Ovarian Carcinoma Develops through Multiple Modes of Chromosomal Evolution

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ABSTRACT

Ovarian carcinoma has the highest mortality of all of the gynecologic cancers. The chromosomal changes in this tumor type are highly complex, and the karyotypes typically show severe aneuploidy. Despite the abundance of cytogenetic information, with ~400 published karyotypes, very little is known about the mode of karyotypic evolution and the possible presence of cytogenetic pathways related to tumor development. In the present investigation we used 387 ovarian carcinoma karyotypes to identify the most frequent genomic imbalances. Tumor cases were then classified with respect to the presence or absence of these imbalances and statistically analyzed to assess the order of appearance of chromosomal imbalances, as well as possible karyotypic pathways and cytogenetic subtypes. We establish the temporal order by which the different imbalances occur and show that at least two cytogenetic pathways exist, one characterized by +7, +8q, and +12, and one by 6q− and 1q−. We show that ovarian carcinomas develop through at least three phases of karyotypic evolution. At the early stages, Phase I, the karyotypic evolution seems to proceed though step-wise acquisition of changes. The transition to Phase II showed signs of an increased chromosomal instability, most probably caused by extensive telomere crisis and the onset of breakage-fusion-bridge cycles. This process was linked to the presence of imbalances characteristic for the 6q−/1q− pathway. The transition to Phase III involved triploidization and was also linked to the presence of the 6q−/1q− pathway.

INTRODUCTION

Ovarian cancer represents one-fourth of the malignancies of the female genital tract but is the most common cause of death among women with gynecologic cancer. The majority of ovarian cancers are found in postmenopausal women, and the median age for ovarian adenocarcinoma, which accounts for 85–90% of all malignant ovarian tumors, is between 60 and 65 years. Close to 75% of the patients have advanced-stage disease at diagnosis. Their prognosis is correlated with numerous clinical and biological factors, of which the tumor stage and the volume of metastatic disease after resection correlate best with outcome (1). Familial or hereditary patterns account for only 1%–5% of all of the ovarian malignancies, most commonly associated with mutations in BRCA1 (2). Most ovarian carcinomas show complex chromosome aberrations with hypodiploid or near-triploid stemline CNVs. The most common numerical changes are losses of chromosomes 4, 8, 11, 13, 14, 15, 17, and 22, and gains of chromosomes 1, 2, 3, 6, 7, 9, 12, and 20 (3, 4). Deletions and unbalanced translocations resulting in loss of chromosomal material are the most common structural abnormalities, typically affecting 1p, 1q, 3p, 3q, 6q, 7p, 10q, 11p, 11q, and 12q (4). CGH analyses have corroborated the essential features of the cytogenetics and revealed frequent gains of 3q, 6p, 7, 8q, and chromosome 20, and losses of 4q, 6q, 12q, 13q, and 16q (5). Little is known about the mechanisms behind these chromosome abnormalities, although recent data suggest that telomere dysfunction and chromosomal BFB events may play a role in the generation of complex chromosomal changes in carcinomas (6).

Several attempts have been made to systematize the cytogenetics of ovarian cancer (4, 7). However, because of the karyotypic complexity an extensive amount of cytogenetic and/or CGH data are needed to reveal possible karyotypic pathways for tumor development or cytogenetic subtypes. In the present investigation we have identified all of the cytogenetically aberrant ovarian carcinomas, altogether 387, present in the Mitelman Database of Chromosome Aberrations in Cancer. To identify the most frequent imbalances we constructed a genomic imbalance map. Tumors were then classified with respect to the presence or absence of these imbalances and statistically analyzed (8, 9) to assess the order of appearance of imbalances, possible karyotypic pathways, and cytogenetic subtypes. These statistics were then compared with clinical data and in vitro measurements of mitotic instability.

MATERIALS AND METHODS

Selection of Data. All of the ovarian carcinomas with abnormal karyotypes were retrieved from the Mitelman Database of Chromosome Aberrations in Cancer. A total of 387 karyotypes were ascertained and used to construct an imbalance map. On the basis of this map, 30 segments affected by imbalances in >15% of the cases were identified (Table 1). Each karyotype was then assessed for the presence or absence of the selected imbalances. The NIPT was then calculated, and the 316 cases with at least one imbalance were selected for additional analysis.

