In Situ c-myc Expression and Genomic Status of the c-myc Locus in Infiltrating Ductal Carcinomas of the Breast

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ABSTRACT

We have studied the expression of the c-myc protooncogene and the cycle-dependent histone 4 gene at the cellular level by RNA:RNA in situ hybridization in 18 primary breast ductal adenocarcinomas. These tumors have previously been examined by Southern and Northern blot analysis for the genomic status of c-myc and its expression, respectively (Escot et al., Proc. Natl. Acad. Sci. USA, 83: 4834–4838, 1986). Positive c-myc hybridization signals were associated with carcinoma cells in all cases, including tumors which had no apparent alterations of the c-myc locus. Steady-state levels of c-myc mRNA appeared heterogeneous in carcinomas with similar histology. High levels of hybridization were found in four of seven tumors with strong amplification of the c-myc locus. Similarly high levels of c-myc hybridization were detected in two of nine cases which had an apparently normal c-myc locus but comparatively low cellularity. In addition to carcinoma cells, dense clusters of infiltrating lymphocytes, present in three tumors, exhibited c-myc hybridization. The expression of the histone 4 gene failed to correlate with levels of c-myc expression. We conclude that in infiltrating ductal carcinomas: (a) the c-myc protooncogene is transcriptionally activated; (b) c-myc amplification is probably underestimated due to heterogeneous cellularity; (c) high-level c-myc amplification is related to high-level expression, but other unknown factors also may play a role; (d) differences in levels of c-myc expression may not only be attributed to differences in the growth fractions; and (e) c-myc mRNA in total RNA from biopsy samples may be contributed by infiltrating lymphocytes.

INTRODUCTION

Deregulation of the c-myc protooncogene by amplification or rearrangement is believed to play an important role in the genesis and progression of a variety of tumors (1, 2). Enhanced c-myc transcription was demonstrated in human tumor cell lines of hematopoietic (3–5) as well as epithelial (6–8) origin. With regard to mammary tumorigenesis, a specific role for the c-myc oncogene is suggested in the murine model by the development of tumors in transgenic mice carrying activated mouse mammary tumor virus/c-myc fusion genes (9). High levels of c-myc mRNA relative to the homologous normal tissues were detected by Northern and dot blot analysis in human breast carcinomas and in a variety of other primary human tumors (10, 11). In addition, genomic amplification of c-myc correlated with clinical aggressiveness in breast carcinomas as in other primary human tumors (12).

We have previously demonstrated that c-myc is amplified or rearranged in, respectively, 33 and 4% of primary breast carcinomas (13). These genetic alterations showed a significant correlation with patients 51 yr of age or older at the time of surgery. We also detected high levels of the 2.4-kilobase c-myc message in 10 of 14 cases (71%) from which RNA could be extracted (13). These included tumors containing amplified as well as apparently normal c-myc genes. Our evaluation of c-myc expression in RNA extracted from the tumor samples was limited by the well-known problem of heterogeneous cellularity in primary carcinoma samples. In fact, extraction of RNA and DNA from samples containing different numbers of tumor cells can cause underestimation of RNA expression or gene amplification due to the dilution of nucleotide sequences from tumor cells with those deriving from inflammatory or stromal cells. The RNA:RNA in situ hybridization technique (14–16) circumvents this problem, providing a correlation between gene expression and histological data. Recent improvements allow the use of this technique to localize endogenous mRNA species, including oncogene transcripts, in tissue sections (17–23). The objective of this study was to examine c-myc expression at the cellular level in tumors for which the status of the c-myc locus was known. In addition, we have compared c-myc expression with that of the cell cycle-dependent H4 gene to determine whether high levels of c-myc RNA reflect the fraction of proliferating cells in tumor biopsies rather than deregulation of the c-myc gene (24).

