Genetic Alterations of c-*myc*, c-*erb*B-2, and c-Ha-*ras* Protooncogenes and Clinical Associations in Human Breast Carcinomas

Irène Garcia, Pierre-Yves Dietrich, Matti Aapro, et al.

Cancer Res 1989;49:6675-6679. Published online December 1, 1989.

[CANCER RESEARCH 49, 6675–6679, December 1, 1989]

Genetic Alterations of c-*myc*, c-*erb*B-2, and c-Ha-*ras* Protooncogenes and Clinical Associations in Human Breast Carcinomas[1]

Irène Garcia,[2] Pierre-Yves Dietrich, Matti Aapro, Geneviève Vauthier, Lucien Vadas, and Eric Engel

Division of Onco-hematology [I. G., P-Y. D., M. A.]; Institute of Medical Genetics [G. V., E. E.] and Central Laboratory [L. V.], University Hospital, Faculty of Medicine, 1211 Geneva 4, Switzerland

ABSTRACT

We have analyzed genomic DNA sequences from 125 prospectively collected single unilateral primary breast carcinoma samples for the presence of alterations of c-*myc*, c-*erb*B-1, c-*erb*B-2, c-Ki-*ras* and c-Ha-*ras* protooncogenes. Amplification of the c-*myc* gene was found in 18% of the samples, and in one sample a non-germ line c-*myc* related DNA fragment or rearrangement was detected. We have found a significant association ($P = 0.0010$) between amplified c-*myc* gene and inflammatory carcinoma, a particularly aggressive breast cancer. The c-*erb*B-2 gene was amplified in 22% of the tumor samples and a rearrangement was observed once. Alteration of the c-*erb*B-2 gene was significantly linked to histological grade III tumors ($P = 0.005$) and the absence of estrogen and progesterone receptors ($P = 0.036$). No amplifications were observed for c-*erb*B-1, c-Ki-*ras*, and c-Ha-*ras* genes. About 40% of breast carcinomas contain either amplified c-*myc* or c-*erb*B-2 protooncogenes, whereas simultaneous amplification of both was seen in only one sample, suggesting the involvement of two distinct molecular mechanisms in breast cancer. Comparison of DNA from peripheral blood and tumor samples indicated loss of one c-Ha-*ras* allele in 29% of patients heterozygous for this polymorphism. A significant correlation ($P = 0.016$) between c-Ha-*ras* locus (11p14) allele loss and patient survival was found. These data suggest that 11p14 allelic loss plays a role in the evolution of human breast cancer, amplification of c-*erb*B-2 gene is associated with increasing stage of malignancy, and alteration of the c-*myc* gene in inflammatory breast carcinoma may contribute to the rapid progression of this human tumor subtype.

INTRODUCTION

Alteration of oncogenes by point mutation, amplification, or rearrangement may be important in the etiology of human tumors. Activation of the c-Ki-*ras* oncogene has been detected in approximately 40% of human colorectal tumors (1, 2) and in more than 95% of the exocrine pancreas carcinomas (3). Amplification of *myc* and *ras* family oncogenes has been observed frequently in tumor cell lines and human tumors (4–11). For example, alterations of c-*myc*, c-*myb*, and c-Ha-*ras* may occur in more than one-third of tumors from different tissue origins and may correlate with progression and metastasis of cancer (12). Detection of specific oncogene alterations may have a prognostic significance in some tumors, such as neuroblastoma where amplification of N-*myc* is associated with rapid tumor progression (13).

Breast carcinoma is the most frequent cancer in women, representing 25% of all female cancers in Geneva (14). Amplification of c-*myc* protooncogene has been found in 33% of breast tumors, with high levels of *myc* expression (15, 16). Alteration of the c-*myc* gene may be correlated with short-term prognosis (17). The c-*erb*B-2 protooncogene (*HER-2* or *neu*), which encodes a transmembrane glycoprotein related to the epidermal growth factor receptor, has been studied extensively in breast cancers (18–30). This gene is amplified and overexpressed in 10–40% of breast cancer samples (20–30). A high number of c-*erb*B-2 gene copies has been associated to poor prognosis (17, 21).

Chromosomal allelic deletions are a second type of genetic alteration found in breast tumors. Allelic deletions of genes in chromosomes 11, 13, and 17 have been reported (31–34). The loss of the c-Ha-*ras* locus in chromosome 11 correlated with breast cancer aggressiveness (31, 32).

The present study attempts to characterize genetic alterations of c-*myc*, c-*erb*B-1, c-*erb*B-2, c-Ki-*ras*, and c-Ha-*ras* protooncogenes occurring in 125 unilateral primary breast carcinomas.

