American Journal of Pathology, Vol. 147, No. 4, October 1995

Genetic Aberrations Detected by Comparative Genomic Hybridization Predict Outcome in Node-Negative Breast Cancer

Jorma J. Isola,*† Olli-P. Kallioniemi,† Lisa W. Chu,* Suzanne A. W. Fuqua,‡ Susan G. Hilsenbeck,‡ C. Kent Osborne,‡ and Frederic M. Waldman*

From the Division of Molecular Cytometry, Department of Laboratory Medicine, * *University of California, San Francisco, San Francisco, California; Institute of Medical Technology,*† *University of Tampere and University Hospital at Tampere, Tampere, Finland; and the Department of Medicine/Oncology,*‡ *University of Texas Health Science Center, San Antonio, Texas*

Breast cancer progression is determined by a complex pattern of multiple genetic aberrations the association of which with patient prognosis is unknown. In this study, we have undertaken a genome-wide screening to detect genetic changes associated with clinical outcome in node-negative breast cancer. Comparative genomic hybridization was used to screen for DNA sequence gains and losses across all human chromosomes in 23 tumors from node-negative breast cancer patients with no disease recurrence after at least 5 years of follow-up and in 25 node-negative patients with recurrence during the first 5 years of follow-up. The total number of genetic aberrations (copy number gains and losses) per tumor was significantly greater in the recurrence group (P = 0.019) and in the subgroup of these patients who died as a result of breast cancer (P = 0.0022). When copy number losses and gains were analyzed separately, only losses were significant (P = 0.013 for recurrence and P = 0.002 for overall survival). Of the individual loci involved, a high level gain of the long arm of chromosome 8 was significantly associated with recurrence (P = 0.01, Fisher's exact test). Furthermore, amplification of DNA sequences at chromosome 20q12–13 was found in 7 cases (15%), 6 of which had early recurrence within 32 months of diagnosis. This genome-wide overview by comparative genomic hybridization suggests that genetically advanced node-negative breast cancers having a high overall number of genetic aberrations may have a poor prognosis and that increased copy number of two specific regions, 8q and 20q13, may confer a more aggressive phenotype. Results of this pilot study suggest that determination of the total number of DNA sequence copy number aberrations may help therapeutic decision making. Specific probes should be developed to test the prognostic value of 8q and 20q12–13 amplifications in large numbers of patients. (Am J Pathol 1995, 147:905–911)

Therapeutic decisions in axillary node-negative breast cancer should be based on the evaluation of the risk of relapse.[1] A number of aberrations affecting specific genes or chromosomal regions have been described and shown to correlate with clinical outcome in breast cancer. For example, c-*erb*B-2, cyclin D/*ems*, and *myc* amplifications are all potential markers of aggressive disease.[2–4] Similarly, several studies have suggested that allelic imbalance or inactivation of tumor suppressor genes at 1q, 7q, 16q, and 17p (p53) are predictive of poor outcome.[5–7] Most studies have focused on one gene or chromosomal locus at a time, thereby providing an extremely restricted view of the genetic aberrations affecting the entire genome.

According to the prevailing hypothesis of genetic progression of cancer, several successive genetic

Supported by Public Health Service grants CA44768, CA58207, CA30195, and CA54174 from the National Cancer Institute, National Institutes of Health. Dr. Isola was supported by personal grants from the Finnish Academy of Sciences and from the Finnish Cultural foundation.

Accepted for publication July 5, 1995.

Address reprint requests to Dr. Jorma Isola, Institute of Medical Technology, University of Tampere, PO Box 607 FIN-33101 Tampere, Finland.

events gradually lead to a cancer cell with a fully malignant phenotype and the capacity for metastasis. Development of genetic instability may play an essential role in this progression by allowing an increased rate of accumulation of genetic changes across the genome with some of these changes affecting critical genes. Complicated multi-locus analyses would be required to determine which collection of genetic changes in highly unstable genomes are most significant. Conventional molecular analyses that target specific individual genes previously implicated in cancer or anonymous markers chosen more or less randomly along chromosome arms one at a time are unlikely to accomplish these tasks. Although classical cytogenetic studies provide an overview of genetic changes,[8] their application to breast cancer is limited by technical difficulties in preparing high quality metaphases as well as in the interpretation of highly complex karyotypes.