MATERIALS AND METHODS

Tissues and DNA and RNA Analysis. Eighteen primary breast carcinomas from our previously published (13) series of 121 cases (all from the Centre Rene Huguenin, St. Cloud, France) were selected on the basis of the availability of tissue adequate for DNA and, where possible, RNA extraction, as well as for frozen sections. All tumors were invasive ductal carcinomas measuring more than 2 cm in diameter and were histologically graded according to the World Health Organization guidelines (25). Estrogen and progestrone receptors were routinely measured by the activated dextran-charcoal method. Clinicopathologic data on these tumors are reported in Table 1. The samples of neoplastic tissue were dissected from the mastectomy specimens immediately after surgery, frozen in liquid nitrogen, and preserved at ~70°C. DNA and RNA extraction from tissue samples as well as electrophoresis, blotting, hybridization conditions, and densitometric measurements, using as reference normal peripheral blood lymphocyte DNAs, were as previously described (13). For each tumor, cellularity was evaluated on hematoxylin and eosin stained paraffin-embedded sections as the approximated percentage of total section surface occupied by tumor cells.

Probes. A 1.0-kilobase human c-myc PstI insert containing a portion of exon 2 and exon 3 (pRye 7.4; kindly given by Dr. C. Croce) (26) and a 1.3-kilobase BamHI-EcoRI murine 28S rRNA insert (kindly donated by Dr. J. Fetherston, University of Kentucky) were subcloned into plasmid pUC9, propagated in E. coli HB101. The murine H4 probe is a 610-bp EcoRI-HindII open reading frame fragment isolated from clone pF0108A (kindly provided by Dr. G. Stein, University of Florida, Gainesville, FL) (27). This fragment was subcloned in Gemini I (Promega Biotech) and also propagated in E. coli HB101. Antisense RNA labeled with [35S]UTP (400 Ci/mmol; Amersham, Arlington Heights, IL) was transcribed from truncated templates using the SP6 system (Amersham) (28) according to the manufacturer’s specifications. The specific activity of the probes was in the range of 7000 cpm/μg.

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2 The abbreviations used are: H4, histone 4; PBS, phosphate-buffered saline.
In Situ Hybridization. The technique used was essentially an adaptation of that reported by Harper et al. (18). Acid-precleaned microscope slides were incubated in poly-L-lysine (100 μg/ml) or purified collagen (100 μg/ml) in 50 mM sodium acetate, pH 5, for 20 min, dipped in two changes of double-distilled water, and dried overnight in a 37°C incubator. The tissue used for frozen sections was derived from the sample utilized for DNA and, when possible, RNA extraction. Frozen sections were cut at 5 μm thickness, thaw mounted onto precoated microscope slides, and rapidly fixed in 4% paraformaldehyde in PBS, pH 7.4, for 15 min at 4°C. The sections were then washed for three times in 2x SSC and PBS, incubated in 0.1 M ammonium acetate, pH 8.0 (10 min), rinsed in 2x SSC and PBS, incubated in 0.1 M Tris-HCl, pH 7.0/0.1 M glycine (30 min), rinsed in 2x SSC, and transferred in 2x SSC/50% formamide at 50°C prior to hybridization. Radiolabeled RNA transcripts were precipitated in ethanol and dried under vacuum. RNA pellets were resuspended in double-distilled sterile water and adjusted to the desired concentration (1 x 10^6 cpm/μl). Routinely, 2 μl of labeled probe per slide were added to 18 μl of hybridization solution to give final concentrations of 50% formamide, 2x SSC, 10 mM fresh diethiothreitol, 1 mg/ml E. coli tRNA, 1 mg/ml sonicated salmon sperm DNA, and 2 mg/ml bovine serum albumin. The probes were heated for 5 min at 65°C in the hybridization solution and then transferred to a multiblock heater at 50°C. The slides were retrieved from the 2x SSC/50% formamide incubation, blotted with lint-free paper, and transferred to a slide warmer at 50°C. Hybridization solution (20 μl) was gently applied to the frozen sections, which were then covered with acid-precleaned siliconized 22-mm square coverslips and sealed with rubber cement. The hybridization was carried on for 4 h at 50°C, then the rubber cement was removed, and the coverslips were allowed to slide off in 2x SSC at 50°C. The sections were washed in 2x SSC at 55°C (10 min) and 2x SSC/50% formamide at 55°C (two washes, 5 and 20 min), rinsed in 2x SSC at room temperature, and blotted dry. RNase A (30 μl; Boehringer, Mannheim, FRG), 100 μg/ml in 0.2 M sodium phosphate, pH 6.5, was applied to the tissue sections. The sections were covered with acid-precleaned coverslips, sealed with rubber cement, and incubated for 30 min at 37°C. After removal of the coverslips, the slides were washed in two changes of 2x SSC at 55°C, then for 5 min in 2x SSC/50% formamide with stirring, and finally overnight in 2x SSC at 55°C with constant stirring. All washes were done in a 200-ml volume for sets of up to 25 slides. The sections were then dehydrated, dried, and dipped in Kodak NTB2 emulsion diluted 1:1 with water. After an exposure of 6 days in light-proof boxes at 4°C, the autoradiographs were developed in Kodak D-19 developer (4 min), washed in two changes of tap water, fixed in Kodak Rapid Fixer (5 min), rinsed in tap water, and counterstained with hematoxylin or hematoxylin and eosin. Stained sections were examined for silver grains.

