Amplification of c-*erb*B-2 and Aggressive Human Breast Tumors?

Little is known about the clinical significance of many genetic alterations that appear in primary human breast carcinoma. These include amplification of the c-*myc* (*1, 2*), *int*-2 (*3*), and c-*erb*B-2 (*2, 4–8*) genes, as well as allelic deletions of genes on chromosomes 11 or 13 (*9, 10*). The frequency and level of amplification of c-*myc* and c-*erb*B-2 proto-oncogenes vary between different groups of patients studied by different laboratories. Amplification of c-*erb*B-2 was reported in 10, 16, 18, and 40% of breast tumors from different groups of patients (*2, 5–8*). In one study this proto-oncogene was suggested as a "prognostic factor" (*5*) and in another as an "imperfect guide" (*7*) for aggressive breast tumors.

We explored the relation between the copy number of the c-*erb*B-2 proto-oncogene and biological characteristics of primary human breast tumors collected at the Centre René Huguenin, St. Cloud, France (*8*). A 2- to 40-fold increase in c-*erb*B-2 (chromosome 17q) was identified in 12 out of 122 tumor DNAs. In contrast, the presence of a normal copy number of the gene encoding the tumor antigen p53 (chromosome 17p) (*11*) in all the tumor DNAs demonstrates that c-*erb*B-2 amplification in certain tumors was not due to increased ploidy of chromosome 17. There was no evidence of an association between increased copy number of c-*erb*B-2 and the biological characteristics of tumors that may be indicative of their degree of malignancy. Among these parameters are the number of involved lymph nodes, hormone receptor status, histopathologic grading, age at diagnosis, and menopausal status (*8*).

Our observations are in some ways inconsistent with those reported by Slamon *et al* (*5*). First, amplification of c-*erb*B-2 was detected in 10% of the breast tumors as compared with 18% and 40%, respectively, in two different groups of patients examined by Slamon *et al.* (*5*). This difference could in part be due to an underestimation of the copy number of the c-*erb*B-2 gene because of the presence of contaminating stromal tissue and infiltrating lymphocytes. Conversely, chromosomal duplication rather than gene amplification, at least in some cases, might give rise to an exaggerated frequency of tumors with c-*erb*B-2 amplification. We ruled out the latter possibility in our study.

A close look at the combined data described here (group A, 122 patients with a follow-up time of 53 months and 62 months for those still surviving; group B, 103 patients with no follow-up; and group C, 86 patients with a median follow-up time of 46 months and 47 months for those still-living patients) provides evidence that the number of tumors with c-*erb*B-2 amplification varies among these groups (Table 1). Only 3 to 4% of patients in groups A and B, as compared with 27% of patients in group C, have a two- to fivefold amplification of the c-*erb*B-2 gene. Thus, in this respect patients in group C differ from those in groups A and B.

Table 1. Comparative analysis of c-*erb*B-2 amplification in breast tumors from different groups of patients.*

Group	Number of tumors		
	Single copy	2 to 5 copies	>5 copies
Total patients			
A (our data)	110 (90%)	4 (3%)	8 (7%)
B (*5*, table 1)	84 (82%)	3 (3%)	16 (15%)
C (*5*, table 2)	52 (60%)	23 (27%)	11 (13%)
Patients with positive lymph nodes			
A (our data)	67 (89%)	3 (4%)	5 (7%)
B (*5*, table 1)	37 (79%)	2 (4%)	8 (17%)
C (*5*, table 2)	52 (60%)	23 (27%)	11 (13%)

*A multiple comparisons procedure based on Pearson's χ^2 statistic (*15*) was used to compare the three groups of patients. Group C differs significantly from group A ($P < 0.001$) and group B ($P < 0.007$), whereas groups A and B appear to not differ ($P > 0.09$) in both sets of patients.

Amplification of c-*erb*B-2 in 40% of the 86 patients in group C does not appear to be due simply to the selection of lymph node–positive patients. Table 1 shows that selecting for patients with positive lymph nodes does not increase the frequency of tumors with c-*erb*B-2 amplification in group A or in group B. Furthermore, for total patients and for patients with positive lymph nodes, the data in group C differ significantly from those in groups A and B, while there is no statistical evidence that groups A and B are different.

