Loss of Heterozygosity Accumulation in Primary Breast Carcinomas and Additionally in Corresponding Distant Metastases Is Associated with Poor Outcome

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ABSTRACT

The occurrence of distant metastases is the most feared manifestation of breast cancer, often occurring years after the primary surgery and associated with poor survival. The dominant metastatic clone is characterized by an accumulation of genetic alterations, but it is not actually known at what stage of the metastatic cascade these alterations have occurred. We investigated allelic losses during breast cancer progression in a series of 17 primary breast carcinomas and 22 corresponding brain, liver, lung, and bone metastases (mean metastasis-free interval, 31 months) by analyzing 19 microsatellite markers on seven breast cancer- or metastasis-related chromosomal regions and correlated the incidence of combined loss of heterozygosity (LOH) with metastasis-free and postmetastatic survival. We found that, in comparison with the corresponding primary tumor, additional LOH events are frequently found in metastases and that the incidence of combined LOH in the primary tumor, plus the occurrence of additional LOH events in the distant metastases, correlated significantly with decreased postmetastatic survival. We hypothesize that the occurrence of additional LOH events is either involved in termination of dormancy of micrometastatic tumor cells at distant organ sites or acquired during further progression of metastases.

INTRODUCTION

The metastatic spread of malignant tumor cells from the primary tumor to distant organ sites is the most life-threatening manifestation of breast cancer disease, and most patients ultimately die because of metastatic dissemination. Metastasis formation is a highly selective and multistep process involving complex interactions between tumor and host cells (1). To metastasize, tumor cells detach from the primary tumor, invade the host stroma, and penetrate into the vessels, where they disseminate, extravasate, and start to grow at susceptible organ sites. All of the steps must be completed successfully for the tumor cell to avoid elimination. Survival of a minor subpopulation of cells with increased metastatic potential is favored within the population of heterogeneous primary tumor cells (2). According to the clonal evolution theory (3), this cell clone, usually the most aggressive one, has an additional selective growth advantage over its nonmetastatic counterparts: the capacity to overgrow the primary tumor and to metastasize (“clonal dominance”; Ref. 4). Metastatic cell clones are characterized by increased genetic instability and the accumulation of genetic alterations affecting various genes such as proto-oncogenes, tumor/metastasis suppressor genes, and genes involved in the DNA repair pathway (5–6). It is not known whether this accumulation of genetic alterations is restricted to the time of selective growth in the primary tumor or is acquired during later stages of the metastatic process.

Many studies on primary breast tumors have reported a positive correlation between high LOH2 frequency in various tumor suppressor gene loci and clinical parameters of increased tumor malignancy (7–14), which suggests that the malignant behavior of the primary tumor is influenced by the LOH frequency. In other studies, no such correlation could be found (15, 16), perhaps because the growth of this metastatic cell clone is influenced by specific genetic alterations in the parental tumor (“seed”) as well as the microenvironment of the host organ (“soil”; Ref. 17).

Comparative LOH analyses of a variety of tumor entities such as non-small cell lung carcinomas and squamous cell carcinomas of the head and neck have shown that metastases are characterized by additional genetic alterations in comparison with the primary tumor (18–21). However, studies on primary breast carcinomas and corresponding distant metastases have in the past been restricted to single cases (22).

The aim of this study was to investigate at what stage of the

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2 The abbreviations used are: LOH, loss of heterozygosity; MFI, metastasis-free interval; PMS, postmetastatic survival; ER, estrogen receptor; PR, progesterone receptor; CM, brain metastasis/metastases; LM, liver metastasis; PM, pulmonary metastasis; BM, bone metastasis.
metastatic cascade genetic alterations occur in a series of sporadic breast carcinomas and to determine whether LOH frequency could be correlated with the clinical course and outcome.