Evaluation of In Situ Hybridization. A quantitation of signals due to c-myc, Hem, and 28S rRNA probes was determined in all cases relative to background levels, detected with plasmid (pSP64) transcripts. Silver grains were counted using an ocular micrometer under light microscope at a magnification of × 1000. For each cell type in each section, a 1.2-mm^2 area with uniform cellular composition (i.e., carcinoma cells, stromal cells, and in some cases, normal epithelial cells and lymphocytes) was evaluated and the number of grains was determined. The grain counts observed with the indicated probes were as follows: pSP64: 11-53 (average, 24 tumors), 7-41 (average, 18 tumors); c-myc: 45-296 (tumor cells), 7-45 (stroma), 41-90 (lymphocytic infiltrate); Hem: 90-367 (tumor cells), 17-72 (stroma), 91-114 (lymphocytic infiltrate); 28S rRNA: 151-425 (mean, 212, tumor cells). Labeling indexes for c-myc, Hem, and 28S rRNA were the ratios of the respective number of silver grains per area to the number of silver grains per similar area in the control slide hybridized to pSP64 transcripts and exposed under the same emulsion in the same experiment.

RESULTS AND DISCUSSION

Cellular c-myc transcripts were detected by the clustering of silver grains over tumor cells in all 18 cases studied (Fig. 1, a and c; Fig. 2, a and b). Labeling indexes for c-myc RNA in carcinoma cells ranged from 2.00 to 16.00, while in tumor stroma with no lymphocytic infiltration, the range was from 0.38 to 1.60 (Tables 2 and 3). These results indicate heterogeneity in the levels of c-myc RNA in tumor cells and in the stroma the level of silver grains was either slightly below or slightly above the background obtained with pSP64 transcripts in the same experiment (negative control). In Cases 13 and 16, c-myc labeling indexes for stroma with dense lymphocytic infiltration were in a positive range (i.e., 2.11 and 3.05) due to labeling of lymphocytes. Positive c-myc hybridization in dense clusters of peritumoral lymphocytes was also clearly demonstrated in Case 9 (Fig. 2, a-d; Table 2). In this case the labeling index determined for lymphocytes was 4.63. Expression of c-myc in such dense lymphocytic infiltrates is consistent with reports that the transcriptional activation of this protooncogene is associated with the mitogenic stimulation of T and B lymphocytes (30, 31).