Table 2. Comparison of diagnostic factors in univariate and multivariate relapse and survival analyses in two studies.*

Factor	Group A (our data)		Group C (*5*, table 4)	
	Relapse	Survival	Relapse	Survival
		Univariate analysis		
Positive lymph nodes (no.)	0.002 (0.107 ± 0.031)	0.01 (0.102 ± 0.035)	0.0002	0.0001
c-*erb*B-2 copies (no.)†	0.51	0.56	<0.0001	0.0011
Progesterone receptor‡	0.04 (−0.269 ± 0.134)	0.01 (−0.396 ± 0.165)	0.05	0.05
Estrogen receptor‡	0.54	0.86	0.10	0.15
Age at surgery	0.26	0.64	0.61	0.22
		Multivariate analysis		
Positive lymph nodes (no.)	0.0001 (0.126 ± 0.033)	0.002 (0.111 ± 0.036)	0.001 (0.085 ± 0.027)	0.0003 (0.094 ± 0.026)
c-*erb*B-2 copies (no.)†	—	—	0.001 (0.138 ± 0.043)	0.02 (0.087 ± 0.039)
Progesterone receptor‡	0.02 (−0.317 ± 0.141)	0.008 (−0.454 ± 0.172)	—	—
Estrogen receptor†	—	—	—	0.03 (−0.516 ± 0.24)

*Entries are *P* values (regression coefficient ± SD). Dash indicates variable not included in the multivariate model. For group A, with the multivariate model, the number of c-*erb*B-2 copies, estrogen receptor, and age at surgery were not significant. †In group A, variable for number of c-*erb*B-2 copies is 1 if 1 copy, 2 if 2 to 5 copies, 3 if 6 to 15 copies, and 4 if ≥16 copies. ‡In the analysis of group A, hormone receptor variable was considered to be 0 if 0 to 10 mol of receptor per milligram of protein were present, 1 if 10 to 50 fmol, 2 if 51 to 150 fmol, and 3 if >150 fmol. This variable in proportional hazard assumption is easier to check graphically than the logged values in group C.

Second, in our study there is no evidence of an association between the copy number of c-*erb*B-2 and the number of positive lymph nodes either by the χ^2 test or by the more powerful Kendall's correlation ($\hat{\tau}_b \pm$ SE of -0.048 ± 0.083) (*12*). Neither of the two separate groups of patients examined by Slamon *et al.* (*5*) provides strong evidence of an association. The reported strong association ($P = 0.002$) obtained by combining data from their two groups poses two major problems. (i) The importance of the P value (0.002) is diminished by the fact that the analysis of the first group was used to formulate the association hypothesis as well as to help confirm it; and (ii) the above analysis of Table 1 shows that the proportions with the amplified gene are quite different in their two groups. Hence their combination is of questionable validity for studying the association between the amplified c-*erb*B-2 gene and the number of positive lymph nodes. The inconsistency between our analysis and that of Slamon *et al.* could be due to the small proportion of patients with amplified c-*erb*B-2 gene in our study (or to the large proportion in their second group). It is, however, not due to the original size of the group, as comparable numbers of patients were available in all groups.

Third, the analysis of survival information on the patients in our study does not confirm the importance of c-*erb*B-2 amplification in breast tumors in predicting overall survival or time to relapse. The only variables important for our study in predicting disease-free and overall survival, both in univariate and multivariate Cox regression analyses (*13*) as well as a robust log rank analysis (*8*), were the number of positive lymph nodes and progesterone receptor status (Table 2). These are the same two variables identified previously by Clark *et al.* (*14*).

At present there cannot be a simple explanation for these contradictory findings. The discrepancies between our results and those of Slamon *et al.* or between the results of various groups of patients studied in the same or different laboratories could reflect differences in genetic background, geographical location, or other nutritional and environmental factors. In this respect, breast cancer patients in the French population seem to have longer disease-free and overall survival periods compared with the American patients (*8*). Our results therefore advise caution in the preliminary assignment of c-*erb*B-2 amplification as an indicator for breast tumor aggressiveness and poor disease prognosis. Moreover, an association of a particular genetic alteration with disease prognosis requires analysis of expanded numbers of tumors from patients representing different geographical and genetic compositions.

