A Tumor Suppressor Gene on Chromosome 1p32–pter Controls the Amplification of MYC Family Genes in Breast Cancer

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Abstract

To investigate the possibility of collaboration between telomeric deletion on the short arm of chromosome 1 and genetic amplification similar to that described in human neuroblastoma, 122 human primary breast tumors were examined by restriction fragment length polymorphism analysis for loss of heterozygosity on 1p32–pter and for the three most frequently amplified genetic regions in breast carcinomas (MYC and ERBB2 protooncogenes and the chromosomal region 11q13). Allelic losses at one or more loci on the telomeric part of the short arm of chromosome 1 was observed in 57 (47%) of 122 informative tumors. MYC, ERBB2, and the 11q13 region were amplified in 23, 20, and 21% of breast tumors, respectively. A correlation was found between loss of heterozygosity on chromosome 1p32–pter and amplification of the MYC (formerly c-myc) protooncogene (P = 0.003), suggesting that these two genetic events may collaborate during tumor progression in human breast cancer.

These results, together with those obtained in human neuroblastoma, suggest that the distal part of the short arm of chromosome 1 harbors an unidentified tumor suppressor gene(s), whose inactivation may be involved in MYC family gene amplification (an example of genetic instability) in tumors of various cellular origins.

Introduction

Carcinogenesis is now considered to be a highly complicated process in which accumulation of gene mutations are required to transform a normal cell into a malignant cell. Any of several mechanisms, such as point mutations, translocations, gene amplifications, and loss or gain of whole chromosomes or part of a chromosome, can activate oncogenes or inactivate tumor suppressor genes and allow a cell to escape from normal growth control.

We have little evidence that particular oncogenes or tumor suppressor genes have an exclusive role in tumorigenesis. It is even conceivable that the malignant phenotype results merely from the cumulative effect of multiple genetic alterations (1). On the other hand, the distinctive combinations of genetic lesions that appear in different tumors raise the possibility that such abnormalities may collaborate in specific ways during tumorigenesis. For example, the human MDM2 protein binds to and functionally inactivates p53 protein (2), while the NF1 tumor suppressor gene protein, which resembles a GTPase-activating protein, presumably interacts directly with a RAS protein (3).

The hypothetical relationship between tumor suppressor gene inactivation and oncogene amplification is supported by the fact that the ability to amplify an endogenous gene behaves as a recessive genetic trait (4). In human neuroblastoma, the protooncogene MYCN may be under the control of a tumor suppressor gene located on the distal short arm of chromosome 1, which is deleted concomitantly with amplification of MYCN (5–7).

In human breast cancer, we have previously identified on the short arm of chromosome 1 two distinct deleted regions, 1p13–p21 and 1p32–pter, suggesting that two tumor suppressor genes may be involved in the development of human breast carcinoma (8). The latter region is potentially the same as that involved in neuroblastoma, suggesting that this region (1p32–pter) harbors a tumor suppressor gene relevant to the induction of more than one tumor type.

We hypothesized that the most frequently amplified genetic regions in breast carcinomas (MYC and ERBB2 protooncogenes and the chromosomal region 11q13), which are examples of genetic instability, may be under the control of a putative suppressor gene located at 1p32–pter, which may be similar to that involved in neuroblastoma.

To verify this hypothesis, we performed RFLP analysis of 122 human breast tumors for evidence of amplification of MYC, ERBB2, CCND1, and INT2 genes and loss of chromosome 1p32–pter using five polymorphic markers located in this region. We only observed an association between 1p32–pter LOH and MYC amplification but not with the two other amplified DNA sequences.

Materials and Methods

Tumor and Blood Samples. Samples were obtained from 122 primary breast tumors surgically removed from patients at the Centre René Huguenin (St. Cloud, France); none of the patients had undergone previous radiotherapy or chemotherapy. Immediately following surgery, the tumor samples were stored in liquid nitrogen until extraction of high-molecular-weight DNA. A blood sample was also taken from each patient.

DNA Analysis. DNA was extracted from tumor tissue and blood leukocytes from each patient, according to standard methods (9). Ten μg of DNA from each sample were digested with the appropriate restriction endonuclease. The resulting fragments were separated by electrophoresis in agarose gel (leukocyte and tumor DNA samples from each patient were run in adjacent lanes) and blotted onto nylon membrane filters, according to standard techniques. The membrane filters were hybridized with nick-translated 32P-labeled probes, washed, and autoradiographed at −80°C for an appropriate period.

DNA Probes. All probes used in this study are listed in Table 1. A detailed description is given in Ref. 10.

Determination of Allele Loss. Paired normal and tumor DNA from each patient was analyzed using probe-enzyme combinations which identify RFLPs in a large proportion of individuals. Normal DNA samples, which were polymorphic at a given locus, were considered "informative," whereas homozygous samples were "uninformative." The signal intensity of fragments was determined by visual examination and confirmed by densitometry. LOH was considered to occur when the intensity of the allele in tumor DNA was less than 50% of that in corresponding normal tissue DNA. This partial loss is likely to be due either to contaminating normal tissue or to tumor heterogeneity.

Determination of Gene Amplification. Restriction enzyme-digested tumor DNAs were compared with matching lymphocyte DNA in the same agarose gels. Blots of these gels were first hybridized with the protooncogene probes. Rehybridization of the same blots with the c-mos (pHM2A) and the...
β-globin (JW151) probes provided a control for the amount of DNA transferred to the nylon membranes and favored gene amplification rather than whole chromosome imbalance. The protooncogene and control gene autoradiographs were measured by visual examination and/or densitometry of Southern blots. A DNA fragment was considered amplified when the intensity of the signal was increased at least 2-fold relative to the control signal.