Positive control slides hybridized to the murine 28S rRNA probe had strong in situ hybridizations, with indexes ranging from 7.75 to 21.63 (Tables 2 and 3). This range of hybridization should reflect different levels of 28S RNA in these cells rather than differences in RNA preservation. In fact, the tumor with the lowest 28S rRNA labeling index (Case 1) had well-defined c-myc labeling (index = 4.39; Table 2). Also, low c-myc labeling indexes were obtained for tumors with high 28S rRNA labeling (i.e., Case 2; Table 2).

All of the cases studied were genomically characterized for the presence of an amplified or rearranged c-myc locus by Southern blot analysis of HindIII-digested carcinoma DNAs. This analysis revealed no gross alterations of the c-myc locus in 9 of the 18 cases (13) (Table 2). If we consider labeling indexes above the arbitrary value of 5.00 as indicative of strong hybridization, three tumors were strongly hybridizing in this group (Case 5, index = 6.91; Case 6, index 5.29; Case 9, index 6.06).
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Fig. 1. RNA-RNA in situ hybridizations demonstrating different levels of c-myc expression in histologically similar grade III infiltrating ductal carcinomas. a–c, frozen sections from a tumor with high level (++) amplification of the c-myc locus (Case 13). a, strong hybridization to the c-myc probe (labeling index 16.00; Table 3) indicated by clustering of silver grains over tumor cells (T); stromal (S) cells are not labeled. b, negative hybridization to plasmid (pSP64) transcripts. c, strong hybridization to the 28S rRNA probe (labeling index 14.38; Table 3). Note the distribution of silver grains over both tumor cells (T) and stromal cells (S) in contrast to the tumor-associated distribution of silver grains in a. d–f, frozen sections from a tumor containing an apparently normal c-myc locus (Case 3). d, section hybridized to the c-myc probe has low hybridization (labeling index 3.44; Table 2) indicated by silver grains scattered over tumor cells (T); stromal cells (S) appear unlabeled. e, section showing no hybridization to plasmid (pSP64) transcripts. f, strong hybridization to the 28S rRNA probe (labeling index 14.63; Table 2) is similar to c, indicating that lower c-myc expression in this tumor is not related to poor RNA preservation. All sections were autoradiographed for 6 days and lightly counterstained with hematoxylin and eosin (× 650).

= 10.87; Table 2). c-myc Labeling indexes for the other six tumors ranged from 2.00 to 4.75. If we take into consideration the cellularity of the cases with apparently normal c-myc loci, only one case had cellularity greater than 50% (Case 3; Table 2). Of the three cases with strong c-myc hybridization, one (Case 5) had cellularity inferior to 25%, the other two cases (Cases 6 and 9) had cellularity inferior to 50%. In addition, Case 9 had a prominent lymphocytic infiltration (Fig. 2, a–d). Thus, in Case 9 lymphocytic DNA could have significantly contributed to the total DNA extracted. Genomic analysis of c-myc in the other nine tumors revealed variable levels of c-myc amplification (13) (Table 3). Of seven tumors with high level (++) c-myc amplification (i.e., 6 to 15 times the level of normal lymphocyte DNAs) (13), four had c-myc labeling indexes superior to 5.00 (Case 10, index = 7.20; Case 13, index = 16; Case 15, index = 6.29; Case 16, index = 6.71). The other three cases with high (++) amplification and the two cases with moderate (+) amplification (i.e., 2 to 5 times the level of normal lymphocyte DNAs) had c-myc labeling indexes ranging from 2.72 to 4.72 (Table 3). Seven of the nine cases with genomic amplification of c-myc had cellularities superior to 50% (Table 3).