To elucidate this question, we performed a comparative LOH analysis using 19 microsatellite markers in a series of 17 primary sporadic breast tumors and 22 corresponding metastases to the brain, liver, lung, or bone with a mean LOH of 31 months. We selected three chromosomal regions (17q21, 13q12–13, and 11q22–23) known to harbor genes associated with breast cancer (23–25) and three putative breast cancer metastasis-related gene loci (17q21.3, 1q32–41, and 16q22.2–23.2;Refs. 26–28) as well as the 17p13.1 locus harboring the p53 gene (“guardian of the genome”; 29), and we correlated the LOH incidence with patients’ MFI sand PMS times.

MATERIALS AND METHODS

Patients and Samples. Peripheral blood and tumor samples from 17 breast cancer patients as well as 22 corresponding metastases to the brain (CM, n = 15), liver (LM, n = 2), lung (PM, n = 2) and bone (BM, n = 3) were collected during surgery. From four patients, up to three different distant metastases were available (Table 1). We excluded lymph node metastases from our examination because they are normally gained during primary surgery, nor did we use local recurrences because they may arise from residual tumor cells. Fourteen of the 17 corresponding primary breast tumor specimens were archival paraffin tissue samples. Histopathological diagnosis and tumor stage (tumor-node-metastasis, stage I-IV) were determined by pathologists according to the WHO criteria. At the time of first diagnosis, 1 breast tumor was classified as stage I, 10 tumors as stage II, 3 tumors as stage III, and 3 tumors as stage IV. All except one tumor (lobular carcinoma) were diagnosed as invasive ductal breast carcinomas, 10% were grade I, 20% were grade II, and 70% were grade III tumors. Two tumors were ER/PR positive, four tumors were ER positive/PR negative, one tumor was ER negative/PR positive, and six tumors were negative for both receptors. The mean interval between primary surgery and surgery of distant metastases (MFI), and the mean PMS times were used as clinically relevant prognostic factors. The mean MFI was 31 months (range, 0–136 months), and the mean observation time of the patients after the first diagnosis of breast cancer was 40 months (range, 7–156 months; Table 1). Patients underwent different adjuvant hormone, radiation, and chemotherapies according to standard treatment guidelines.

DNA Extraction. Immediately after surgery, microscopically identified tumor tissue was snap-frozen in liquid nitrogen and stored at −80°C. For paraffin-embedded samples, tumor regions were harvested by the microdissection of 10-μm tissue sections. Extraction of genomic DNA from frozen or paraffin-embedded tumor samples and peripheral blood leukocytes was performed using the QiAamp blood and tissue kit (QIAGEN) according to the manufacturer’s instructions.

Microsatellite Analysis. Allelic loss (LOH) at three breast cancer-related loci (chromosome 11q22–23, 13q12–13, 17q21), three metastasis-related chromosomal regions (chromosome 1q32–41, 16q22.2–23.2, 17q21.3), and the p53 locus (17p13.1) was examined using 19 polymorphic microsatellite markers (listed in Table 1). The β-actin locus on 15q11 was used as control.

Microsatellite analysis was performed as described previously (30). Briefly, paired peripheral blood leukocyte and tumor DNAs were amplified by PCR in a Gene Amp 9600 thermocycler (Perkin-Elmer). PCR products were analyzed by gel electrophoresis on an automated laser fluorescence (A.L.F.) sequencer (Pharmacia Biotech; Fig. 1), and calculation of allelic loss was determined semiquantitatively using the Fragment Manager software. Resulting values of ≥1.5 (imbalance factor), reflecting allele reduction of one-third or more in tumor DNA, were defined as the LOH cutoff point. LOH of one chromosomal locus was defined if at least one of the markers analyzed for this region showed LOH.

Statistical Methods. The combined LOH at several gene loci was chosen as study criteria based on the assumption that the accumulation of genetic alterations in several gene loci has a major influence on the metastatic process. We used a combination of the seven chromosomal regions together to evaluate a general influence; for gene-specific influence, subgroups were formed as defined in Table 3. Groups were proven to be homogeneous regarding their tumor stage using χ² test. Differences in the distribution of LOH between the different groups of samples were tested by using the one-tailed χ² test or Fisher’s exact test. Significance of differences between the MFI sand PMS times of patients were tested by Student’s t test (MFI) and the log-rank test (PMS), respectively. PMS rates of patients were estimated by the Kaplan-Meier method.