Temporal Analysis. The TO was determined essentially as described in Höglund et al. (9). Briefly, all of the tumors with a given imbalance were selected and the distributions of the NIPT plotted. The modes of these distributions were used as an estimate of TO. For each distribution the 25th and the 75th percentiles were calculated.

HCA and MDS. HCA and MDS were performed on an imbalance distance matrix based on Euclidean distances produced by using imbalances as the variables and the tumors as the observations. An HCA group objects into clusters and then organizes the clusters in a hierarchical tree according to similarity (10). A consequence of HCA is that one object may only be part of one cluster. Ward’s method was used for cluster formation. MDS organizes a higher dimensional matrix in a lower dimensional space so as to maintain as much as possible of the original distances (10), and, hence, MDS extracts the major features of a distance matrix. HCA and MDS were performed using the Statistica software package (Statsoft, Tulsa, OH).

PCA. To search for possible patterns of correlations between the imbalances, PCA was performed using the Statistica software package (Statsoft). PCA is a standard multivariate method used frequently to search for underlying structures in data sets (11). In short, principal components are linear combinations of the original variables, orthogonal, and ordered with respect to their variance so that the first principal component has the largest variance. To analyze imbalances, these were used as variables and the individual tumors as the observations; this will group imbalances seen frequently in the same tumors. The factor models arrived at were evaluated at two levels: total

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4 The abbreviations used are: CN, chromosome number; BFB, breakage-fusion-breakage; NIPT, number of imbalances per tumor; TO, time of occurrence; PCA, principal component analysis; HCA, hierarchical cluster analysis; MDS, multidimensional scaling; FIGO, International Federation of Gynecology and Obstetrics; CIN, chromosomal instability; CGH, comparative genome hybridization.
The CNs (Fig. 1a) revealed a highly heterogeneous tumor population. The polyploid (CN >57) group constituted 36% of the tumors with CNs ranging from 58 to 127. Hyperdiploid (CN = 48–57) tumors were seen in 28%, and hypodiploid (CN <45) in 22%, whereas the peridiploid tumors (CN = 45–47) was a minority, seen in 14% of the cases. The distribution of the NIPT (Fig. 1b) showed a sharp decline in frequencies of tumors with increasing NIPT and was similar to a geometrical distribution for NIPT = 1–8. However, above NIPT = 8 the frequencies were constant or slightly increasing, suggesting a multimodal distribution. To investigate the possible multimodality additionally, the NIPT distributions of tumors containing specific imbalances were analyzed separately. Tumors with 1p−, 1q−, 7p−, 10p−, −13, −15, −16, −17, and −18, and with +1q, showed clear bi- or trimodal distributions. In Fig. 1c, cases containing these imbalances have been pooled and the NIPT distribution plotted. Three subsets of tumors are revealed by this analysis, those with 1−7, those with 8−16, and those with >16 imbalances. We next pooled and analyzed the hyper-, hypo-, and the peridiploid tumors. These near-diploid cases were dominated by tumors with 1−7 changes, showed a shoulder at NIPT = 8−15, and had very few tumors with >15 imbalances (Fig. 1d). The polyploid tumors showed a clear bimodal distribution, with tumors having 1−4 changes or >16 changes as the dominating subtypes (Fig. 1e). The data thus suggest that ovarian carcinomas may be divided into three classes of karyotypes representing three different phases of karyotypic evolution. Phase I is seen in both poly- and nonpolyploid tumors, and is characterized by a few imbalances. Phase II is mainly seen in near-diploid tumors and show 7−16 imbalances, and Phase III is mostly seen in polyploid tumors and contains >16 imbalances.