Statistical Analysis. χ² test was used for statistical analysis of the results.

Results

We used RFLP analysis to examine normal DNA (peripheral blood lymphocytes) and autologous tumor DNA from 122 breast cancer patients for LOH on the distal part of the short arm of chromosome 1 and for amplification of MYC and ERBB2 protooncogenes and the chromosomal region 11q13 (loci INT2 and CCND1).

We used five markers on the distal short arm of chromosome 1 to identify all patients informative for at least one locus. This permitted us to study LOH on 1p32–pter in all 122 patients included for gene amplification detection. The incidence of informative patients at a given locus ranged from 42% (locus D1S76) to 85% (locus D1S7). The frequencies of LOH for each of these five loci are summarized in Table 1. The highest frequency of allele losses was observed with the D1S75 locus (44%) located at 1p33–p35. LOH occurred in at least one chromosomal region Ilql3 (loci INT2 and CCND1).

We tested for links between genetic amplification at any of the three chromosomal regions studied and 1pter LOH (Table 2) and found an association between MYC amplification and 1p32–pter LOH (χ² = 8.91; P = 0.003); amplification was found in 20 of 57 cases (35%) presenting 1pter LOH, compared with only 8 of 65 (12%) without 1pter LOH.

Discussion

Genetic alterations leading to activation of protooncogenes or inactivation of tumor suppressor genes are associated with the development and progression of malignancies. Certain chromosomal abnormalities have a clear role in several models of tumor development, whereas others correlate with pathological indices of poor clinical outcome. Our hypothesis is that some of these genetic alterations may act in cooperation with each other. In human neuroblastoma, several groups (5–7) have suggested that two genetic events, distal deletion on the short arm of chromosome 1 and amplification of the MYCN protooncogene, may be related. The fact that we have recently identified a similar partial telomeric deletion on chromosome 1p in human breast tumors (8) led us to investigate the possibility of collaboration between this deleted region and the frequently amplified chromosomal regions in human breast cancer (MYC, ERBB2, and 11q13 region). It is noteworthy that the MYCN protooncogene, frequently amplified in human neuroblastoma, was not amplified in breast tumors, and neither was the MYCL1 gene (17).

Therefore, we tested for links between 1peter LOH and amplification on any of the three amplified chromosomal regions. The frequencies of 1peter LOH and genetic amplifications observed in this series of primary breast tumors were in good agreement with those previously described by us (8, 11–13) and others (14–16, 18). We observed a significant association between 1peter LOH and amplification of the MYC gene (P = 0.003), whereas the other two amplifications were not associated with 1peter LOH (Table 2).

We did not observe such imbalance between MYC gene amplification and 7q31 LOH, a second frequently deleted region in human breast cancer (data not shown). It is important to note that five of the
eight tumors which showed a MYC amplification without 1pter LOH were uninformative for the two more telomeric loci D1S76 and D1S80, suggesting that the putative tumor suppressor gene could be located in this telomeric region. The three other tumors should bear small-scale alterations within the putative gene. The hypothesis of a gene distal to loci MYC, D1S8, and D1S57 and proximal to loci D1S76 and D1S80 (because these two loci did not show higher LOH frequencies than the first three) is also supported by the fact that we did not find such an association in a previous study using only the MYC locus (17). Moreover, Borg et al. (19) have observed, in a smaller series of patients, a trend towards an association between LOH at D1S57 (1p35–p33) and/or D1Z2 (1p36) and amplification of the MYC gene.

The observed ability of 1pter LOH and amplification of the MYC gene to collaborate in the transformation process shows that each acts on the cell phenotype in a distinct and complementary way. Surprisingly, the amplified genes which collaborate with 1pter LOH in human neuroblastoma (MYCN) and in human breast cancer (MYC) belong to the same gene family. Inactivation of the putative suppressor gene located at 1pter could be a specific factor of instability for genes of the MYC family. It would thus be interesting to test such an association in other cancers which bear both 1pter LOH and specific amplified loci, especially those of the MYC family. Human colorectal and head and neck cancers are good candidates.

The discovery of cancer gene collaboration has lent substance to the simple model of multistep tumorigenesis. Each step in the tumorigenic process would be due to a mutation leading to activation of one or other cellular gene; the activated oncogenes would then work together to induce the full neoplastic phenotype. Our study suggests that LOH on chromosome 1pter precedes MYC amplification. Indeed, tumors with MYC amplification only represented a subset of tumors with chromosome 1pter deletion. We have previously detected an association between 11p LOH (locus RAS) and MYC amplification (20). However, 11p LOH most frequently appears in poorly differentiated adenoscarcinomas (defined as histological grade III and lack of estrogen receptor and progestosterone receptor; Ref. 21), and this alteration might occur at a later stage of tumorigenesis than 1pter LOH and MYC amplification. We did not detect an association between 11p LOH and 1p LOH (data not shown), which suggests that the order of these three changes may be 1p LOH, MYC amplification, and 11p LOH.

It is now clear that accumulation of genetic alterations is required to transform the normal breast cell into a cancer cell (22). Our data and others (23, 24) suggest sequential genetic alterations during tumor progression in breast cancer as in colorectal cancer (1). For example, Sato et al. (23) has identified a concordance of LOH on 16q and 17p in human breast cancer. However, it is unlikely that a single pattern of sequential changes will account for all breast tumors, given their biochemical, histological, and clinical heterogeneity.

In conclusion, this study suggests that a tumor suppressor gene located in the telomeric part of the short arm of chromosome 1 could control the amplification of MYC family genes in various cancers, including human neuroblastoma and breast cancer. Identification of this putative suppressor gene will require further LOH studies using fine-scale genetic mapping with RFLP or microsatellite markers and construction of a physical map of this 1p32–pter region.

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References

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