RESULTS

Incidence of Allelic Losses in Primary Breast Tumors and Distant Metastases. LOH frequencies at the different chromosomal regions in 17 primary breast tumors and 22 distant metastases are listed in Tables 1 and 2. At each locus examined, the incidence of allelic loss was higher in the group of distant metastases compared with the group of primary tumors. By the χ² test, allelic loss in metastases was significantly higher at 17p13.1, 13q12–13, and 16q22.2–23.2 (P < 0.05), whereas at 17q21, 11q22–23, 17q21.3, and 1q32–41 the observed increases did not reach statistical significance. We also observed an insignificant increase at the β-actin control locus.

Correlation of Combined LOH with MFI and PMS. To investigate whether the incidence of LOH in either the primary tumor group or the group of distant metastases may be used as a prognostic factor, we correlated combined LOH frequencies of each group with patients’ MFI s and PMS times. In the group of primary breast tumors, we observed a correlation of increased combined LOH events with decreased MFI s as well as with decreased PMS for all of the loci groups examined, although results did not reach statistical significance. Unexpectedly, in the group of distant metastases, we found an inverse correlation of LOH events with the MFI s (mean MFI of patients with high LOH, 32.9 months versus 30.8 months for patients with LOH, P = 0.707). But again, patient samples exhibiting a high combined LOH in the metastases had a shorter (although not significantly) PMS than those with low LOH.
Correlation of Combined LOH Frequency in Primary Tumors plus Additional LOH Events in Distant Metastases and Clinical Course. On the basis of our observation of a correlation of increased numbers of LOH events with decreased patient survival times and according to the established theory that the metastatic potential of a tumor cell seems to be characterized by the accumulation of genetic alterations, we hypothesized that both the incidence of LOH in the primary lesion plus the occurrence of additional LOH events in the distant metastases may determine the malignant behavior of metastatic tumors. For statistical evaluation, we defined two patient groups for each of the four gene loci combinations based on their LOH status in primary tumors plus the occurrence of additional LOH events in their distant metastatic tumor samples; group 1 consisted of patients with low LOH, whereas group 2 comprised patients with high LOH according to the definition in Table 3.

Table 1  LOH in 17 sporadic breast carcinomas and 22 corresponding distant metastases in seven chromosomal regions, harboring breast cancer or metastasis-related genes and one control locus (β-actin)

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Table 1. LOH in 17 sporadic breast carcinomas and 22 corresponding distant metastases in seven chromosomal regions, harboring breast cancer or metastasis-related genes and one control locus (β-actin).

- **Casea**: Oligonucleotide primer sequences are according to the Genethon genetic linkage map and Genome Database (GDB; http://gdbwww.gdb.org).
- **Stageb**: Primary breast tumors were staged at the time of first surgery according to tumor-node-metastasis classification.
- **MFIc (mo)**: Time between first diagnosis of breast cancer and occurrence of the distant metastases.
- **PMSc (mo)**: Survival time after surgery of distant metastases.
- **17p13.1p53**: TP53
- **17q21**: BRCA1
- **13q12–13**: BRCA2
- **11q22-23**: ATM
- **17q21.3**: NM23
- **16q22-22.3**: KISS-1
- **1q32-41**: b-actin
- **15q11**: b-actin