**Temporal Appearance of the Imbalances.** To analyze the early steps in the karyotypic evolution, the near-diploid Phase I tumors were selected, as this subset was homogeneous with respect to the mode of karyotypic evolution and as they were the least complex. In addition, the analysis was limited to the imbalances seen in at least 5% of the near-diploid Phase I cases. To estimate the temporal order of imbalances, tumor cases with a given imbalance were selected and the NIPT distributions produced. The modal values of these distributions were used as a measure of the TO (Fig. 2; Ref. 9). Early imbalances (TO = 1−2) were 6q−, +7, +8q, and 1q−; intermediate imbalances (TO = 3−4) were +12, −8, and +1q; and the remaining were late (TO = 5−6). Imbalances not included in this analysis, and thus mostly seen in Phase II and Phase III tumors, were considered late and with no specific TO
attached. Of the gains, the early +8q and +12 showed significant \((P < 0.01)\) associations with FIGO stage I and the late +6p with stage IV. Similarly, +12 was associated \((P < 0.01)\) with histopathological grade 1 and the late 6p with grade 3. The majority of the late losses, −4, −5, 7p−, 7q−, −8, 10p−, 11p−, −13, −14, −15, −16, −17, −19, and −22, were significantly \((P < 0.01)\) associated with grade 3.

**Cytogenetic Pathways.** To make a first analysis of the associations among the imbalances, a distance matrix based on Euclidean metrics was produced. The HCA of this matrix revealed two major clusters of imbalances, one consisting of the gains +3q, +7, +8q, +12, and +20, and one of all losses and 1q (Fig. 3a). The MDS analysis of the same distance matrix produced one central cluster of late losses, whereas the early losses were positioned at one side of the graph (Fig. 3b). The gains were scattered at some distance from the central cluster and at the opposite side of the early losses. The PCA of the imbalances produced one tight cluster of the gains +3q, +7, +8q, +12, and +20, whereas the remaining imbalances formed a more dispersed cluster with no obvious substructures (data not shown). Thus, these analyses suggest two subtypes of tumors, one dominated by gains and one by losses. As no time axis was obvious in the previous PCA, the TO values were included as an observation and the PCA repeated. In the resulting two-dimensional configuration (Fig. 3c), the first principal component correlates with the TO values and, thus, represents a time axis. The second component separated all of the gains, except +1q, from the losses. The PCA thus suggests the presence of two cytogenetic pathways; one hyperdiploid initiated by either +7 or +8q, followed by +12 and then by +20 and +3q as late imbalances, and one hypodiploid pathway initiated by 6q−, followed by 1q− or +1q, and then by the remaining losses (Fig. 3c). Of the two pathways, the latter seems to lead to more aggressive tumors, as
6q−/1q− tumors were over-represented in FIGO stages III and IV (P = 0.002) as well as in grade 2 and 3 tumors (P = 0.012). The two-factor model explained 67% of the variability, and the communalities ranged from 0.23 to 0.85. However, a separate three-dimensional PCA of the hypodiploid pathway revealed that the 6q−/1q− pathway could be resolved into one initiated by 6q− followed by +1q and −8 as intermediate changes, and another initiated by 1q− followed by 1p− and then by −8 (Fig. 3d). These two pathways converged to a set of common late imbalances. The dichotomy of the hypo- and diploid pathway was also evident from the MDS analysis, where 6q− and 1q− were well separated (Fig. 3b). The correlation matrix on which the PCA was based revealed significant associations (P < 0.01) between many of the gains, including the early imbalances +7 and +8q but no negative correlations.

To additionally evaluate the relationships among the pathways, tumor cases were analyzed by PCA. In this analysis the tumors were used as variables and the imbalances as the observations; this will group tumors with similar sets of imbalances. The NIPT for each individual tumor was used as an observation in addition to the imbalances (9). This revealed four major and three minor clusters (Fig. 4a). Classifying imbalances (9) were identified by testing each imbalance for its presence in all of the members of a given PCA cluster. Loss of 6q and loss of 1q behaved as classifying imbalances, and identified one major cluster each, whereas the three imbalances +7, +8q, and +12 were needed to determine the remaining major cluster (Fig. 4b).

Phase II Near-Diploid Tumors. The MDS of the near-diploid Phase II cases revealed two major clusters of imbalances, one tight cluster consisting of the gains, and one cluster consisting of the late imbalances.

Fig. 3. The identification of cytogenetic pathways. Included in the analysis are imbalances present in >5% of the near-diploid Phase I tumors. a, HCA based on Euclidean distances and Ward’s agglomeration algorithm. b, MDS based on Euclidean distances. c, a two-dimensional PCA of the imbalances. The TO for each imbalance was included as an observation for each imbalance. d, a three-dimensional PCA of the hypodiploid cases.