MATERIALS AND METHODS

Human Tissue Samples. One hundred twenty-five single unilateral breast carcinoma samples were obtained prospectively from female patients immediately after surgical excision in several clinics collaborating with the University Hospital of Geneva, Switzerland. These include 102 patients with infiltrating ductal tumors and 23 with various breast cancer histologies. Peripheral blood samples (10–20 ml) from 57 patients were collected in tubes containing EDTA. Breast tumor samples were frozen after surgical removal and stored at −80°C.

Histology and Hormonal Receptor Determination. Histological examination was performed by independent pathologists, mainly from the Division of Pathology, University Medical Center, and classified according to standard criteria (35). Determination of estrogen and progesterone receptors was performed by a routine dextran-charcoal method (36).

DNA Analysis. Total genomic DNA was extracted from fresh tumors or from the pellet obtained after mechanical disruption of frozen tissues, suspended in 10 mM Tris (pH 7.5)-1.5 mM EDTA-10% glycerol, and centrifugation at 25,000 rpm. The supernatant containing the cytosolic fraction was used for biochemical hormone receptor determination. The nuclear fraction was suspended in 10 mM Tris-HCl (pH 7.5)-1 mM EDTA-100 mM NaCl, treated with proteinase K (200 mg/ml) for 16–18 h, and extracted with phenol-chloroform before ethanol precipitation. DNA samples were treated with restriction enzymes, fractionated by electrophoresis in a 0.8% agarose gel, transferred to nylon Hybond-N membranes (Amersham), and cross-linked by UV irradiation.

Hybridization Conditions. Prehybridizations were carried out at 42°C for at least 6 h in heat-sealed plastic bags with 50% (v/v) formamide, 3 × SSC,[3] 10 × Denhardt's solution (0.2% Ficoll-0.2% polyvinylpyrrolidone-0.2% bovine serum albumin), and 20 μg of salmon sperm DNA per ml. Hybridizations with ^{32}P-nick-translated probes were done at 42°C for 24 h in 50% formamide, 3 × SSC, 1 × Denhardt's solution, 10 μg/ml polyadenylic acid, 8% dextran sulfate, and 20 μg salmon sperm DNA per ml. Five ng of ^{32}P-nick-translated probe per ml were added to the hybridization solution. Unbound probe was washed from the membranes by treating twice at room temperature for 30 min with 2 × SSC, then twice at 65°C for 30 min with 1 × SSC/0.1% sodium dodecyl sulfate, and finally twice with 0.1 × SSC/0.1% sodium dodecyl sulfate at 65°C for 30 min. Membranes were exposed to Kodak X-Omat XAR-5 at −70°C for 1–4 days. After autoradiographic exposure the membranes were treated for 30 min in 0.4 M NaOH and then neutralized in 0.2 M Tris-HCl (pH 7.5)-0.1 × SSC to remove the probe.

Received 7/13/89; accepted 9/5/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Grants 3.856-0.85 and 3.842.0.85 from the Swiss National Funds for Scientific Research and Grant 250.86.4 from the Swiss Cancer League.

[2] To whom requests for reprints should be addressed, at Division of Onco-hematology, University Hospital, Faculty of Medicine, 1211 Geneva, Switzerland.

[3] SSC, standard saline-citrate; cDNA, complementary DNA; G-CSF, granulocyte-colony-stimulating factor.

The same membrane was rehybridized 4–5 times with different probes.

Probes and Measurement of Protooncogene Amplification. The probes we have used are: c-*myc*, a subclone of the genomic c-*myc* gene containing a 1.8-kilobase *Cla*I-*Eco*RI fragment comprising exon III (37) and a 1.5-kilobase *Sac*I fragment including exon II of the c-*myc* gene; c-*mos*, a 2.75-kilobase fragment from human placental DNA inserted in pSP65 (38); c-*erb*B-1, a 2.5-kilobase *Hin*dIII-*Eco*RI human DNA fragment from the recombinant plasmid pHE-B (39) subcloned in pSP65; c-*erb*B-2, a 0.714-kilobase *Bam*HI-*Eco*RI human cDNA fragment inserted into pSP64 (19); G-CSF, a *Eco*RI-*Rsa*I cDNA fragment containing the G-CSF gene (40); c-Ki-*ras*, a *Pst*I-*Bam*HI fragment derived from c-Ki-*ras* cDNA (9); c-Ha-*ras*, a 3.0-kilobase *Sac*I fragment from c-Ha-*ras* human gene pEJ plasmid (5); β-globin, a 4.4-kilobase *Pst*I fragment cloned in pBR322 (41). Protooncogene amplification was first measured by densitometry of the hybridizing signals due to protooncogene probe and control probe in tumors and patient's or normal donor's DNA. Control probes were c-*mos* for c-*myc* gene and G-CSF for c-*erb*B-2 to distinguish between gene amplification and the presence of extra copies of chromosomes 8q and 17q, respectively. In DNA samples with gene amplification, serial dilutions of the tumor DNA were performed to obtain a Southern hybridization signal of about one copy as reported by Slamon *et al.* (21). Quantification of gene amplification was done by scanning densitometry of autoradiograph bands using a Bio-Rad VD620 soft laser densitometer.

