown). This implies that H-ras amplification is not associated with loss of heterozygosity at this locus. The HER-2/neu locus, however, is amplified in 8 of 25 (32%) tumors examined (Table 3; Fig. 3), in agreement with Slamon et al. (3). There is also no statistically significant relationship (P = 0.88) between HER-2/neu amplification and increasing tumor grade (Table 3). In fact, the tumor with the highest degree of amplification (tumor 5) is a grade I lesion, and the one with the second highest HER-2/neu copy number (tumor 32) is a grade II. Although somewhat unexpected, these results are not incompatible with the notion that HER-2/neu amplification is associated with poor clinical outcome (3). It is possible that the above 2 tumors with HER-2/neu amplification will show a more rapid clinical course than other tumors of similar grades. Long-term follow-up data on the above 2 patients, however, are not yet available.

![Fig. 2. Relationship between histological grades and specific losses of heterozygosity in human ovarian carcinomas. Upper left panel, total number of loci with losses of heterozygosity over the total number of informative loci examined for each tumor grade. Other 3 panels, total number of tumors with losses of heterozygosity on chromosome 3, 6, or 11 over total number of tumors informative for these chromosomes for each grade.](image-url)
cDNA probe for the HER-2/neu protooncogene. After autoradiography the
endonuclease and analyzed by Southern blotting using pMACHV. which is a
patients 5, 8, 12, and 32. DNAs were digested with the EcoRestriction
ian carcinomas. DNA was extracted from blood (b) and tumor (t) specimens from
chromosome, however, were not as high as those reported by
studies (1,2) showing losses of heterozygosity at nonrandom
zymosity in our tumor population. Chromosome 17p, which
the above results support the notion that inactivation of
specific genes on chromosomes 3, 6, and 11 constitute impor-
tant steps leading to the development of ovarian epithelial
tumors. Our data also suggest that the putative tumor suppressor
gene on the short arm of chromosome 3 is closer to the
BH302 (erbA) locus than to the H3H2 locus. The same chro-
mosomal region has been implicated in several other tumor types (23–26).

We have also examined the state of amplification of the HER-2/neu and H-ras protooncogenes in our population of
ovarian tumors. None of the tumors, including those with loss of
heterozygosity at the H-ras locus, showed H-ras amplification.
Thus, the retained H-ras allele in these tumors was not
amplified. We did not examine the presence of point mutations
in the H-ras gene because such mutations were reported to be
rare in ovarian tumors (7, 8). The HER-2/neu locus was am-
plified in approximately one-third of the tumors, in agreement
with Slamon et al. (3). We were unable to demonstrate any
relationship between HER-2/neu amplification and the pres-
ence of losses of heterozygosity at any of the loci that we
examined.

We obtained additional information regarding the potential
consequences of the above genetic alterations on tumor phe-
notype when we stratified our data according to histological
grades. As expected, the total number of observed abnormalities
detected was generally higher in tumors of higher histological
grades and abnormalities at specific loci often showed a similar
relationship when examined individually. For example, losses
of heterozygosity on either chromosomes 3 or 11, which
represent 2 of the most frequent abnormalities that we detected in
ovarian tumors, were not seen in any of our grade I lesions,
although they were present in most grade III tumors. Thus,
both abnormalities may be incompatible with the well differen-
tiated (grade I) phenotype. Allelic losses on chromosome 6,
however, were seen in several grade I tumors and the frequencies
of such losses were not significantly higher in higher grade
lesions. Likewise, there was no apparent association between
HER-2/neu amplification and increased tumor grade in the
cases that we examined. We conclude that inactivation of a
gene on chromosome 6 or HER-2/neu amplification, although
associated with malignant behavior, is consistent with the well
differentiated (grade I) phenotype. In contrast, inactivation of
genes on chromosome 3 or 11 are associated with more ana-
plastic or poorly differentiated (grade III) phenotypes. These
data constitute the essence of the provisional model of ovarian
tumor development illustrated in Fig. 4. The top photomicro-
graph in Fig. 4 is a histological picture of normal adult human
ovary. In Fig. 4, the left arrow indicates a well differentiated
(grade I) tumor that may have developed in part as a result of
either HER-2/neu amplification or loss of a gene function on
chromosome 6. The right arrow in Fig. 4 indicates a poorly
differentiated (grade III) tumor that developed as a result of
gene inactivation on either chromosome 3 or 11. Progression
from a well differentiated lesion to a more poorly differentiated
one may likewise be the result of allelic losses on any of these
2 chromosomes (Fig. 4, bottom arrow). Similar associations
between specific molecular abnormalities and specific degrees
of malignant transformation have been reported from other
laboratories using other tumor models such as colorectal car-
cinomas (27), astrocytomas (14, 15), bladder carcinomas (28),
and breast carcinomas (13). Such findings, in addition to pro-
viding potentially useful information on the possible cellular
functions affected by specific cancer-related genes, may even-
tually lead to the development of new classifications for specific
tumor types, based on molecular rather than histological
criteria.

We used a histological grading system as a measure of the
degree of anaplasia in our tumor population. Our goal was to

**Fig. 3. Detection of HER-2/neu protooncogene amplification in human ovar-
ian carcinomas. DNA was extracted from blood (b) and tumor (t) specimens from
patients 5, 8, 12, and 32. DNAs were digested with the EcoRⅠ restriction
endonuclease and analyzed by Southern blotting using pMACHV, which is a
cDNA probe for the HER-2/neu protooncogene. After autoradiography the
membrane was reprobed with pYNZ22, which is a cloned DNA segment comple-
mentary to a DNA sequence on the same chromosome as HER-2/neu.**

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**HER-2/neu:**

|    |    |    |    |    |
|----|----|----|----|
| 23.1 | 9.4 |

**pYNZ22:**

|    |    |    |    |    |
|----|----|----|----|
| 23.1 | 9.4 |
identify associations between specific molecular genetic abnormalities and specific tumor phenotypic changes. The grading system that we have adopted is a measure of the ability of tumor cells to form differentiated structures such as glands and papillae. Loss of such abilities in high grade tumors results in striking morphological changes readily apparent even to someone inexperienced with tumor histology (see Fig. 4). These morphological changes are most likely the result of fundamental molecular changes important in tumor progression. We have not looked at associations between clinical outcome and specific molecular changes, although high grade tumors are often clinically more aggressive. A relationship between HER-2/neu amplification and poor prognosis was demonstrated by Slamon et al. (3) for ovarian as well as breast carcinomas. It is therefore possible that the tumors with such amplifications in the present study will behave more aggressively than other tumors of similar histological grades. The prognostic consequences of any of the other molecular changes detected in ovarian tumors are still unclear.

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REFERENCES


Distinction of Low Grade from High Grade Human Ovarian Carcinomas on the Basis of Losses of Heterozygosity on Chromosomes 3, 6, and 11 and HER-2/neu Gene Amplification


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