Fig. 4. PCA of the tumor cases. a, light blue, NIPT = 1; blue, NIPT = 2; violet, NIPT = 3; red, NIPT = 4; brown, NIPT = 5; black, NIPT >5. b, red, tumors with 6q− but not 1q−, +7, +8q, or +12; violet, tumors with 6q− and 1q− but not +7, +8q, or +12; green, tumors with 1q− but not 6q−, +7, +8q, or +12; yellow, tumors with 1q− and +7, +8q, or +12; blue tumors with +7, +8q, or +12, but not 6q− and 1q−; light blue, tumors with 6q− and +7, +8q, or +12; black, tumor without, 6q−, 1q−, +7, +8q, and +12.
losses and of +1q (Fig. 5a). The fact that +2 and +6p clustered with
the other gains indicates that these imbalances function as very late
imbalances in the +7/+8q/+12 pathway. The early losses 1q− and
6q− were, as in the MDS of the Phase I tumors, placed at some
distance from each other, and, thus, the 1q− and the 6q− pathways
are still separated in Phase II tumors. The frequency of the individual
imbalances in Phase II tumors were calculated (Table 1) and com-
pared with the corresponding frequencies in Phase I cases. The ma-
jority of the imbalances showed a significant (P < 0.01) increase in
frequency, as determined by X2 tests, with the exception of +7, +8q,
+12, +20, 1q−, and 1p−. The fact that +7, +8q, and 1q− did not
increase in frequency, whereas 6q− did, may indicate that the 6q−
pathway is more prone to transition into Phase II. This was also
supported by the finding that only 25% of the near-diploid +7/+8q/
+12, and the 1q− cases, respectively, were Phase II tumors, whereas
41% of the near-diploid 6q− cases belonged to this class. The hyper-
and the hypodiploid pathways were also evaluated by comparing the
frequencies of the imbalances in the respective pathways. This was
accomplished by selecting tumors with the characteristic changes
+7, +8q, or +12, and 6q− or 1q−, and then calculate the frequencies
of the imbalances. An extensive overlap between the pathways was then
observed (Table 2). However, the cases were dominated by 6q−/1q−
and by 6q−/1q− or 6q−, respectively, were Phase II tumors, whereas
41% of the near-diploid 6q− cases belonged to this class.

Polyploid Ovarian Carcinomas. The polyploid Phase I cases
were selected and the frequencies for the individual imbalances cal-
culated. Imbalances showing particularly high frequencies were 1q−,
1p−, and 6q−, seen in 32, 30, and 28% of the cases, respectively,
whereas imbalances characteristic for the +7/+8q/+12 pathway were
seen at significantly (P < 0.001) lower frequencies (0–6%). The
Phase III cases were then selected and the frequencies for the
individual imbalances calculated. Imbalances showing particularly high
frequencies were −4, −8, −15, −17, −18, −22, and −X, all seen in
>80% of the cases. Gains of chromosomes 7 and 12 were seen at
frequencies comparable with what was seen in near-diploid Phase I
and II tumors indicating that the +7/+8q/+12 path is not under-
represented in the polyploid Phase III tumors (Table 2). The under-
representation of +6q may be because of the frequent -8 in polyploid
tumors. The MDS of the polyploid Phase III cases revealed two major
clusters of imbalances, one consisting of gains and one of losses
(Fig. 5b). This shows that the +7/+8q/+12 and the 6q−/1q− path-
ways are separated to some extent also in Phase III polyploid tumors.

Terminal Deletions. The most common type of abnormality was
deletions, either caused by terminal deletions or unbalanced translo-
cations. To study these losses in more detail, the chromosome arms

Table 2 Pathway-specific imbalance frequencies

<table>
<thead>
<tr>
<th>Imbalances</th>
<th>+7/+8q/+12</th>
<th>6q−/1q−</th>
<th>+7/+8q/+12</th>
<th>6q−/1q−</th>
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<td>+1q</td>
<td>12</td>
<td>7</td>
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<td>35</td>
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<td>+2</td>
<td>8</td>
<td>0</td>
<td>27</td>
<td>13</td>
</tr>
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<td>3</td>
<td>52</td>
<td>30</td>
</tr>
<tr>
<td>+6p</td>
<td>6</td>
<td>8</td>
<td>15</td>
<td>13</td>
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<td>+7</td>
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<td>4</td>
<td>36</td>
<td>23</td>
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<tr>
<td>+8q</td>
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<td>3</td>
<td>36</td>
<td>18</td>
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<tr>
<td>+12</td>
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<td>64</td>
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<td>+20</td>
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<td>10</td>
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<td>67</td>
<td>63</td>
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</table>