Clinical Observations. Patient data were collected from the collaborating pathologists' and surgeons' reports. Patient survival is being prospectively observed.

Statistical Analysis. The classical prognostic parameters for breast carcinoma were compared with the oncogene alterations by the χ^2 or Fisher exact test (2-tailed) using a commercially available software package (BMDP).

RESULTS

c-*myc* Protooncogene Amplification in Human Breast Cancers. We have looked for the presence of genetic alterations of the c-*myc* protooncogene in 125 breast cancers. DNAs from tumor samples were digested with *Eco*RI restriction enzyme and the 12.5-kilobase *Eco*RI c-*myc* hybridizing fragment was analyzed for abnormal amount and the presence of variant restriction fragments. Filters hybridized with c-*myc* were probed with c-*erb*B-2 and c-*erb*B-1 probes (Fig. 1) and with control probes, c-*mos*, G-CSF, and β-globin genes (data not shown). DNAs were digested with *Bam*HI restriction enzyme and hybridized with the same probes. Amplification of the c-*myc* protooncogene was observed in 18% of samples (Table 1). In infiltrating ductal carcinoma 15 of 102 samples (15%) contained more than one c-*myc* gene. The extent of amplification was between 2- and 10-fold. The frequency of c-*myc* amplification in inflammatory carcinoma was significantly higher than that in other tumors ($P = 0.0010$; Table 2).

There was no correlation between amplification of c-*myc* protooncogene and histological grade, estrogen-progesterone receptors, or lymph node metastases.

Rearrangement of the c-*myc* gene was detected in one infiltrating ductal cancer. By Southern hybridization with c-*myc* exon III, we observed an additional 9.5-kilobase *Eco*RI restriction fragment and an additional 8.2-kilobase *Hin*dIII fragment. These new bands were absent in DNA from the patient's lymphocytes, indicating that the alteration is specific for the tumor tissue. Use of the *Pvu*II restriction enzyme did not reveal any additional bands in this tumor (Fig. 2). The intensity of the additional *Eco*RI or *Hin*dIII bands was similar to the intensity of the normal c-*myc* bands, indicating that tumor tissue was homogeneous. Amplification of c-*erb*B-2 oncogene was also found in this tumor sample as was loss of a c-Ha-*ras* allele.

c-*erb*B-1 and c-*erb*B-2 Protooncogenes in Breast Tumors. No alterations of the c-*erb*B-1 protooncogene were observed in the samples analyzed. In contrast, amplification of c-*erb*B-2 sequences were found in 27 of 125 tumors (22.0%). Alteration of c-*erb*B-2 protooncogene was found only in infiltrating ductal breast carcinomas (25%) and one comedocarcinoma. No alterations were observed in tumors of better clinical prognosis as papillary, mucinous, or lobular (Table 1). We found that 13 of 125 tumors (11%) had 2 to 5 copies of the gene, and 11% had 6 to more than 20 copies (Table 3).

Alteration of c-*erb*B-2 was significantly ($P = 0.005$) more frequent in advanced histological grade III breast tumors. Significant association ($P = 0.036$) was observed between the presence of amplified c-*erb*B-2 and the absence of estrogen and progesterone receptors. There was no significant association between lymph node metastases and c-*erb*B-2 amplification.

In one infiltrating ductal carcinoma non-germ line restriction fragments were found with the c-*erb*B-2 probe. Additional hybridizing bands were detected: an 8.4-kilobase *Eco*RI; an 8.3-kilobase *Bam*HI fragment; a 4.6-kilobase *Pvu*II fragment; and a 3.2-kilobase *Sac*I fragment (Fig. 3). Unfortunately, DNA from peripheral lymphocytes was not available from this patient.

c-Ki-*ras* and c-Ha-*ras* Genetic Alterations in Human Primary Breast Carcinomas. One hundred nineteen primary breast carcinomas were evaluated for genetic alterations of the c-Ki-*ras* and c-Ha-*ras* protooncogenes. No gene amplifications or rearrangements were seen.