- **Patient alive.**
- **Patient died from myocardial infarction.**

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Fig. 1  Illustration of LOH. Examples of computer print-outs of polyacrylamide gel analyses of PCR products by an automated DNA sequencer. Shown are PCR products from selected breast tumors (second row), distant metastases (third to fifth rows), and corresponding blood samples (first row), amplified with different microsatellite primers. The microsatellite alleles are represented by two (heterozygote) peaks. LOH at one allele (arrows) corresponds to a significant reduction in one of the two peaks (imbalance factor, ≥1.5). Homozygosity (one allele only) is not shown in these
The combination of seven chromosomal regions (Table 3B) for the correlation of these defined criteria with the PMS (Table 3B) for the combination of seven chromosomal regions (P = 0.0017) and the combination of p53 with the three breast cancer-related regions (P = 0.0203) as well as the breast cancer regions alone (P = 0.0241). Patients with high combined LOH events showed a significant reduced PMS in comparison with those with low LOH (Fig. 2A and B). In correlation with the MFI, these three groups showed positive trends without reaching statistical significance (Table 3A). In contrast, the three metastasis-related chromosomal regions revealed a surprising inverse correlation with the MFI (P = 0.431) and an insignificant correlation with the PMS times (P = 0.0805). These results suggest that the occurrence of combined genetic alterations in the three putatively metastasis-related chromosomal regions has no major influence on the aggressive behavior of metastasis in breast carcinomas.

Accumulation of Allelic Losses during Tumor Progression in Corresponding Tumors. We found that concurrent as well as different LOH patterns in primary and corresponding metastatic tumor samples were observed at a similar frequency (Table 1). However, in 14 of the 17 cases, a different LOH pattern was present in at least one of the seven regions examined. We never observed the phenomenon that LOH was present in the primary tumor but absent in the corresponding distant metastatic lesion, which, therefore, excluded random events. Two patients (48 and 692, both stage IV) were operated on simultaneously for breast carcinomas and distant metastases. Both tumor sets revealed additional LOH events in the metastatic lesions, which suggested that the additional allelic losses had been acquired by the metastatic clone after detachment from the parental tumor.

Up to three distant metastases, removed at different time intervals, were available from four patients (see Table 1): the first metastatic lesion, which, therefore, excluded random events. We never observed the phenomenon that LOH was present in the primary tumor but absent in the corresponding distant metastatic lesion, which, therefore, excluded random events. Two patients (48 and 692, both stage IV) were operated on simultaneously for breast carcinomas and distant metastases. Both tumor sets revealed additional LOH events in the metastatic lesions, which suggested that the additional allelic losses had been acquired by the metastatic clone after detachment from the parental tumor.

Up to three distant metastases, removed at different time intervals, were available from four patients (see Table 1): the first metastatic lesion, which, therefore, excluded random events. We never observed the phenomenon that LOH was present in the primary tumor but absent in the corresponding distant metastatic lesion, which, therefore, excluded random events. Two patients (48 and 692, both stage IV) were operated on simultaneously for breast carcinomas and distant metastases. Both tumor sets revealed additional LOH events in the metastatic lesions, which suggested that the additional allelic losses had been acquired by the metastatic clone after detachment from the parental tumor.

The results of this statistical evaluation are summarized in Table 3. Briefly, we found significant differences in the correlation of these defined criteria with the PMS (Table 3B) for the combination of seven chromosomal regions (P = 0.0017) and the combination of p53 with the three breast cancer-related regions (P = 0.0203) as well as the breast cancer regions alone (P = 0.0241). Patients with high combined LOH events showed a significant reduced PMS in comparison with those with low LOH (Fig. 2A and B). In correlation with the MFI, these three groups showed positive trends without reaching statistical significance (Table 3A). In contrast, the three metastasis-related chromosomal regions revealed a surprising inverse correlation with the MFI (P = 0.431) and an insignificant correlation with the PMS times (P = 0.0805). These results suggest that the occurrence of combined genetic alterations in the three putatively metastasis-related chromosomal regions has no major influence on the aggressive behavior of metastasis in breast carcinomas.

Accumulation of Allelic Losses during Tumor Progression in Corresponding Tumors. We found that concurrent as well as different LOH patterns in primary and corresponding metastatic tumor samples were observed at a similar frequency (Table 1). However, in 14 of the 17 cases, a different LOH pattern was present in at least one of the seven regions examined. We never observed the phenomenon that LOH was present in the primary tumor but absent in the corresponding distant metastatic lesion, which, therefore, excluded random events. Two patients (48 and 692, both stage IV) were operated on simultaneously for breast carcinomas and distant metastases. Both tumor sets revealed additional LOH events in the metastatic lesions, which suggested that the additional allelic losses had been acquired by the metastatic clone after detachment from the parental tumor.