* Frequencies given in percentage. Frequencies in bold indicate observed ±5% dif-
fences in frequencies between the pathways.
* For abbreviations see Table 1.
* +7/+8q/+12 indicates the hyperdiploid pathway, 6q−/1q− indicates the hypodip-
dloid pathway.
arms, with a high frequency of 3p14/3p21 breaks, and 3p14–21 interstitial losses in Phases II.

**In Vitro Analysis of Chromosome Bridging.** Interphase remnants of anaphase bridges were detected in preparations from four well-differentiated ovarian adenocarcinomas with simple karyotypes (NIPT <5), including +7, +8q, or +12 but none of the common losses, and in five moderately or poorly differentiated tumors with karyotypes dominated by losses, including 1p−, 3q−, 6q−, 7p−, or −8, and with NIPT 3−19. The frequency of bridge remnants were <2% in all tumors of the former group. In the latter group four of five tumors showed bridge remnants in 5–20% of interphase cells; the remaining case showed remnants in <1% of cells.

**DISCUSSION**

In the present study we used the accumulated cytogenetic data from ovarian carcinomas to investigate the karyotypic profile of this tumor type. After identifying the most common imbalances, several statistical methods were applied to extract the most important features of the karyotypic evolution. The distribution of the CNs was heterogeneous. The large proportion of hypodiploid tumors and the even larger proportion of hyperdiploid tumors show that chromosome evolution in ovarian carcinomas may follow several different modes. A consequence of this may be the finding that the NIPT distribution was multimodal. A more detailed analysis of the NIPT of tumors with selected imbalances suggested the presence of at least three modes of karyotypic evolution: Phase I, with 1−7 imbalances, Phase II, with 8−15 imbalances, and Phase III, with >15 imbalances. Phase I tumors showed a geometrical-like distribution characteristic for a step-wise acquisition of imbalances (8) similar to the distributions found for bladder, breast, colorectal, kidney, and neural tumors (8, 14−16). This finding suggests that Phase I ovarian tumors evolve through mechanisms similar to those operating in these less complex tumor types, whereas different mechanisms of karyotypic evolution operate in Phase II and III tumors. We have recently identified a similar subdivision of head and neck squamous cell carcinomas into three karyotypic phases. Interestingly, these were also characterized by having NIPT values ranging from 1−7, 8−15, and 16−24, respectively.

In previous investigations we have used the total tumor population when analyzing the temporal order of imbalances (8, 14−16). However, this is only applicable when the distribution of NIPT is monomodal and, hence, is expected to follow one single mode of karyotypic evolution. To evaluate the temporal order among the imbalances in ovarian carcinoma, we therefore limited the analysis to the near-diploid Phase I tumors. We used the modal value of the NIPT distribution of tumors with a given imbalance as a measure for the TO of that imbalance (9). In this way we have arrived previously at chronological orders of karyotypic events that correlate with histopathological staging (14). The temporal analysis revealed 1q−, 6q−, −7, and −8q to be early imbalances, −4, −8, +1q, +12, and +20 to be intermediate, and the remaining imbalances to be late. The determined temporal order of imbalances correlated very well with stage and grade where early imbalances were predominantly seen in low-stage and low-grade tumors, whereas late imbalances were seen in high-stage and high-grade tumors. Hence, the deduced temporal order of imbalances most likely reflects the order occurring *in vivo*.