The c-Ha-*ras* locus has a restriction fragment length polymorphism with a *Bam*HI restriction enzyme (42). This polymorphism is generated by the variable length of a segment at

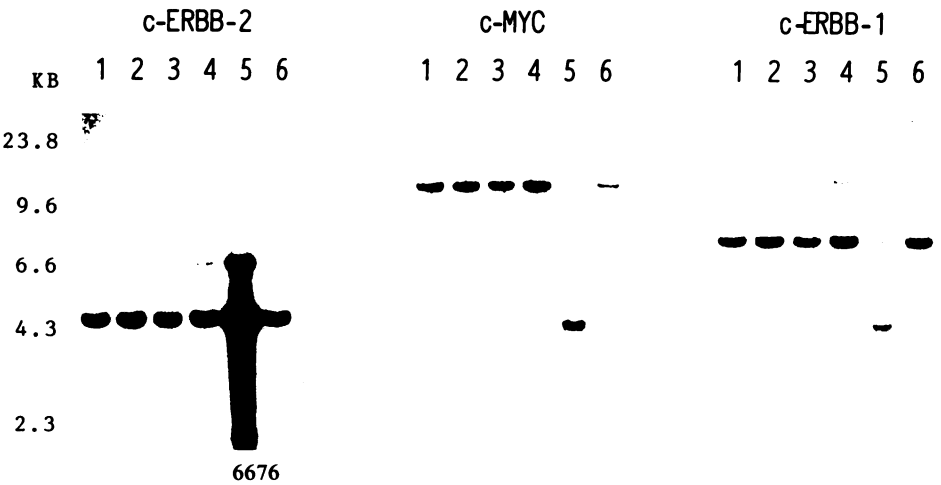

Fig. 1. Southern blot analysis of breast cancer DNA for alteration of c-*erb*B-2, c-*myc*, and c-*erb*B-1. Tumor DNA (10 μg) was digested with *Eco*RI restriction enzyme and hybridized first to the c-*erb*B-2 probe, then to the c-*myc* probe, and finally to the c-*erb*B-1 probe. This shows amplification of c-*erb*B-2 (*Lane 5*, 18-fold). Some background is observed on the c-*erb*B-2 band of tumor 5 after washing and hybridizing with c-*myc* or c-*erb*B-1. *KB*, kilobase.

Table 1 *Unilateral breast carcinoma histology and frequency of gene alterations of c-myc and c-erbB-2*

Type of carcinoma	No. of patients	Amplified	
		c-*myc*	c-*erb*B-2
Infiltrating ductal	102	15 (15)[a]	26 (25)
Lobular	4	2	0
Comedo	2	0	1
Mucinous	6	2	0
Papillary	4	0	0
Carcinoma *in situ*	3	1	0
Medullary	4	2	0
Total	125	22 (18)	27 (22)

[a] Numbers in parentheses, percentage.

Table 2 *Amplification of c-myc protooncogene in relation to clinical parameters in single unilateral primary breast cancer patients*

	1 copy	2–10 copies	Total	*P*
Histological grade				
I	20	6	26	
II	58	10	68	NS[a]
III	25	6	31	
	103	22	125	
Estrogen/progesterone receptors				
E+P+	60	11	71	
E+P−	10	3	13	NS
E−P+	6	2	8	
E−P−	27	6	33	
			125	
Tumor size				
T1	37	10	47	
T2	50	7	57	
T3	7	0	7	
T4	7	0	7	
Inflammatory	2	5	7	0.0010
			125	
Positive nodes				
0	55	11	66	
≤3	19	1	20	NS
>3	27	8	35	
			121	

[a] NS, not significant; E, estrogen; P, progesterone.

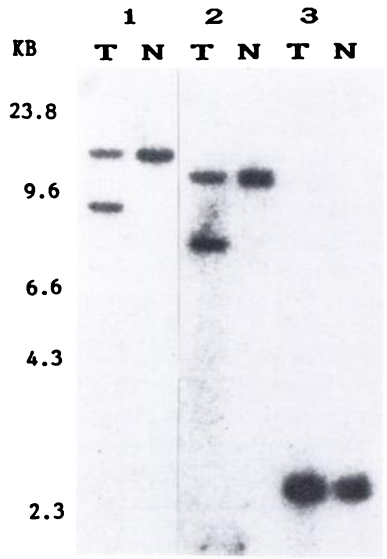

Fig. 2. Southern blot analysis of human breast cancer DNA (Nb 46) for alteration of c-*myc*. DNA (10 μg) from tumors (*T*) or peripheral blood cells (*N*) was digested with *Eco*RI (*1*), *Hin*dIII (*2*), and *Pvu*II (*3*) restriction enzymes. Additional restriction fragments are found in tumor tissue DNA after *Eco*RI. [9.5 kilobases (*KB*)] and *Hin*dIII [8.2 kilobases] digestion.

Table 3 *Correlation of c-erbB-2 protooncogene amplification with clinical parameters for single unilateral primary carcinoma patients*

	1 copy	2–5 copies	6–20 copies	Total	*P*
Histological grade					
I	25	0	1	26	
II	52	11	5	68	
III	21	2	8	31	0.005
	98	13	14	125	
Estrogen/progesterone receptors					
E+P+[a]	60	8	3	71	
E+P−	10	2	1	13	
E−P+	7	0	1	8	
E−P−	21	3	9	33	0.036
				125	
Tumor size					
T1	36	5	6	47	
T2	47	6	4	57	
T3	5	1	1	7	NS
T4	5	1	1	7	
Inflammatory	5	0	2	7	
				125	
Positive nodes					
0	52	8	6	66	
≤3	15	1	4	20	NS
>3	28	4	3	35	
				121	

[a] E, estrogen; P, progesterone; NS, not significant.