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Table 2  Incidence of LOH in primary breast tumors and corresponding distant metastases

<table>
<thead>
<tr>
<th>Chromosomal region</th>
<th>Candidate gene locus</th>
<th>Primary breast carcinomas, %</th>
<th>Distant metastases, %</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>17p13.1</td>
<td>p53</td>
<td>35 (5/15)</td>
<td>68 (13/19)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>17q21</td>
<td>BRCA1</td>
<td>27 (4/15)</td>
<td>44 (8/18)</td>
<td>NS*</td>
</tr>
<tr>
<td>13q12–13</td>
<td>BRCA2</td>
<td>13 (2/16)</td>
<td>67 (14/21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>11q22–23</td>
<td>ATM</td>
<td>27 (4/15)</td>
<td>55 (13/20)</td>
<td>NS</td>
</tr>
<tr>
<td>17q21.3</td>
<td>NM23</td>
<td>38 (6/16)</td>
<td>57 (12/21)</td>
<td>NS</td>
</tr>
<tr>
<td>16q22.2–23.2</td>
<td>unknown</td>
<td>41 (7/17)</td>
<td>73 (16/22)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1q32–41</td>
<td>KISS-1</td>
<td>41 (7/17)</td>
<td>55 (12/22)</td>
<td>NS</td>
</tr>
<tr>
<td>15q11</td>
<td>β-actin</td>
<td>23 (3/13)</td>
<td>38 (7/18)</td>
<td>NS</td>
</tr>
</tbody>
</table>

a x² test/Fisher’s exact test, significant if P ≤ 0.05.
b Cases with LOH/cases informative.
c NS, not significant.

Table 3  Correlation of the combination of LOH incidence in primary tumors plus additional LOH in distant metastases with MFI and PMS

<table>
<thead>
<tr>
<th>Primary tumors and metastases</th>
<th>Seven loci a</th>
<th>p53 + breast cancer regions b</th>
<th>Breast cancer-related regions c</th>
<th>Metastasis-related regions d</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Mean MFI, in months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (low LOH)</td>
<td>34.8</td>
<td>34.7</td>
<td>35.2</td>
<td>25.5</td>
</tr>
<tr>
<td>Group 2 (high LOH)</td>
<td>19.0</td>
<td>22.4</td>
<td>25.8</td>
<td>42.3</td>
</tr>
<tr>
<td>P*</td>
<td>0.526</td>
<td>0.247</td>
<td>0.731</td>
<td>0.431</td>
</tr>
<tr>
<td>B. Mean PMS, in months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1 (low LOH)</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Group 2 (high LOH)</td>
<td>6.0</td>
<td>8.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>P f</td>
<td>0.0017</td>
<td>0.0203</td>
<td>0.0241</td>
<td>0.0805</td>
</tr>
</tbody>
</table>

a Combined LOH at 17p13.1, 17q21, 13q12–13, 11q22–23, 1q32–41, 16q22.2–23.2, and 17q21.3; group 1: ≥1 LOH in primary tumor plus ≥1 additional LOH in metastases; group 2: ≥2 LOH in primary tumor plus ≥2 additional LOH in metastases.
b Combined LOH at 17p13.1, 17q21, 13q12–13, and 11q22–23; group 1: heterozygous for p53 in primary tumor independent of LOH events in metastasis; group 2: LOH in p53 in primary tumors plus ≥1 additional LOH in the three breast cancer regions in metastases.
c Combined LOH at 17q21, 13q12–13, and 11q22–23; group 1: no LOH in primary tumor and no additional LOH in metastases; group 2: ≥1 LOH in primary tumor plus ≥1 additional LOH in metastases.
d Combined LOH at 1q32–41, 16q22.2–23.2, and 17q21.3; group 1: no LOH in primary tumor and no additional LOH in metastases; group 2: ≥1 LOH in primary tumor plus ≥1 additional LOH in metastases.

* Significant if P ≤ 0.05, Student’s t test.
' Significant if P ≤ 0.05, log-rank test.