Our newly developed molecular cytogenetic technique, comparative genomic hybridization (CGH), provides a unique opportunity to detect and map clonal DNA sequence copy number changes across the genome in a single hybridization.[9–11,14] CGH is based on a competitive *in situ* hybridization of normal metaphase spreads by two differentially labeled whole genomic DNAs, one derived from the tumor tissue and another from a normal reference. Regions of altered DNA sequence copy number (losses, gains, and amplifications) in the tumor are highlighted and quantitated as color ratio changes along metaphase chromosomes.

In this pilot study we have taken advantage of the ability of CGH to detect DNA sequence copy number changes along all human chromosomes to test the hypothesis that genetically advanced node-negative tumors, those having an increased number of genetic changes, have a worse prognosis than tumors with few genetic changes. We also have used CGH to identify specific chromosomal loci that are associated with a worse prognosis.

Materials and Methods

Patients

We studied primary tumor specimens from forty-eight patients who had invasive breast carcinoma, were axillary lymph node negative, and had a primary tumor size of 2 to 4 cm. The specimens were taken from the San Antonio Tumor/Data Network, a breast cancer tumor bank with associated histopathological, clinical, laboratory, and follow-up data. The specimens used in this study were derived from fresh-frozen (−70°C) nuclear pellet fractions made from tumor tissue homogenates after routine ligand binding hormone receptor assays performed at the time of diagnosis by San Antonio's clinical laboratory. Forty-two of the tumors were of infiltrating ductal type, three were medullary, two were mucinous, and one was of other histological subtype. The sample for this study was selected so that one-half of the patients (n = 23) had a good outcome and were disease free after at least 5 years of follow-up. The other half (n = 25) had recurred in less than 5 years and had a poor outcome. Median follow-up time in the good outcome group was 80 months (range, 61 to 108). Median disease-free survival in the poor outcome group was 16.5 months (range, 1 to 54). The distributions of patients' age (mean ± SD: good, 59.3 ± 14 years; poor, 56.1 ± 14 years), primary tumor size (2.6 ± 0.6 cm versus 2.7 ± 1.0 cm), and estrogen receptor positivity (53% versus 47%) were not significantly different in the two groups. Three patients in the good group and four in the poor group received adjuvant therapy. Three patients had a positive family history.

Comparative Genomic Hybridization

Probe Preparation

Frozen nuclear pellets (10 to 20 mg) were suspended in DNA extraction buffer (0.1 mg/ml proteinase K, 100 mmol/L NaCl, 10 mmol/L Tris-HCl, pH 8, 25 mmol/L EDTA, pH 8, 0.5% sodium dodecyl sulfate) and incubated with shaking at 50°C overnight. High molecular weight DNA was extracted with phenol-chlorofom-isoamyl alcohol and precipitated with ethanol. Tumor DNA samples were labeled with biotin-14-dATP by the Bionick labeling system (BRL, Gaithersburg, MD). The reaction time and the amount of DNAse and DNA polymerase I were adjusted to obtain a probe fragment size of 600 to 3000 bp (detected by nondenaturing agarose gels stained with ethidium bromide). Normal reference DNA was extracted from mononuclear cells obtained from peripheral blood of healthy female volunteers and labeled with digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN) with the Bionick kit.