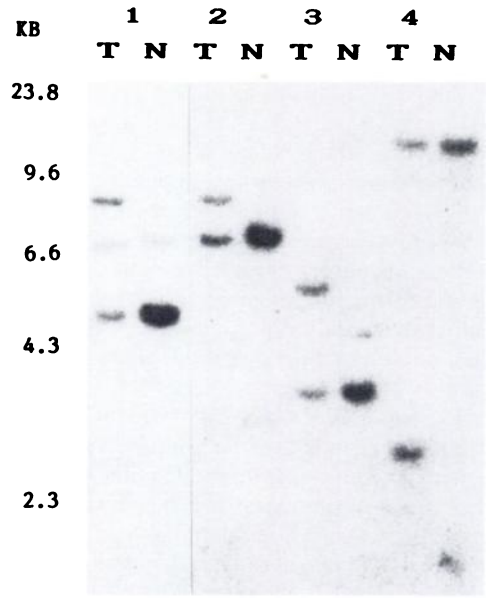

Fig. 3. Southern blot analysis of human breast cancer DNA (Nb 54) for the alteration of c-*erb*B-2 protooncogene. Ten μg of tumor (*T*) DNA and DNA from peripheral blood cells of another patient (*N*) were digested with *Eco*RI (*1*), *Bam*HI (*2*), *Pvu*II (*3*), and *Sac*I (*4*) restriction enzymes. New restriction fragments are observed in tumor tissue DNA after *Eco*RI [8.4 kilobases (*KB*)], *Bam*HI [8.3 kilobases], *Pvu*II [4.6 kilobases], and *Sac*I [3.2 kilobases] treatment.

the 3′ region containing 28-base pair tandem repetitive sequences. We have compared peripheral blood DNA to tumor DNA after digestion with *Bam*HI restriction enzyme and hybridization with c-Ha-*ras* probe. Peripheral leukocyte DNA from the same patient was available in 57 cases. We found 29 patients homozygous at this site and 28 patients heterozygous, having two alleles. In 7 of 28 heterozygous individuals one c-Ha-*ras* allele was lost in the tumor DNA (Fig. 4). Four of these 7 patients died 10 to 30 months after surgery. Only 2 of 21 heterozygous patients with allele loss died during this period of time. There is a significant link ($P = 0.016$) between loss of c-Ha-*ras* allele and patient death. In two of the dead patients with

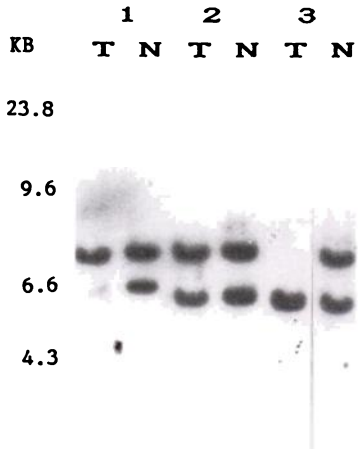

Fig. 4. Autoradiograph of tumor (*T*) and blood cell (*N*) DNA from three breast cancer patients, digested with *Bam*HI and hybridized with c-Ha-*ras* probe. Tumor tissue from patient 1 shows a fine band which comes from normal tissue and presents about 10% of the intensity of the remaining allele. Tumor tissue from patient 2 shows no allele loss and patient 3 tumor tissue shows complete absence of one allele. *KB*, kilobase.

allele loss (one infiltrating ductal, one inflammatory), we observed complex abnormalities. Both tumors contained c-*erb*B-2 amplification (more than 6 copies) together with c-*myc* rearrangement and amplification, respectively.

DISCUSSION

We have analyzed the genomic organization of several protooncogenes in human breast carcinoma samples to better understand the influence of these genetic abnormalities in the evolution of breast carcinoma patients. This analysis was performed with prospectively collected samples, which represent about three-fourths of all patients operated on in Geneva. Our data should thus be truly representative of the value of such analyses in a nonselected patient population.

Amplification of c-*myc* gene in breast and other tumors has been correlated with clinical aggressiveness (12, 17). We found alteration of this gene in 18% of the samples. This is comparable to the frequencies found in previous studies (15, 17, 43). Although the number of samples is low, we detected a significant link ($P = 0.0010$) between alteration of c-*myc* gene and inflammatory breast carcinomas. Because inflammatory breast tumors are an aggressive breast cancer subtype, we could expect frequent genomic changes in these tumor DNAs. Indeed all 7 such samples show gene change and in 5 of 7 tumors the c-*myc* protooncogene was amplified. The 2 tumor samples not presenting amplification of c-*myc* showed amplification of c-*erb*B-2 gene and the loss of a c-Ha-*ras* allele, respectively. Our data thus suggest that in this type of breast cancer the frequency of easily detectable genomic alterations is 100%.