High-level c-myc transcription is related to structural alterations of the c-myc locus in several types of tumors (7, 13, 26, 32–34). However, there are studies suggesting that elevated c-myc mRNA levels may be found in the absence of apparent genetic alterations in colon carcinomas (35) and in some breast carcinomas (13). Possibly, c-myc expression may be influenced by alterations of distant regulatory genes. In the present study, we cannot exclude genetic rearrangements outside the restric-
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Fig. 2. RNA:RNA in situ hybridization of a Grade III infiltrating ductal carcinoma (Case 9) containing an apparently normal c-myc locus. In a, low-power view of a frozen section (hybridized to the c-myc probe) shows a dense lymphocytic infiltrate (L) in the stroma (S) surrounding clusters of tumor cells (T) and scattered normal mammary ductules (D). Dilution of tumor DNA with lymphocytic DNA may have caused an underestimation of c-myc amplification in tumor cells (T') that, as shown in b, demonstrate strong c-myc expression (labeling index 10.87, Table 2). In c, the expression of c-myc is also detectable in lymphocytes (L; labeling index 4.63, Table 2), whereas the adjacent normal mammary ductules (D) may be labeled at a very low level only (c-myc labeling index 1.80, Table 2). The section hybridized to pSP64 transcripts (d) does not reveal hybridization in either tumor cells (T) or lymphocytes (L). All sections were autoradiographed for 6 days and lightly counterstained with hematoxylin and eosin (a, ×150; b-d, ×650).

We found that, in breast carcinoma, c-myc was expressed irrespective of its genomic amplification. However, the observed tumor cellularity in several cases strongly suggests that amplification was underestimated. This was particularly true in those cases that contained an apparently normal c-myc locus and had high levels of c-myc RNA (i.e., Cases 5, 6, and 9; Table 2). The c-myc labeling indexes in cases with low-level amplification and high cellularity were in the range of most cases with apparently normal c-myc locus (see Tables 2 and 3, Cases 14 and 18). This may reflect an experimental limitation of in situ hybridization to detect the quantitative effects of low-level c-myc amplification on c-myc RNA levels. Cases with high-level amplification had strong c-myc hybridization, with the noticeable exception of Cases 12 and 17. These two tumors did not exhibit evidence of degradative loss of RNA (see 28S rRNA and H4 labeling). Since we are measuring steady-state levels of RNA, these findings could be consistent with a high rate of c-myc RNA turnover in these two tumors.

In five tumors (Cases 7, 8, 10, 13, and 16), the expression of the c-myc protooncogene was also studied by Northern blot analysis of polyadenylated RNA. The three carcinomas (Cases 10, 13, and 16) in which the 2.4-kilobase c-myc mRNA was detected demonstrated strong in situ c-myc hybridization (labeling indexes 7.20, 16.00, and 6.71; Table 3). Tumors 7 and 8, in which the 2.4-kilobase c-myc message was undetectable, had low in situ c-myc hybridization (indexes 3.00 and 2.89; Table 2). The cellularity of Cases 7 and 8 was less than 50% and 25%, respectively. Thus, it appears that the inability to
Table 2 Tumor cellularity and expression by RNA:RNA in situ hybridization of c-myc, Hs, and 28S rRNA in 9 ductal carcinomas in which c-myc appeared normal