IQBAL UNNISA ALI
Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD 20892
GREGORY CAMPBELL
Laboratory of Statistical and Mathematical Methodology, Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD 20892
ROSETTE LIDEREAU
Centre René Huguenin, 5 rue Gaston Latouche, 92211 St. Cloud, France
ROBERT CALLAHAN
Laboratory of Tumor Immunology and Biology, National Institutes of Health

REFERENCES

1. C. Escot *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4838 (1986).
2. J. M. Varley, J. E. Swallow, W. J. Brammar, J. L. Whittaker, R. A. Walker, *Oncogene* **1**, 423 (1987).
3. R. Lidereau *et al.*, *Oncogene Res.* **2**, (1988).
4. C. R. King, M. H. Kraus, S. A. Aaronson, *Science* **229**, 974 (1985).
5. D. J. Slamon *et al.*, *ibid.* **235** 177 (1987).
6. M. van de Vijver *et al.*, *Mol. Cell. Biol.* 7, 2019 (1987).
7. D. Zhou, H. Battifora, J. Yokota, T. Yamamoto, M. J. Cline, *Cancer Res.* **47**, 6123 (1987).
8. I. U. Ali, G. Campbell, R. Lidereau, R. Callahan, *Oncogene Res.*, in press.
9. I. U. Ali, R. Lidereau, C. Theillet, R. Callahan, *Science* **238**, 185 (1987).
10. C. Lundberg, L. Scoog, W. R. Cavanee, M. Nordenskjoeld, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2372 (1987).
11. M. Isobe, B. S. Emanual, D. Givol, M. Oren, C. M. Croce, *Nature* **320**, 84 (1986).
12. M. G. Kendall, *Rank Correlation Methods* (Hafner, New York, ed. 3, 1962).
13. D. R. Cox, *J. Statist. Soc.* **34**, 187 (1972).
14. G. M. Clark, W. L. McGuire, C. A. Hubay, O. H. Pearson, J. S. Marshall, *N. Engl. J. Med.* **309**, 1343 (1983).
15. J. P. Shaffer, *J. Am. Statist. Assoc.* **81**, 826 (1986).

23 February 1988; accepted 29 February 1988

Response: The association between amplification of the HER-2/*neu* gene, also called c-*erb*B2, and poor prognosis in human breast cancer was first reported approximately 18 months ago (*1*). Since that report, a number of studies regarding amplification of this gene in primary human breast cancers have been published (*2*–*7*). Ali *et al.* present results indicating that there is "no evidence of an association between increased copy number of c-*erb*B2 and the biological characteristics of tumors that may be indicative of their degree of malignancy. Among these parameters are numbers of involved lymph nodes, hormone receptor status, histopathologic grading, age at diagnosis, and menopausal status" (*8*). They go on to say that "Our observations are in some ways inconsistent with those reported by Slamon *et al.*, referring to our initial study (*1*).

A major discrepancy between our data (*1*) and those reported by Ali *et al.* (*8*) is the incidence of amplification of the HER-2/*neu* gene in human breast cancer. There are several possible explanations for this discrepancy. First, tissue from human malignancies is made up of heterogeneous cell populations. This is particularly true of breast cancer, where stromal elements can account for as much as 50% of the tumor mass. Given this, it is easy to see how gene amplification might be underestimated rather than overestimated due to dilution of the tumor cell DNA with DNA from nonmalignant cells. Still the potential for overestimates in amplification incidence as a result of technical variability exists and must be addressed. In our initial study, we evaluated 86 tumors with clinical follow-up in a blinded fashion and, of those tumors, 11 had amplification of HER-2/*neu* to levels of five copies or greater (*1*). It was this latter group that showed the greatest difference in prognosis when compared with a single copy group (*1*). One possibility is that our initial data were in error regarding the incidence of amplification. To reevaluate this group for the possibility of error, we repeated the Southern blot analysis and updated the follow-up on the cases from which the tumors were obtained. In no case did we see a change in DNA copy status on repeat analysis. In addition, with a new overall median follow-up of 53 months (60 months for those patients still alive), the association between gene amplification and disease free survival as well as overall survival has not only persisted, but the significance level has increased ($P = 0.0017$ and $P = 0.0035$, respectively, determined by mutivariate analysis) compared with our previously published data (*1*). Using lymph node status, estrogen receptor, progesterone receptor, size of tumor, age of patient, and HER-2/*neu* copy number as factors in multivariate studies, we found that gene amplification remained superior to other prognostic factors with the exception of positive lymph nodes in predicting clinical outcome. Therefore, the association between HER-2/*neu* amplification and poor prognosis holds for this group of tumors. Reviewing our data, Ali *et al.* state that the incidence of HER-2/*neu* amplification is significantly different in lymph-node positive group with follow-up compared with the lymph-node positive group without follow-up (*8*). They do not point out, however, that the latter group represented a relatively small number of cases (approximately half of those in the former group). Thus, the difference may be real or may