No statistically significant correlations were found between c-*myc* gene amplification and other clinical parameters in our results. Varley *et al.* (17) detected amplification of c-*myc* in 8 of 37 (22%) infiltrating ductal breast tumors and no correlations with the class, grade, estrogen receptor status, and age of patients at surgery, but they report a significant association between an altered c-*myc* gene and a poor prognosis. We detected a non-germline c-*myc*-related DNA fragment or rearrangement in one tumor sample. Our data suggest that the genetic alteration, probably a deletion of about 3.4 kilobases, could happen at the 5′ region of the c-*myc* gene. The same sample showed amplified c-*erb*B-2 gene and deletion of a c-Ha-*ras* allele.

We have investigated alterations in the structure of the c-*erb*B-2 oncogene in the same tumor samples. We detected amplification in 27 of 125 samples (22.0%) and one tumor with a c-*erb*B-2 gene rearrangement. In our patients, time after surgery is still short (average, 27 months) to discuss any clinical meaning of single c-*erb*B-2 or c-*myc* protooncogene amplification. Nevertheless, we found good correlation between grade III tumors and high copy number of c-*erb*B-2 oncogene confirming previous reports (24, 28). Association of c-*erb*B-2 gene amplification and the absence of estrogen and progesterone receptors was detected. From our data and those of others (24, 28) it is still impossible to conclude if amplification of c-*erb*B-2 (*neu*) gene is an independent prognostic factor once tumor grade is considered. No significant correlations were observed for the presence of positive lymph nodes. Recently, Van de Vijver *et al.* (29) have also reported no association of *neu* overexpression with lymph node status in a group of 189 tumors from patients with stage II breast cancer. Association with comedo-type ductal carcinoma *in situ* was reported by the same authors (29). Our results suggest that c-*erb*B-2 gene alteration is associated with increasing stage of malignancy, whereas c-*myc* may play a role in rapidly proliferating inflammatory carcinomas.

Amplification of c-*erb*B-2 together with c-*myc* was observed in only 1 case, compared to 16% in another report (25). Our data and those from Varley *et al.* (17) rather suggest that alteration of either the c-*myc* or the c-*erb*B-2 genes occurs in two different groups of breast carcinomas the clinical outcome of which remains to be determined. Oncogene products of c-*myc* and c-*erb*B-2 seem to cooperate rarely in the malignant phenotype of mammary cancers.

Loss of chromosomal regions inactivating tumor suppressor genes may be an additional step in the evolution of human tumors. Allele loss on the short arm of chromosome 17 was found in 61% of breast tumors analyzed indicating that this region may contain a breast cancer susceptibility gene (34). Previously, loss of a c-Ha-*ras* allele on the short arm of chromosome 11 (11p14) has been shown to be associated with aggressive human primary carcinomas. We found loss of this locus in 29% of breast tumors. The 57% of patients with allele loss died 10 to 30 months after surgery as compared to 2 of 21 without allele loss ($P = 0.016$). Interestingly, two of the patients with allele loss were the only two cases showing more than one simultaneous genetic alteration. The deletion of a c-Ha-*ras* locus may indicate an additional step leading to rapid evolution of breast cancer.

Human breast cancer is a heterogeneous and complex neoplastic disease where many factors may influence biological behavior. Activation of several oncogenes may occur at the same time as deletion of genetic material, inducing loss of recessive antioncogene alleles. Several combinations of genetic defects must be responsible for tumor progression. To find out which are those primarily causing tumor formation and those related to its evolution we need more extensive studies including investigation of the alteration of many other oncogenes, growth factors, hormone receptors, and cytoskeletal proteins.

ACKNOWLEDGMENTS

The help of E. Cottin and C. Charrin and the collaboration of Dr. J. Weintraub, Professor F. Krauer and colleagues, and many physicians and pathologists is acknowledged. We thank Drs. J. Eliason and P.

Sappino for comments on the manuscript and acknowledge the secretarial skill of C. Amez-Droz.