Hybridization

Labeled genomic DNAs were hybridized onto normal lymphocyte metaphase preparations essentially as described elsewhere.[9,10] In brief, 200 ng of labeled tumor DNA, 80 ng of labeled reference DNA, and 10 μg (10 μl) of unlabeled Cot-1 DNA were

precipitated with ethanol. The DNAs were dissolved in 10 μl of hybridization mixture (50% formamide, 10% dextran sulfate, 2X standard saline citrate (SSC), pH 7.0) and denatured (5 minutes at 70°C) immediately before applying onto slides. Slides were denatured in 70% formamide, 2X SSC (pH 7) at 74°C for 3 minutes, dehydrated in graded ethanols, digested with proteinase K (0.1 mg/ml in 20 mmol/L Tris, 2 mmol/L CaCl, pH 7.5) at room temperature for 7.5 minutes, and dehydrated again. Hybridization was done under sealed coverslips for 2 days at 37°C in a moist chamber. After hybridization, the slides were washed and signals were detected by immunofluorescence with avidin-fluorescein isothiocyanate and anti-digoxigenin rhodamine. Samples were counterstained with 4,6-diamino-2-phenylindole (DAPI) in an anti-fade solution.

Controls

Validation of CGH by comparisons with karyotyping and loss of heterozygosity analyses has been described elsewhere.[11] Each CGH experiment included slides hybridized with biotin-labeled female leukocyte DNA as a negative (normal cell) control sample and slides hybridized with biotin-labeled MCF-7 breast cancer cell line DNA as a positive control. Eight randomly selected tumor samples were also labeled with digoxigenin and hybridized with biotin-labeled normal reference DNA to control for differences in hybridization between biotin- and digoxigenin-labeled probes (inverse labeling CGH). Comparison between these two hybridizations confirmed the consistency of the aberrations seen at all loci. However, aberrations involving chromosomes 1p-ter, 16p, 17p, 19, and 22 could sometimes not be verified in inverse labeling control experiments. It was therefore decided that these regions were excluded from statistical analyses. These regions are known to be rich in guanidine and cytosine, which in our experience affect hybridization efficiency. Hybridization of biotin- and digoxigenin-labeled probes does not happen with equal efficiency in guanidine- and cytosine-rich regions as is the case elsewhere in the genome.

Digital Image Analysis

CGH hybridizations were analyzed with a Zeiss Axioplan microscope equipped with a 63× Neo-Fluar objective (Zeiss, Jena, Germany). Three separate images of the metaphases on three different wavelengths (matching green fluorescein, red rhodamine, and blue DAPI fluorescence) were captured with a black and white cooled CCD camera (Microimager 1400, Xillix Technologies, Vancouver, Canada) and processed with a digital image analysis system developed specifically for CGH.[12] In brief, the green and red fluorescence intensities were determined from p-telomere to q-telomere by integrating intensities (gray values in images) at 1-pixel intervals across the chromosome medial axis. After background subtraction, the green-to-red fluorescence intensity ratio profiles were calculated for each chromosome. The green-to-red ratios were normalized so that the ratio for the entire metaphase spread was set to 1.0. Data from four replicates of each chromosome were collected for all samples, and the mean green-to-red profile and its standard deviation were plotted for all chromosomes. The set of these ratio profiles comprise the copy number karyotype.[11,12]

Criteria used to define DNA sequence copy number alterations were based on negative (normal to normal DNA) and positive (human cancer cell lines) control hybridizations.[10] An increased DNA sequence copy number was defined as a chromosomal region with a green/red ratio ≥ 1.2 and a copy number decrease (loss or deletion) as a region with a green/red ratio <0.85. Subregional (in contrast to whole arm) copy number increases were called amplifications. High-level copy number increases with a green/red ratio ≥ 1.4 were defined as a distinct type of aberration. The final evaluation of the green/red ratio profiles was done in combination with visual inspection of digital images. This was done to exclude the effect of artifacts causing clearly outlying observations. Less than 10% of technically bad digital images were rejected and replaced by new ones. Copy number changes were required to be present on both sister chromatids in all replicate chromosomes. Subregional locations for chromosomal changes were determined based on DAPI banding pattern. The effect of normal cell contamination of the tumor sample has been previously studied by mixing MCF-7 cell line and lymphocyte DNA.[11] On average, copy number aberrations were detectable if the changes were present in more than 40 to 50% of the cells in the sample.