BM and CM of patient 660 revealed a different LOH pattern, which suggested an independent development; and, in addition, both metastatic lesions accumulated LOH events compared with the primary tumor. Patient 677 developed a local recurrence in the brain (CM/II) 7 months after the removal of the first metastatic lesion (CM/I). The first metastasis (CM/I) had accumulated two additional LOH events in comparison with the primary breast tumor, whereas in the local recurrence (CM/II), four additional LOH events were obvious. Assuming that the recurrence may have developed from residual tumor cells, this example suggests that these cells are susceptible to further genetic instability during the time...
course. In contrast, the brain and lung metastases of patient 1137 revealed an identical LOH pattern and only one additional LOH event compared with the primary tumor. Patient 1401 was operated on for a first breast cancer CM (CM/I) 3 months before she developed a local recurrence (CM/II) and a further CM contralaterally (CM/III). The recurrent lesion and the contralateral metastasis had an identical LOH pattern, whereas, again, an increase of LOH during progressive disease was observed. We conclude that residual cancer cells gave rise to the local recurrence and acquired one additional LOH event during the time course, whereas the independent lesion located in the opposite brain hemisphere.

\[\text{Fig. 2} \quad \text{PMS curves of patients with low and high LOH.} \]

A. Decreased PMS of patients with \( \geq 2 \) LOH in primary breast tumor plus additional \( \geq 2 \) LOH in the distant metastases at 17p13.1, 17q21, 13q12–13, 11q22–23, 1q32–41, 16q22.2–23.2, and 17q21.3 (group 2) in comparison with those following this criteria (group 1; log-rank test, \( P = 0.0017 \)). *

\( \text{censored} \)

\[ P = 0.0017 \]

B. Decreased PMS of patients with LOH at 17p13.1 in primary tumor plus additional \( \leq 1 \) LOH in their metastases at 17q21, 13q12–13, or 11q22–23 (group 2) compared with those that are heterozygous at 17p13.1 independent of the LOH events found in distant metastases in the three breast cancer-related regions (group 1; log-rank test, \( P = 0.0203 \)).

\( \text{censored} \)

\[ P = 0.0203 \]
probably derived from the recurrent lesion, revealing an identical LOH pattern (Fig. 1A).

DISCUSSION

In this LOH analysis on a series of primary sporadic breast carcinomas and corresponding distant metastases, we found that in comparison with their primary tumor distant metastatic lesions showed an accumulation of additional genetic alterations. These observations confirm the data of several studies analyzing a variety of tumor entities and reporting higher LOH frequencies in metastases than in primary tumors (18–22, 27, 31–34). Most of these studies compared primary tumors and noncorresponding metastases (27, 31–33), whereas reports of comparative LOH analyses of primary tumors with their corresponding distant metastases are rare, are based on small patient numbers, and—to our knowledge—have not been performed in breast carcinomas (18, 20, 21, 34). In contrast, the comparison of LOH frequencies in primary breast tumors and corresponding axillary lymph node metastases showed an identical LOH pattern in all of the cases (35–38). This discrepancy may be due to the fact that lymph-node metastases are mainly harvested during primary operations, whereas distant metastases are characterized by hematogenous spread and a distinct time interval. In this comparative analysis of 17 primary breast tumors and 22 corresponding distant metastases, we observed that the incidence of LOH in the group of distant metastases was always higher than in primary tumors. It was significantly increased in three of the seven breast cancer- and metastasis-related chromosomal regions examined (17q21, 13q12–13, and 16q22.2–23.2; Table 2). The correlation of high combined LOH incidence with decreased MFI and PMS showed a positive trend but failed to reach statistical significance in the primary tumor group, which suggests that the LOH frequency in primary tumors does not seem to be the only relevant factor for the malignant behavior of
metastatic tumors. This result confirms the discordant observations of previous LOH studies in primary breast carcinomas, some of which reported a positive correlation of single or combined LOH frequencies with decreased survival or clinicopathological markers of enhanced malignancy (7–14, 39–41) and others unable to confirm these findings (15, 16).