REFERENCES

1. Bos, J. L., Fearon, E. R., Hamilton, S. R., Verlaan-de Vries, M., van Boom, J. H., van der Eb, A. J., and Vogelstein, B. Prevalence of *ras* gene mutations in human colorectal cancers. Nature (Lond)., *327:* 293–297, 1987.
2. Forrester, K., Almoguera, C., Han, K., Grizzle, W. E., and Perucho, M. Detection of high incidence of K-*ras* oncogenes during human colon tumorigenesis. Nature (Lond.), *327:* 298–303, 1987.
3. Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N., and Perrucho, M. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. Cell, *53:* 549–554, 1988.
4. Goldfarb, M., Shimizu, K., Perucho, M., and Wigler, M. Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells. Nature (Lond.), *296:* 404–409, 1982.
5. Shih, C., and Weinberg, R. A. Isolation of a transforming sequence from a human bladder carcinoma cell line. Cell, *29:* 161–169, 1982.
6. Shimizu, K., Birnbaum, D., Ruley, M. A., Fasano, O., Suard, Y., Edlund, L., Taparowsky, E., Goldfarb, M., and Wigler, M. Structure of the Ki-*ras* gene of the human lung carcinoma cell line Calu-1. Nature (Lond.), *304:* 497–500, 1983.
7. Pulciani, S., Santos, E., Lauver, A. V., Long, L. K., Aaronson, S. A., and Barbacid, M. Oncogenes in solid human tumors. Nature (Lond.), *300:* 539–542, 1982.
8. Little, C. D., Nau, M. N., Carney, D. N., Gazdar, A. F., and Minna, J. D. Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. Nature (Lond.), *306:* 194–196, 1983.
9. McCoy, M. S., Toole, J. J., Cunningham, J. M., Chang, E. H., Lowy, D. R., and Weinberg, R. A. Characterization of a human colon/lung carcinoma oncogene. Nature (Lond.), *302:* 79–81, 1983.
10. Wong, A. J., Ruppert, J. M., Eggleston, J., Hamilton, S. R., Baylin, S. B., and Vogelstein, B. Gene amplification of c-*myc* and N-*myc* in small cell carcinoma of the lung. Science (Wash. DC), *233:* 461–464, 1986.
11. Graham, K. A., Richardson, C. L., Minden, M. D., Trent, M. J. M., and Buick, R. N. Varying degrees of amplification of the N-*ras* oncogene in the human breast cancer cell line MCF-7. Cancer Res., *45:* 2201–2205, 1985.
12. Yokota, J., Tsunetsugu-Yokota, Y., Battifora, H., Le Fevre, C., and Cline, M. J. Alterations of *myc, myb,* and *ras*^{Ha} protooncogenes in cancers are frequent and show clinical correlation. Science (Wash. DC), *231:* 261–265, 1986.
13. Seeger, R. C., Brodeur, G. M., Sather, H., Dalton, A., Siegel, S. E., Wong, K. Y., and Hammond, D. Association of multiple copies of the N-*myc* oncogene with rapid progression of neuroblastomas. N. Engl. J. Med., *313:* 1111–1116, 1985.
14. Geneva Tumor Registry. Incidence of Cancer in Geneva, 1970–1983. Geneva: Geneva State Press, 1984.
15. Escot, C., Theillet, C., Lidereau, R., Spyratos, F., Champeme, M. H., Gest, J., and Callahan, R. Genetic alteration of the c-*myc* protooncogene (*MYC*) in human primary breast carcinomas. Proc. Natl. Acad. Sci. USA, *83:* 4834–4838, 1986.
16. Mariani-Constantini, R., Escot, C., Theillet, C., Gentile, A., Merlo, G., Lidereau, R., and Callahan, R. *In situ* c-*myc* expression and genomic status of the c-*myc* in infiltrating ductal carcinomas of the breast. Cancer Res., *48:* 199–205, 1988.
17. Varley, J. M., Swallow, J. E., Brammar, W. J., Whittaker, J. L., and Walker, R. A. Alterations to either c-*erb*B-2 (*neu*) or c-*myc* protooncogenes in breast carcinomas correlate with poor short-term prognosis. Oncogene, *1:* 423–430, 1987.
18. King, C. R., Kraus, M. H., and Aaronson, S. A. Amplification of a novel c-*erb*-related gene in a human mammary carcinoma. Science (Wash. DC), *229:* 974–976, 1985.
19. Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Miyajima, N., Saito, T., and Toyoshima, K. Similarity of protein encoded by the human c-*erb*B-2 gene to epidermal growth factor receptor. Nature (Lond.), *319:* 230–234, 1986.
20. Semba, K., Kamata, N., Toyoshima, K., and Yamamoto, T. A v-*erb*B-related protooncogene, c-*erb*B-2, is distinct from the c-*erb*B-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. Proc. Natl. Acad. Sci. USA, *82:* 6497–6501, 1985.
21. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. Science (Wash. DC), *23:* 177–182, 1987.
22. Venter, D. J., Kumar, S., Tuzi, N. L., and Gullick, W. J. Overexpression of the c-*erb*B-2 oncoprotein in human breast carcinomas: immuno-histological assessment correlates with gene amplification. Lancet, *2:* 69–72, 1987.