Statistics

In this pilot study, we examined both aberrations affecting specific regions (ie, loss of 8p) and the total number of aberrations (classified into losses and gains of DNA sequences). In the latter analysis, data for 1p-ter 16p, 17p, 19, and 22 were deemed unreliable and excluded (see above), leaving 1–12 p and q, 13–17q, 18, and 20 p and q, 21q, and Xp and q as

possible sites of aberration. Whole chromosome losses were counted as a single event. Two (or more) clearly distinct subregional copy number changes affecting the same chromosome arm were counted as separate events.

Differences in the total number of DNA sequence copy number aberrations by outcome group were evaluated by the Wilcoxon rank sum test. Spearman's rank correlation was used to examine the association of the total number of gains and losses. Fisher's exact test was used to detect associations between the presence of individual aberrations and outcome. Because of the small sample size, only the 10 most common aberrations of specific chromosomal regions were analyzed. All *P* values are two-tailed.

Results

Total Number of Genetic Aberrations and Patient Outcome

For the entire group of 48 breast cancers, the median number of losses was 3 (range, 0 to 14), the median number of copy number increases was 3 (range, 0 to 12), and the median number of aberrations of either type was 7 (range, 0 to 16). Only 5 tumors (10%), all in the good outcome group, showed no copy number aberrations by CGH. The total number of aberrations per tumor was significantly higher in the recurrence group ($P = 0.019$ by Wilcoxon rank sum test, Figure 1A). When copy number losses and gains were analyzed separately, only losses were significant ($P = 0.013$). The median number of losses was 4 in the recurrence group and 2 in patients with no recurrence.

An analysis based on survival status (alive >60 months or dead ≤60 months) rather than recurrence status at 60 months, and omitting 8 cases censored with less than 60 months of follow-up, yielded similar results (Figure 1B). There were more total aberrations (gains and losses, $P = 0.0056$) and more losses ($P = 0.002$) in the group with short survival (n = 17). This group comprises a subsample of the recurrent cases having an especially poor outcome. There was also a trend toward higher numbers of copy number gains in this group ($P = 0.09$).

Finally, the number of copy number losses and gains were correlated with each other for the combined groups (Spearman correlation, $r_{sp} = 0.51$, $P < 0.001$). Interestingly, within the good outcome group, the correlation was significant ($r_{sp} = 0.72$, $P < 0.001$), whereas there was no relationship between copy number losses and gains in the poor outcome group ($r_{sp} = 0.26$, $P = 0.21$).

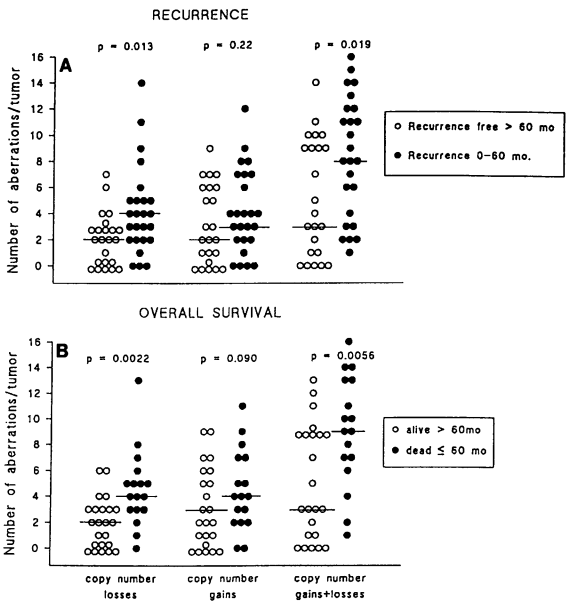

Figure 1. *Distributions of the total number of genetic aberrations (losses, copy number increases, or their sum) in node-negative breast cancer patients with (n = 23) or without recurrence (n = 25) during a 5-year follow-up* (**A**). **B:** *The same distribution when patients were grouped by overall breast-cancer-specific survival. Seventeen patients died as a result of breast cancer, whereas twenty-three patients whose follow-up extended up to 60 months, were alive. Horizontal lines indicate medians. P values refer to the Wilcoxon rank sum test.*