<table>
<thead>
<tr>
<th>Case</th>
<th>% tumor cellularity</th>
<th>c-myc (tumor)</th>
<th>c-myc (stroma)</th>
<th>Hs (tumor)</th>
<th>Hs (stroma)</th>
<th>28S rRNA (tumor)</th>
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<tbody>
<tr>
<td>1</td>
<td>≤25</td>
<td>4.39</td>
<td>1.14</td>
<td>ND</td>
<td>ND</td>
<td>7.75</td>
</tr>
<tr>
<td>2</td>
<td>≤25</td>
<td>2.00</td>
<td>ND</td>
<td>12.00</td>
<td>2.24</td>
<td>16.15</td>
</tr>
<tr>
<td>3</td>
<td>≤75</td>
<td>3.44</td>
<td>0.38</td>
<td>15.81</td>
<td>1.36</td>
<td>14.63</td>
</tr>
<tr>
<td>4</td>
<td>≤50</td>
<td>4.75</td>
<td>1.31</td>
<td>12.08</td>
<td>ND</td>
<td>17.50</td>
</tr>
<tr>
<td>5</td>
<td>≤25</td>
<td>6.91</td>
<td>1.60</td>
<td>15.61</td>
<td>2.42</td>
<td>18.34</td>
</tr>
<tr>
<td>6</td>
<td>≤50</td>
<td>5.29</td>
<td>1.93</td>
<td>ND</td>
<td>ND</td>
<td>8.90</td>
</tr>
<tr>
<td>7</td>
<td>≤50</td>
<td>3.00</td>
<td>0.80</td>
<td>ND</td>
<td>ND</td>
<td>17.40</td>
</tr>
<tr>
<td>8</td>
<td>≤25</td>
<td>2.89</td>
<td>23.41</td>
<td>ND</td>
<td>ND</td>
<td>17.52</td>
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<tr>
<td>9</td>
<td>≤50</td>
<td>10.87</td>
<td>8.59</td>
<td>2.23</td>
<td>1.26</td>
<td>13.09</td>
</tr>
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</table>

* Cellularity is ~% of section surface occupied by tumor cells in paraffin-embedded hematoxylin and eosin stained sections (measuring from ~ 1.5 x 1.0 cm to ~ 2.5 x 2.0 cm).
* c-myc Labeling index for normal mammary epithelium = 1.75.
* Labeling index, i.e., the ratio of the mean number of silver grains obtained with the indicated probe in a 1.2-mm² area to the number of silver grains in a 1.2-mm² area obtained with the pSP64 control probe.
* ND, not done.
* Labeling indexes for normal mammary epithelium, c-myc = 1.80, Hs = 1.72; labeling indexes for lymphocytes, c-myc = 4.63, Hs = 4.91.

Table 3 Tumor cellularity and expression by RNA:RNA in situ hybridization of c-myc, Hs, and 28S rRNA in 9 ductal carcinomas with amplified c-myc locus

<table>
<thead>
<tr>
<th>Case</th>
<th>% tumor cellularity</th>
<th>c-myc Amplification</th>
<th>c-myc (Tumor)</th>
<th>c-myc (Stroma)</th>
<th>Hs (tumor)</th>
<th>Hs (stroma)</th>
<th>28S rRNA (tumor)</th>
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<tr>
<td>10</td>
<td>≤50</td>
<td>++</td>
<td>7.20</td>
<td>0.72</td>
<td>ND</td>
<td>ND</td>
<td>10.27</td>
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<tr>
<td>11</td>
<td>≤100</td>
<td>++</td>
<td>4.72</td>
<td>1.01</td>
<td>12.00</td>
<td>3.84</td>
<td>12.64</td>
</tr>
<tr>
<td>12</td>
<td>≤50</td>
<td>++</td>
<td>2.77</td>
<td>0.73</td>
<td>9.92</td>
<td>2.85</td>
<td>16.16</td>
</tr>
<tr>
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<td>++</td>
<td>16.00</td>
<td>2.11</td>
<td>21.14</td>
<td>1.90</td>
<td>14.38</td>
</tr>
<tr>
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<td>≤75</td>
<td>+</td>
<td>2.74</td>
<td>0.77</td>
<td>19.29</td>
<td>5.22</td>
<td>15.74</td>
</tr>
<tr>
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<td>≤100</td>
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<td>6.29</td>
<td>1.31</td>
<td>16.90</td>
<td>6.54</td>
<td>21.63</td>
</tr>
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<td>≤100</td>
<td>++</td>
<td>6.71</td>
<td>3.05</td>
<td>2.82</td>
<td>2.03</td>
<td>10.44</td>
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<td>1.39</td>
<td>3.41</td>
<td>1.41</td>
<td>13.06</td>
</tr>
<tr>
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<td>+</td>
<td>3.95</td>
<td>1.00</td>
<td>7.50</td>
<td>6.00</td>
<td>11.10</td>
</tr>
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</table>