Comparison of the LOH patterns of the corresponding tumor samples revealed that 14 of the 17 patients acquired additional LOH events in their metastatic lesions. When combined LOH in primary tumors plus the occurrence of additional LOH events in the distant metastases were correlated with the defined clinical parameters, we observed a significant correlation with decreased PMS ($P = 0.0017$; Table 3B and Fig. 2A). These results suggest that the increase of genetic instability during metastasis seems to accelerate disease progression. Evaluation of a gene-specific influence showed that the combined inactivation of the breast cancer-related regions alone ($P = 0.0241$; Table 3B), as well as the combination of LOH at the $p53$ locus in primary tumors with maintenance during progression plus the acquisition of additional LOH events in metastases in the three breast cancer-related regions (Fig. 2B; $P = 0.0203$) correlated significantly with decreased postmetastatic patient survival. This result can be brought in accordance with recent studies that suggest a mutual dependency of $p53$ and BRCA abnormalities in breast carcinogenesis (42).

According to the clonal evolution theory (3), distant metastases arise from a selected clone in the primary tumor that has acquired and accumulated a variety of genetic alterations necessary for its metastatic behavior. This clone will finally overgrow the primary tumor because of a growth advantage over its counterparts and, therefore, become the dominant clone in the late stage of tumor development (4). Following this theory, one would suspect an identical LOH pattern in the primary tumor and distant metastases, at least in the later stages of tumor growth and progression. The discordant LOH pattern in our tumor series may be due to the fact that the metastatic clone in the primary tumor is not yet the dominant one. In this case, the LOH pattern of the distant metastasis might be considered as a representative of the most aggressive clone of the primary tumor; and, based on previous data (11–13, 39), one could expect a correlation of high combined LOH frequencies in the distant metastases with decreased MFIs. Our contrary results of an inverse correlation suggest that the increase of LOH events in the distant metastases may be due to the accumulation of additional genetic alterations in the metastatic cell clone after detachment from the parental tumor.

To further specify the time point in the metastatic cascade at which these additional genetic alterations occur, we developed the following hypothetical model based on data of patients presenting with multiple recurrent distant metastases:

(a) a characteristic feature of breast cancer disease is the development of metastases many years after resection of the primary neoplasm. According to Paget’s “seed and soil” theory (17), the growth at susceptible distant organ sites is influenced by environmental host factors as well as the aggressive potential of the metastatic clone itself. Reduced patient immunity or the release of angiogenic factors (43) have a major influence on the sudden growth of dormant cells. However, the question remains: Why in some patients does a single metastasis arise at a certain organ site, whereas others develop multiple lesions at the same time? The occurrence of additional genetic events in a single one of the multiple quiescent, metastatic clones may represent a critical event, being involved in the sudden termination of the dormancy state and consecutively favoring the development of a single metastatic lesion. In contrast, an enhanced influence of host factors may rather favor the sudden growth of multiple metastatic clones at distant organ sites (for illustration, see Fig. 3).

(b) alternatively or additionally, the increased LOH incidence in metastatic lesions may be acquired during the progressive growth of the distant metastatic tumor cell clones (see patients 1401 and 677) and would be responsible for the increased malignant behavior of metastases and poor patient outcome (Fig. 2; for illustration, see Fig. 3).

In conclusion, the results of this comparative study of primary breast carcinomas and corresponding distant metastases suggest that the aggressive behavior of a metastatic breast tumor seems to be determined by the incidence of genetic alterations accumulated during selective growth in the primary tumor plus the acquisition of additional genetic alterations further down in the metastatic cascade (Fig. 3). In addition, our data suggest a specific influence of combined LOH—at the three breast cancer-associated chromosomal regions on $17q21$, $13q12–13$, and $11q22–23$ alone or in combination with allelic loss at the $p53$ locus—on the malignant behavior of breast cancer metastases.

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REFERENCES


Loss of Heterozygosity Accumulation in Primary Breast Carcinomas and Additionally in Corresponding Distant Metastases Is Associated with Poor Outcome

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