Aberrations of Specific Regions and Patient Outcome

A total of 55 different genetic aberrations were found and are listed in Table 1, in order of decreasing prevalence. Copy number aberrations are shown on an arm-by-arm basis and for subregional gains (amplifications), the minimal overlapping region is also indicated. The 10 most common aberrations comprised 40% of all changes. An example of a digital image of a hybridization showing a genetically advanced node-negative breast cancer and the corresponding green to red ratio profiles generated by the image analysis software are shown in Figure 2.

Associations of the 10 most common aberrations with early recurrence are shown in Table 1. Two of these were associated with outcome. Increased copy number at 8q (Table 1, $8q^+$) was common in both groups and was not significantly associated with either group ($P = 0.38$ by Fisher's exact test). However, high level copy number increases of 8q (green/red ratio ≥1.4) were classified as a distinct aberration (Table 1, footnote), which were significantly associated with recurrence ($P = 0.011$). High level gains of 8q were often associated with loss of 8p (11 of 14 cases), suggesting that isochromosome formation often led to this type of increased copy

Table I. *Genetic Aberrations in 48 Node-Negative Breast Cancers Detected by Comparative Genomic Hybridization*

Aberration	Prevalence (%)	Recurrent cases/ total aberrant
1q+	50	13/24
8q+*	48	14/23
8p−	31	9/15
16q−	27	8/13
4q+	19	3/9
11p−	17	6/8
3q+	17	3/8
13q−	15	5/7
12q−	15	3/7
20q12−13+	15	6/7†
3p−, 5p+, 6q−, 11p+, 11q−, 11q+,‡ 13q+, 15q−	13	
2p+, 4p−, 5q−, 7p+, 12p+, 14q−, 14q+, Xp−	10	
2p−, 9p+, 12q+, 17q22−24++	8	
2q+, 5q+, 6p−, 6q+, 7q+, 9p−, 9q−, 10p−, 10q−, 18p−, 18q−, 20p+, 21q+	6	
1p+, 3p+, 5p−, 7p−, 8p+, 10p+, 12p−, 15q+, 21q−, Xq−, Xq+	4	
2q−, 3q−, 6q−, 7q−, 10q+, 17q12+, 20q−, Xp+	2	

−, copy number loss (subregional or whole arm); +, copy number gains (subregional or whole arm).
*Consisting of 9 low level (green/red ratio 1.2 to 1.4) and 14 high level gains (green/red ratio >1.4); 3 of the former 9 cases (*P* value nonsignificant) and 11 of the latter 14 cases had recurrence in 5 years (*P* = 0.011).
†*P* = 0.044.
‡11q13 amplification was associated with loss of the distal q arm in 5 cases.

number at 8q. Amplification restricted to the *myc* region (8q24) was seen in only 2 of these 14 cases.

Second, subregional copy number increase (amplification) at 20q12–13 was also significantly more common in the poor outcome group (*P* = 0.044, Table 1). This aberration was found in only 7 of 48 cases; 6 of these cases were in the recurrence group and all relapsed within 32 months of diagnosis. None of the other 8 common aberrations were associated with outcome.