23. van de Vijver, M., Bersselaar, R., Devilee, P., Cornelisse, C., Peterse, J., and Nusse, R. Amplification of the *neu* (c-*erb*B-2) oncogene in human mammary tumors is relatively frequent and is often accompanied by amplification of the linked c-*erb*A oncogene. Mol. Cell. Biol., *7:* 2019–2023, 1987.
24. Zhou, D., Battifora, H., Yokata, J., Yamamoto, T., and Cline, M. J. Association of multiple copies of the c-*erb*B-2 oncogene with spread of breast cancer. Cancer Res., *47:* 6123–6125, 1987.
25. Cline, M. J., Battifora, H., and Yokota, J. Protooncogene abnormalities in human breast cancer: Correlations with anatomic features and clinical course of disease. J. Clin. Oncol., *5:* 999–1006, 1987.
26. Ali, I. U., Campbell, G., Lidereau, R., and Callahan, R. Amplification of c-*erb*B-2 and aggressive human breast tumors? Science (Wash. DC), *240:* 1795–1796, 1988.
27. Slamon, D. J., and Clark, G. M. Amplification of c-*erb*B-2 and aggressive human breast tumors: response. Science (Wash. DC), *240:* 1796–1798, 1988.
28. Berger, M. S., Locher, G. W., Saurer, S., Gullick, W. J., Waterfield, M. D., Groner, B., and Hynes, N. E. Correlation of c-*erb*B-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. Cancer Res., *48:* 1238–1243, 1988.
29. van de Vijver, M. J., Peterse, J. L., Mooi, W. J., Wisman, P., Lomans, J., Dalesio, O., and Nusse, R. *Neu*-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma *in situ* and limited prognostic value in stage II breast cancer. N. Engl. J. Med., *319:* 1239–1245, 1988.
30. Slamon, D. J., Godolphin, W., Lovell, A. J., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J. Stuart, S. G., Udove, J., Ullrich, A., and Press, M. F. Studies of the HER-2/neu protooncogene in human breast and ovarian cancer. Science (Wash. DC), *244:* 707–712, 1989.
31. Theillet, C., Lidereau, R., Escot, C., Hutzell, P., Brunet, M., Gest, J., Schlom, J., and Callahan, R. Loss of a c-H-*ras*-1 allele and aggressive human primary breast carcinomas. Cancer Res., *46:* 4776–4781, 1986.
32. Ali, I. U., Lidereau, R., Theillet, C., and Callahan, R. Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science (Wash. DC), *238:* 185–188, 1987.
33. Lundberg, C., Skoog, L., Cavenee, W. K., and Nordenskjöld, M. Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation of chromosome 13. Proc. Natl. Acad. Sci. USA, *84:* 2372–2376, 1987.
34. Mackay, J., Elder, P. A., Steel, C. M., Forrest, A. P. M., and Evans, H. J. Allele loss on short arm of chromosome 17 in breast cancers. Lancet, *2:* 1384–1385, 1988.
35. de Vita, V. T., Hellman, S., and Rosenberg, S. A. Cancer, Principles and Practice of Oncology, Ed. 2, Chap. 34, pp. 1119–1129, 1985.
36. Zava, D. T., Ballmoss, A. W., Goldhirsch, A., Roos, W., Takahashi, A., Eppenberger, U., Arrenbrecht, S., Martz, G., Losa, G., Gomez, F., and Guelpa, C. A quality control study to assess the inter-laboratory variability of routine estrogen and progesterone receptor assays. Eur. J. Clin. Oncol., *18:* 713–721, 1982.
37. Rothberg, P. G., Erisman, M. D., Diehl, R. E., Rovigatti, U. G., and Astrin, S. M. Structure and expression of the oncogene c-*myc* in fresh tumor material from patients with hematopoietic malignancies. Mol. Cell. Biol., *4:* 1096–1103, 1984.
38. Watson, R., Oskarsson, M., and Van de Woude, G. Human DNA sequence homologous to the transforming gene (*mos*) of Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA, *79:* 4078–4082, 1982.
39. Jansson, M., Philipson, L., and Vennström, B. Isolation and characterization of multiple human genes homologous to the oncogenes of avian erythroblastosis virus. EMBO J., *2:* 561–565, 1983.
40. Nagata, S., Tsuchiya, M., Asano, S, Kaziro, Y., Yamazaki, T., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, M., Nomura, H., and Ono, M. Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. Nature (Lond.), *319:* 415–418, 1986.
41. Lawn, R. M., Efstratiadis, A., O'Connell, C., and Maniatis, T. The nucleotide sequence of the human β-globin gene. Cell, *21:* 647–651, 1980.
42. Krontiris, T. G., Di Martino, N. A., Colb, M., and Parkinson, D. R. Unique allelic restriction fragments of the Ha-*ras* locus in leukocytes and tumor DNAs of cancer patients. Nature (Lond.), *313:* 369–374, 1985.
43. Bonilla, M., Ramirez, M., Lopez-Cueto, J., and Gariglio, P. *In vivo* amplification and rearrangement of c-*myc* oncogene in human breast tumors. J. Natl. Cancer Inst., *80:* 665–671, 1988.