* Cellularity is ~% of section surface occupied by tumor cells in paraffin-embedded hematoxylin and eosin stained sections (measuring from ~ 1.5 x 1.0 cm to ~ 2.5 x 2.0 cm).
* ++, 6 to 15 times the level of normal lymphocyte DNAs; +, 2 to 5 times the level of normal lymphocyte DNAs.
* Labeling index, i.e., the ratio of the mean number of silver grains obtained with the indicated probe in a 1.2-mm² area to the mean number of silver grains in a 1.2-mm² area obtained with the pSP64 control probe.
* ND, not done.
* Stromata with dense lymphocytic infiltration.

detect c-myc message by Northern blotting reflected both low-level expression and dilution of c-myc RNA.

The level of c-myc RNA in normal mammary epithelium could not be compared with that found in carcinomas due to lack of adequate samples. In two tumor samples (Cases 1 and 9), residual mammary ductules were entrapped in the neoplastic tissue. These ductules failed to exhibit significant hybridization to c-myc (normal epithelium: Case 1, index = 1.75; Case 2, index = 1.80; Table 2, Fig. 2c). It should be pointed out that these samples are not representative of normal disease-free...
mammary epithelium nor of the range of nonneoplastic changes of the human breast.

The expression of c-myc has been reported to be under cell type-specific and developmental control in embryonal human and murine epithelial (22, 36). In addition, c-myc expression was found to be dependent upon the cellular growth state in placental cytrophoblasts (20) and in human colon carcinomas (24). In tissue culture experiments, murine lymphocytes and fibroblasts were found to have low c-myc RNA levels in serum-deprived quiescent cells and high levels in exponentially growing cells (30, 31, 37). These and other results suggested that the expression of c-myc is cell cycle regulated and occurs during the G1 phase of the cell cycle (38). In separate studies c-myc RNA (39) and protein (40) levels in cells maintained in the presence of serum growth factors have been reported to be constant throughout the cell cycle. Based on these results, it was postulated that c-myc expression reflects the competency of cells to enter and progress through the cell cycle. Thus, c-myc expression could be maintained independent of the position of the cell cycle.

To evaluate the possibility that differences in c-myc expression observed in our breast carcinoma samples could be secondary to differences in the fraction of cycling cells (41), we hybridized sections from the same cases to the S phase-specific H2A gene (27, 42, 43). We reasoned that if c-myc expression was strictly related to the fraction of cycling cells and to the G1 phase of the cycle, one would assume that levels of c-myc should be similar in tumors with similar expression of an S phase-specific gene. H2A labeling indexes were heterogeneous but generally high in these tumor samples (Tables 2 and 3). There appeared to be no well-defined trend toward an association between levels of c-myc or 28S rRNA hybridization and H2A hybridization (Tables 2 and 3). This is exemplified by Cases 13 and 14 (Fig. 3) which had similar H2A labeling indexes (i.e., 21.14 and 19.29) but very different c-myc levels (i.e., 16.00 and 2.74; Table 3). Thus, the differences in the fraction of cycling cells in the tumor are not sufficient to account for their differences in c-myc hybridization levels.

We conclude from these studies that (a) the c-myc protooncogene is consistently expressed in human ductal carcinomas of the breast, including cases which may result negative by Northern blot analysis due to low expression and/or low cellularity; (b) high levels of c-myc expression tend to be associated with high-level genomic amplification of c-myc; (c) dense peritumoral lymphocytic aggregates represent a potential source of c-myc transcripts in biopsy samples of breast carcinomas; (d) ductal carcinomas of similar grade and histologic appearance may vary widely in the steady-state levels of c-myc RNA; and (e) in ductal carcinomas, c-myc and the S phase-specific gene H2A are not coordinately expressed. Based on these results, a further study of c-myc expression in normal mammary tissues, in benign lesions of the breast, and in other histologic types of breast tumors is warranted.

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