Discussion

The present study reports the first test of prognostic significance using genome-wide screening for DNA sequence copy number aberrations in node-negative breast cancers. This overview provided by CGH makes it possible to define which specific regions are aberrant and how many different aberrations there are. No other currently available method allows estimation of the total number of genetic aberrations in each breast tumor. Classical cytogenetic analysis is technically difficult for solid tumors, whereas allelotyping is usually based on analyses of only a few loci per chromosome arm.[7,8]

The total number of aberrations and especially regions with decreased DNA sequence copy number (losses of chromosome arms or parts therein) was significantly higher in cases with early recurrence as compared with patients with no recurrence in 5 years. This result supports the hypothesis that deletions and losses play a critical role in tumor progression by uncovering mutated tumor suppressor genes at several regions. These results suggest that detection of genetically advanced tumors by characterization of a genetic grade for primary tumors may be prognostically important, possibly independent of tumor size, which was fixed (2 to 4 cm) in this study of node-negative patients. Our finding that none of the individual DNA sequence losses were significantly associated with clinical outcome may reflect small numbers of cases when each aberration is evaluated individually. However, it is likely that there are several genetic pathways that result in promotion of metastasis. A large total number of copy number aberrations may simply reflect an increased chance for crucial hits.

The total number of copy number gains per tumor was not associated with clinical outcome. This finding is compatible with one previous study based on classical cytogenetics, in which the presence of homogeneously staining regions (that are suggestive for the presence of gene amplifications) in 84 breast cancers was not associated with patient outcome.[8] Many common aberrations may be important for tumor formation and occur early on during tumor development. These aberrations may not be as predictive for clinical outcome as those that are associated with subsequent steps in tumor progression. For instance, the copy number increase involving chromosome 1q was the most common (50%) genetic aberration in this set of early stage (node-negative) breast cancers, yet it was not associated with outcome.

Genetic aberrations affecting chromosome 8 were also common (30%). Of these, high level copy number increases at 8q were significantly associated with recurrence. This high level gain of 8q was often

