Identification of Multiple Breast Cancers of Multicentric Origin by Histological Observations and Distribution of Allele Loss on Chromosome 16q

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ABSTRACT

Breast cancer is often detected as multiple lesions clinically and/or histopathologically. To examine if the origin of such lesions can be identified objectively by comparison of their loss of heterozygosity (LOH) patterns, LOH on chromosome 16q was analyzed in a total of 60 cases of multiple breast cancer by Southern blot analysis. Based on continuity among tumors and satellite nodule features, 30 cases of unilateral multiple cancer were classified morphologically into 3 groups: A, multicentric origin (11 cases); B, multifocal invasion of one intraductal carcinoma (15 cases); and C, intramammary metastases (4 cases). As controls, group D, synchronously bilateral breast cancers (11 cases), and group E, sets of a primary tumor and a lymph node metastasis (19 cases), were also examined. On a highly probable assumption that LOH on 16q occurs randomly, each group was compared with the morphological classification and was suggested to be of diagnostic value.

INTRODUCTION

The incidence of multiple cancers in the unilateral breast is reported to vary from 9 to 75% (1-12). These cases may include not only true multicentric cancers but also multiple invasive cancers originating from one DCIS3 by multifocal invasion and intramammary metastasis. Several features have been proposed as morphological criteria for multicentric origin. These include lack of continuity among tumors via the DCIS component (4, 5), distance (≥5.0 cm) among tumors (6), the occurrence of tumors in different quadrants (7-10), and the presence of a DCIS component in each tumor. In addition, tumors with the appearance of satellite nodules around one main tumor can be judged as metastatic lesions. Although it appears possible in most cases to judge the origin of unilateral multiple breast cancer based solely on a combination of morphological findings, it is uncertain whether morphological observations are compatible with the data obtained by objective methods which might reveal accurately whether multiple tumors are of multicentric origin or have originated from a single tumor through metastasis. In cancers of multicentric origin, residual mammary glands appear to carry a high risk of second primary cancers, whereas in those that have originated from one tumor, the nature of the tumor would be highly invasive or metastatic. Therefore, accurate determination of origin would be important for making decisions about the treatment of unilateral multiple breast cancer.

Recently, examination of gene alterations has been applied to the determination of the origin of multiple cancers, e.g., the exogenous viral DNA integration pattern, LOH on certain chromosome regions, the mutation pattern in certain tumor suppressor genes, and the X-chromosome inactivation pattern (13-17). With these approaches, differences in the pattern of gene alterations among tumors have been regarded as evidence for their independent origin. However, gene alterations can be identical even among multiple tumors of independent origin. Furthermore, it is possible that the predominant clone of cancer cells and/or the pattern of gene alterations may alter between primary and metastatic sites in an identical tumor. This prompted us to introduce a probabilistic model for evaluating the origin of multiple cancers and to examine the concordance of gene alteration patterns between primary and metastatic lesions.

In the present study, using surgically resected tumor tissue specimens, we examined the possibility of predicting the origin of unilateral multiple breast cancers from the pattern of their gene alterations. For this purpose, cases of unilateral multiple cancer were first classified into three groups on the basis of morphological features, and the degree of concordance of LOH on 16q among multiple tumors was compared in each group. LOH on 16q has been shown to occur in approximately 50% of breast cancer cases and is suggested to be a random event at their early developmental stage (18, 19). Therefore, the number of cases with a concordant LOH pattern on 16q would be expected to follow a normal distribution model in a multicentric cancer group. The number of cases with a concordant LOH pattern on 16q among tumors was compared between the data obtained by RFLP analysis and the value estimated from a normal distribution model in each morphologically defined multiple cancer group.

PATIENTS AND METHODS

Cases and Histological Criteria. Among 745 patients with primary breast carcinoma who underwent mastectomy at the National Cancer Center Hospital between October 1991 and June 1994, unilateral multiple cancers were observed in 69 patients (9.3%). From 30 of these patients, we obtained fresh tissue specimens of unilaterally multiple tumors. In each case, the presence of an intraductal component in each tumor nodule and continuity among tumor nodules via the intraductal component were examined by histological observation of serially dissected tissue sections. The origin was judged morphologically to be multicentric when (a) multiple cancers were not combined together via the DCIS component and (b) they did not show satellite lesions, which appear as tumor nodules located around a main tumor, sometimes occurring in the skin or adipose tissue, being much smaller in size, one-third or less of the diameter of the main tumor, and having no intraductal component. When the DCIS components of multiple tumors were confirmed or strongly suggested to have a connection with each other microscopically, they were judged to be multifocal invasions of one DCIS. The presence of DCIS in each invasive carcinoma was considered to be a supportive, but not conclusive, indicator of its primary nature. Whether or not the tumors were located within a single quadrant was not taken into account when judging their origin.

According to the above criteria, 11 cases were classified as group A, multicentric origin; 15 as group B, multifocal invasion of one DCIS, in which multiple invasive carcinomas were combined together via the DCIS.
RESULTS

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high, 82% (9/11) and 91% (10/11), respectively. The pattern of LOH on 16q among tumors was identical in 5 (45%) of 11 cases in each of groups A and D (Table 1). In group A, LOH was detected in one tumor of multiple cancers in five cases and in all tumors in four cases: it occurred in the same allele in three cases and in different alleles in one case, but was not detected in any of the tumors in two cases. In group D, LOH was detected in either of bilateral tumors in five cases and in both tumors in another five cases: it occurred in the same allele in four cases and in different alleles in one case, and one case did not reveal LOH in any of the tumors.