To screen for possible cytogenetic pathways, we reasoned that imbalances frequently present in the same cases would belong to the same pathway. Thus, the pattern of correlations among imbalances would reveal possible cytogenetic pathways. To condense the large correlation matrix produced by the 30 imbalances, and to extract the central features of the matrix, we performed PCA. We have shown previously the adequacy of this method by comparing it with multiple correspondence analysis (15), a method similar to PCA but based on $X^2$ statistics. However, to obtain additional information of the struc-

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ture of the imbalance data, HCA and MDS were performed on a matrix of Euclidean distances between the imbalances. These analyses revealed a striking clustering of gains and of losses, with the exception of +1q that was placed together with the losses. Thus, the ovarian carcinomas form two separate cytogenetic subgroups, one hyperdiploid and one hypodiploid. To obtain a more comprehensive model of the cytogenetic pathways we performed a PCA in which the TO for each imbalance was included as an observation. This organizes the imbalances along the first principal component according to the TO values and, thus, by including the second and third components, a three-dimensional representation of the cytogenetic pathways will be obtained (9). The first analysis suggested two pathways, one hyperdiploid, initiated by +7, +8q, or +12, and one hypodiploid initiated by 6q− followed by 1q−. However, the separate three-dimensional analysis of the hypodiploid pathway revealed the presence of two pathways, one initiated by 6q−, followed by +1q and −8, and one initiated by 1q−, followed by 1p− and −8. This conclusion was supported by the fact that 6q− and 1q− were placed in different clusters and at some distance in the HCA and MDS analyses, respectively. As the two early imbalances in the hyperdiploid pathway, +7 and +8q, showed positive correlation they do not represent two exclusive starting points. Both of these imbalances were followed by the much more frequent +12, followed by +20 and +3q, and by +6p and +2 in Phase II tumors. The particular importance of 1q−, 6q−, +7, +8q, and +12 in the development of ovarian carcinoma was also evident by the PCA of the tumor cases in which these were shown to be classifying imbalances. The fact that the third latter imbalances were needed to determine one of the major clusters indicates that they may substitute for each other and that they, together, distinguish one subtype of ovarian carcinomas. The +7/+8q/+12 pathway was found to be less aggressive, as the Phase I tumors belonging to this group showed a significantly lower proportion of stage III/IV and grade 2/3 tumors, compared with the Phase I 6q−/1q− tumors. This is in accordance with previous studies that have demonstrated that gain of the former chromosomes, in the absence of more complex changes, is a characteristic of borderline and low-grade ovarian carcinomas (17, 18).

The MDS of the imbalances present in Phase II tumors showed clustering of gains and of losses and, thus, the two major pathways seem to be separated to some extent in these tumors as well. In addition, 6q− and 1q− were positioned at some distance emphasizing the separation of the two alternative hypodiploid pathways also at Phase II. The frequencies of the individual imbalances in +7/+8q/+12 Phase I and in 6q−/1q− Phase I cases showed clear and distinct differences between the pathways (Table 2), but these differences were less distinct at Phase II. In fact, a high degree of overlap was seen, i.e., 6q− and 1q− were frequently seen together with +7, +8q, or +12. This could indicate that some Phase II tumors develop from Phase I through a recapitulation of Phase I. Another possibility is that Phase II tumors represent an altogether different subgroup of tumors, with a higher rate of acquiring imbalances, and in which both pathways may operate simultaneously, albeit with a slightly higher affinity for either of the hypo- or the hyperdiploid routes in individual cases. This would explain both the apparent separation of gains from losses in the MDS and the less distinct characteristics of the pathways at Phase II. However, a closer inspection of the Phase II tumors revealed that +7/+8q/+12 tumors without the concomitant presence of 6q− or 1q− were underrepresented, whereas 6q−/1q− tumors without +7, +8q, or +12 were not. This implies that +7/+8q/+12 tumors do not progress to Phase II as frequently as 6q−/1q− tumors do. The common simultaneous presence of +7/+8q/+12 and 6q−/1q− then indicates that the acquisition of the latter set of imbalances is a crucial step for progression from low-grade and borderline carcinomas to high-grade ovarian cancer. Hence, Phase II tumors seem to originate either from Phase I 6q−/1q− or from Phase I +7/+8q/+12 tumors having also acquired 6q− and/or 1q−. The present analyses thereby indicate at least two distinct pathways for ovarian epithelial carcinogenesis: one initiated by chromosomal gain in low-grade/borderline lesions, which in rare cases dedifferentiate into high-grade tumors through the acquisition of chromosomal losses, including 6q− and/or 1q−, and another pathway initiated by 6q−/1q− with a higher propensity to progress to Phase II (Fig. 7).