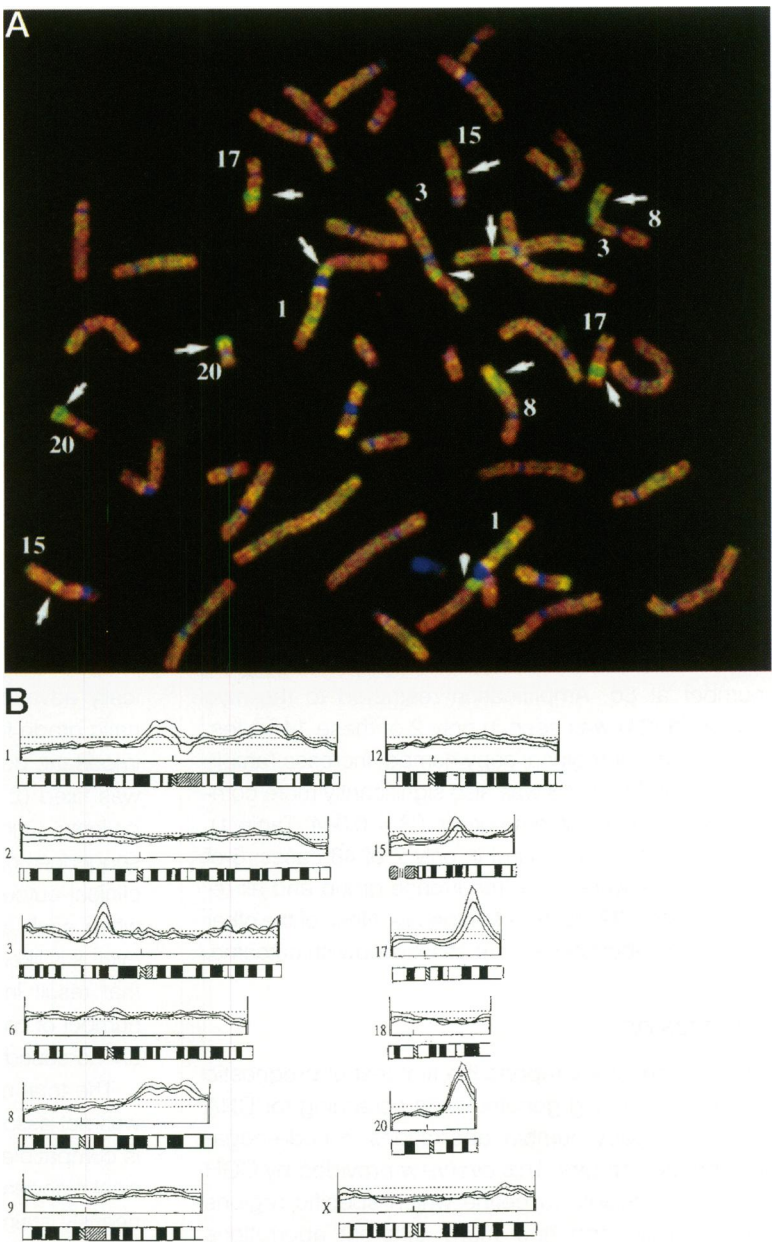

Figure 2. *An example of a digital image of a CGH experiment illustrating genetic aberrations in a node-negative breast carcinoma. DNA extracted from the tumor tissue (labeled in green) and normal reference DNA (labeled in red) were hybridized simultaneously to a normal metaphase spread (counterstained with DAPI). Chromosomal regions that were over-represented in the tumor are visualized in a predominantly greenish color, whereas regions that were lost are highlighted in a red color (relative lack of green). For this figure, three different single color images of the same metaphase were acquired (matching DAPI, fluorescein, and rhodamine stains), overlaid in pseudo-colors color-balanced, and contrast-stretched for display of the color differences. Clearly visible subregional gains (1p13, 1q, 3p14, 8q21–23, 15q21, 17q22–24, 20q13) are indicated in the figure by arrows on each chromosome homolog. Also shown are the green-to-red ratio profiles generated by the image analysis program of all aberrant chromosomes of the same sample. Aberrations detected with the profiles are copy number losses in chromosomes 2q, 6q, 8p, 9p, 12p, 15q, 18q, Xp, and Xq. Dashed lines indicate normal range of the green-to-red ratios (0.85 to 1.2). Chromosome numbers are indicated in the p arm.*

(in 11 of 14 cases) associated with loss of 8p. This is suggestive of isochromosome formation and raises the question of whether the loss of 8p or the gain of 8q is the chromosomal change selected for. As there were 8q gains that were not associated with loss of 8p and as 8p loss itself was not statistically significantly associated with prognosis, we believe that increased copy number of one or more genes at 8q is significant for tumor progression. The region of the *myc* oncogene at 8q24 was highly amplified in only two cases, suggesting that *myc* alone may not explain the biological consequences of high level 8q gain.

Several other previously known oncogene amplifications were also found. Amplification of the 11q13 region (containing the candidate oncogene cyclin D/*ems*1) was found in 6 of 48 cases. Two distinct amplifications at 17q were found: one at 17q12-q21 (corresponding to the *erb*B-2 locus) and another, more distal region at 17q22-q24. The latter region does not contain any known oncogenes. The low frequency of *ERB*B-2 amplification is probably a result of the fact that the amplicon is small and detectable only when highly amplified. Other amplifications at regions associated with known oncogenes (12q14, 8p12, 14q, and 3q21)

were also found, as previously described with a separate set of tumors.[10]

A striking prognostic correlation was found for amplification at 20q12–13. Recurrences were found in 6 of 7 20q12–13-positive cases, all in less than 32 months. This amplification has been previously reported in cell lines and in primary tumors,[10] and recent detailed studies of this region have defined approximately a 1.5-megabase critical region and excluded all candidate genes in this site.[15] The prognostic association found in this study emphasizes the importance of this amplification and the need to identify the target oncogene.