In groups B, C, and E, the incidence of LOH on 16q was 40% (6/15), 25% (1/4), and 42% (8/19), respectively. The LOH pattern among multiple tumors or between primary and metastatic lesions was always identical in groups B (15/15), C (4/4), and E (19/19) (Table 1).

Three tumors were examined in two group A cases and five group B cases. In each case, the possibility that the LOH pattern was the same among 3 tumors was estimated to be only 25% if the LOH had occurred randomly in each tumor with a $P = 0.5$. In fact, in both of the two group A cases, one tumor revealed LOH but the other two did not. For approximation, these two group A cases were considered to carry two tumors which differed in their LOH patterns. In all five group B cases, the LOH pattern was always identical among three tumors, and two tumors were considered to have an identical LOH pattern for statistical analysis.

The observed $x$ values in groups A and D (five each) were within the range of $\mu \pm 2\sigma$ for the estimated $X$ value based on a normal distribution, whereas the observed $x$ lay outside $\mu \pm 2\sigma$ in groups B and E (Table 1). In group C, the LOH pattern was concordant in all four cases, but the number of cases was too small to conclude whether this was statistically significant.

**DISCUSSION**

It was reasonable to conclude that the two tumors in each case in groups A and D were of multicentric origin, because LOH was distributed randomly among the tumors and they were considered to have occurred independently. On the other hand, the multiple tumors in each of the cases in groups B, C, and E were considered to have originated from a single tumor because the pattern of LOH was always concordant among the tumors in each individual case.

The allele pattern on 16q was always concordant among multiple invasive carcinoma foci judged morphologically to represent multifocal invasion of one DCIS. These data support the notion that LOH on 16q occurs at the preinvasive stage and usually does not alter during the process of stromal tumor invasion. Judging from the identical allele pattern between tumor pairs in individual cases in groups C and E, the allele pattern on 16q was also strongly suggested to be almost always concordant between the primary tumor and intramammary/axillary lymph node metastases. Such consistency of LOH on 16q in an individual tumor, regardless of invasion and regional metastases, and its random but high incidence allows us to conclude that examination of LOH on 16q is effective for revealing the origin of multiple breast cancers.

When the pattern of LOH differs among multiple cancers in a unilateral breast, the tumors could be judged to have originated independently. However, in one-half of the cases, the pattern of LOH on 16q was concordant even if they had occurred independently. Clonal analysis by examination of the DNA methylation pattern on the X chromosome involves the same problem (16, 17). Therefore, to estimate accurately the origin of multiple breast cancers at the individual level, combined examination of several gene alterations, which occur frequently in breast cancers, and X chromosome inactivation pattern, is necessary. LOH on the loci 11p, 17p, 17q, and 7q and mutation of the p53 gene occur in >20% of breast cancer cases (25–28). Although most of these alterations are known to occur specifically in breast cancers with high-grade atypia (grade 3; Refs. 25–28) and their occurrence differs among tumors of various histological grades, their examination combined with LOH on 16q would give significant additional information on the origin of multiple cancers in individual cases.

The validity of the morphological criteria used in the present study for the origin of unilateral multiple cancers was supported by the results obtained from analysis at the DNA level. These criteria, based simply on the continuity between tumors via the DCIS component and a satellite nodule feature, were shown to be sufficient for differentiation between two groups with different distributions of LOH on 16q.

Some authorities consider that the multiple cancers combined via a DCIS component and/or comprising main and satellite nodules are multifocal cancer, in contrast to multicentric cancer, which denotes the independent occurrence of multiple cancers (2, 17). On the basis of this definition, the majority of multiple unilateral breast cancers are not truly of multicentric origin. Our results also support this concept, indicating that the incidence of multicentric unilateral breast cancer might be lower than that reported.

In the 11 cases in group D, the occurrence of LOH on 16q was distributed randomly between lesions on each side. Synchronously bilateral breast cancers were thus confirmed to have occurred independently. The majority of bilateral breast cancers occur metachronously. Although the possibility of involvement of LOH on 16q in hematogenous metastases has still not been ruled out (29), the model used in this study would be applicable for determining the origin of such metachronous bilateral cancers.

It is natural that the degree of predisposition not only to multiple breast cancer in patients, but also to primary breast cancer in their families, might differ between groups showing multiple cancers of true multicentric origin and those of monofocal origin. Bilateral breast cancer in premenopausal patients has been shown to be a risk factor for familial predisposition to breast cancers (30, 31). Both bilateral occurrence and true unilaterally multicentric occurrence should have the same significance with regard to genetic predisposition to breast carcinogenesis. Therefore, diagnosis of unilateral breast cancers of multicentric origin, based on histological observations and/or examination of the LOH pattern, might be helpful for identifying patients with a high risk of a second future primary breast cancer and/or primary breast cancer in their family members.

The incidence of LOH on 16q in at least one of a tumor pair was 82 and 91% in groups A and D, respectively, but only 40%, 25%, and 42% in groups B, C, and E, respectively. On the condition that LOH on 16q occurs randomly in 50% of breast cancers, the occurrence of LOH on 16q in at least one of a group of multicentric cancers is
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REFERENCES


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