More than a third of the ovarian carcinomas showed triploid CNs. The NIPT distribution of these tumors clearly distinguished two categories, Phase I cases with a few imbalances and Phase III cases with >15 imbalances. The Phase I cases showed significantly lower frequencies of +7, +8q, and +12, than of 6q− and 1q−. If the acquisition of imbalances precedes the polyploidization step, which we find reasonable to assume, the higher frequencies of 6q− and 1q− suggest that the hypodiploid cases are more prone to undergo polyploidization than the hyperdiploid. In contrast, the Phase III tumors showed +7 and +12 in frequencies comparable with the frequencies seen in near-diploid Phase I and Phase II cases. Considering that the hyperdiploid +7/+8q/+12 cases have a low tendency to enter a polyploidization step, this would suggest that the Phase III cases originate from the near-diploid Phase II tumors. A major difference between the Phase I and Phase II tumors was the high level of cytogenetic overlap in the latter group, i.e., tumors tended to have imbalances from both pathways. Hence, the common denominator in the two suggested that polyploidization events are the presence of imbalances belonging to the 6q−/1q− pathway.

In Phase I tumors, frequent losses of the most telomeric bands were seen. In Phases II and III, this was replaced by a random pattern of breaks along the chromosome arms, and eventually by the loss of the entire arm. An exception was 3p, where breaks at 3p14/3p21 were the dominating events. A telomeric-centromeric shift of breakpoints has been associated with CIN caused by telomere shortening, eventually leading to a telomere crisis (19). The telomere dysfunction may, in turn, trigger the evolution of highly complex karyotypes through repeated BFB events. Measurements of terminal restriction fragment lengths have demonstrated that telomeres in the majority of ovarian carcinomas are indeed shorter than in normal ovary surface epithelium (20) and that the shortening is more pronounced at higher FIGO stages (21). Also, the presence of BFB events has been demonstrated in both primary tumors and established cell lines from ovarian adenocarcinomas (13, 22). In this study we complemented the statistical analyses with measurements of anaphase bridging in a few ovarian carcinomas. Evidence of bridging events was found only in complex tumors with losses but not in those with simple karyotypes including +7, +12, or...
+8q. This indicates that at least two distinct mechanisms for chromosomal evolution are operating in ovarian carcinomas: on the one hand, a thus far unknown mechanism, possibly related to mitotic checkpoint failure (23), generating a smaller number of changes in a step-wise manner in low and moderate grade tumors, and on the other, BFB events leading to the evolution of multiple chromosomal changes, mostly seen as chromosomal deletions in high-grade tumors. The progression from hyperdiploid Phase I to Phase II indicates that the latter mechanism may sometimes be superimposed on the former during progression toward a more aggressive phenotype. BFB events have been implicated in mitotic failure and polyploidization in head and neck carcinomas (24). Thus, the absence of BFB instability in hyperdiploid tumors may possibly also explain why these rarely, compared with the hypodiploid Phase I and Phase II tumors, undergo polyploidization. The unique deletion pattern of 3p may, finally, indicate that another, third mechanism of chromosome mutation operates at these stages. The breakpoints in these tumors were concentrated to 3p14/3p21, containing the FRA3B fragile site (25).

In conclusion, the karyotypic evolution in ovarian carcinomas is characterized by at least two different cytogenetic pathways (Fig. 7). One characterized by chromosomal gains, the +7/8q,+12 pathway, corresponding to low-stage and low-grade tumors, and a second, the 6q−/1q− pathway, characterized by losses and showing moderate levels of stage and grade. At the early stages the karyotypic evolution show similarities to many other tumor types by the seemingly stepwise gain of changes, resulting in the Phase I tumors. The transition to Phase II involved the acquisition of an increased CIN, most probably caused by extensive telomere crisis and the onset of BFB cycles, a process linked to the presence of imbalances characteristic for the 6q−/1q− pathway. Hence, low-stage and borderline +7/8q,+12 tumors could not progress unless they displayed mixed-pathway characteristics. The onset of triploidization was linked to the presence of the 6q−/1q− pathway, and possibly to the predisposition of such tumors to engage in BFB events. Thus, the key element in the progression of ovarian carcinomas is the 6q−/1q− pathway.

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Ovarian Carcinoma Develops through Multiple Modes of Chromosomal Evolution

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