In summary, this study has shown that node-negative breast cancers that are genetically advanced and contain a large number of chromosomal losses are at an increased risk for recurrence. CGH is the method of choice to further define the prognostic significance of genetic grade as it provides information of the entire genome. It should also be pointed out that recent developments of the hybridization methods (for example, the use of directly fluorochrome-conjugated DNA as probes) make it possible to avoid the interpretation problems associated with particular chromosome regions that were identified in this study. Additional developments in laboratory techniques, such as automation of image analysis and the ability to use archival paraffin blocks as starting material[16] will probably make it possible to carry out a large confirmatory study (~500 patients). At the same time, other techniques, such as fluorescence *in situ* hybridization or allelic imbalance studies, can be applied to define in more detail the regions implicated in this study and to study their biological and prognostic roles in a large number of patients.

Acknowledgments

The authors thank Ms. Louise Riedell for assistance with chromosome identification and Ms. Sandy DeVries for technical assistance.

References

1. McGuire WL, Clark GM: Prognostic factors and treatment decisions in axillary node-negative breast cancer. N Engl J Med 1992, 326:1756–1761
2. Slamon DJ, Godolphin W, Jones LA, Holt J, Wong S, Keith D, Levin W, Stuart S, Udove J, Ullrich A: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989, 244:707–712
3. Schuuring E, Verhoeven E, van Tinteren H, Peterse JL, Nunnink B, Thonnisen F, Devilee P, Cornelisse C, van-de Vijver M, Mooi W: Amplification of genes within the chromosome 11q13 region is indicative of poor prognosis in patients with operable breast cancer. Cancer Res 1992, 52:5229–5234
4. Borg A, Baldetorp B, Ferno M, Olsson H, Sigurdsson H: c-*myc* amplification is an independent prognostic factor in postmenopausal breast cancer. Int J Cancer 1992, 51:687–691
5. Bieche I, Champeme MH, Matifas F, Hacene K, Callahan R, Lidereau R: Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer. Lancet 1992, 339:139–143
6. Borg A, Zhang QX, Olsson H, Wenngren E: Chromosome 1 alterations in breast cancer: allelic loss on 1p and 1q is related to lymphogenic metastases and poor prognosis. Genes Chromosomes & Cancer 1992, 5:311–320
7. Callahan R, Cropp CS, Merlo GR, Liscia DS, Cappa AP, Lidereau R: Somatic mutations and human breast cancer: a status report. Cancer 1992, 69:1582–1588
8. Zafrani B, Gerbault-Seureau M, Mosseri V, Dutrillaux B: Cytogenetic study of breast cancer: clinicopathologic significance of homogeneously staining regions in 84 patients. Hum Pathol 1992, 23:542–547
9. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992, 258:818–821.
10. Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Gray JW, Pinkel D, Waldman FM: Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. Proc Natl Acad Sci, USA 1994, 91:2152–2160
11. Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman F, Gray JW, Pinkel D: Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994, 10:231–243
12. Piper J, Rutovitz D, Sudar D, Kallioniemi A, Kallioniemi OP, Waldman F, Gray J, Pinckel D: Computer image analysis of comparative genomic hybridization. Cytometry 1995, 19:10–26
13. Cornelisse CJ, Kuipers-Dijkshoorn N, van Vliet M, Hermans J, Devilee P: Fractional allelic imbalance in human breast cancer increases with tetraploidization and chromosome loss. Int J Cancer 1992, 50:544–548
14. Reid T, Petersen I, Holtgrevegretz H, Speicher M, Cremer T: Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. Cancer Res 1994, 54:1801–1806
15. Tanner M, Tirkkonen M, Kallioniemi A, Collins C, Stokke T, Karhu R, Kowbel D, Shadrawan F, Hintz M, Kuo W-L, Waldman F, Isola J, Gray J, Kallioniemi OP: Increased copy number at 20q13 in breast cancer: defining the critical region and exclusion of candidate genes. Cancer Res 1994, 54:2457–2460
16. Isola J, deVries S, Chu L, Ghazvini S, Waldman F: Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. Am J Pathol 1994, 145: 1301–1308