Low-Level c-myc Amplification in Human Colonic Carcinoma Cell Lines and Tumors: A Frequent, p53-independent Mutation Associated with Improved Outcome in a Randomized Multi-institutional Trial

Leonard H. Augustin, Scott Wadler, Georgia Corner, Christine Richards, Louise Ryan, Asha S. Murtani, Sen Pathak, Al Benson, Daniel Haller, and Barbara G. Heerdt

INTRODUCTION

The c-myc gene, located on human chromosome 8 at q21, is the normal homologue of a transforming sequence first identified in the avian MC29 virus (1, 2). The first report of a role for c-myc in colonic carcinoma was a 10-fold elevation in expression of the gene in a carcinoma cell line (3). The first indication of c-myc amplification was not related to incidence of p53 mutations. Although the c-myc gene is overexpressed in approximately 70% of human colonic cancers, previous studies have not detected frequent gene amplification or rearrangement of c-myc in these tumors, although such amplification has been reported in chemically induced rodent colon cancer and quantitative analysis of gene copy number has shown the gene to be amplified at a low level in mucinous and poorly differentiated human colon carcinomas. Using rigorously controlled blot methodology, we have established that the c-myc gene, located at 8q21, exhibited amplification of 87% to 35-fold in 7 of 10 human colonic carcinoma cell lines. This was highly significant even at a low level of amplification in HT29 cells (P < 0.0001). Cytogenetic analysis by G-banding did not detect aneuploidy involving chromosome 8q, suggesting that the amplification for the c-myc gene on 8q was relatively specific, and this was consistent with a lack of amplification detected for the c-mos gene on 8q24, which was assayed similarly. The same methodology then revealed amplification of c-myc from 1.5-fold to 5-fold in 32% of tumors from 149 patients entered into a multi-institutional Phase III study of adjuvant therapy for colon cancer. c-myc status was not related to time to recurrence or death, but low levels of c-myc amplification identified a subset of patients who showed a statistically significant increase in disease-free survival, and a corresponding trend to longer overall survival, in response to adjuvant therapy with 5-fluorouracil plus levamisole. Presence of c-myc amplification was not related to incidence of p53 mutations.

ABSTRACT

Human colonic cancer is associated with multiple genetic deletions, mutations, and alterations in gene expression; in contrast, gene amplification has not been recognized as a prominent characteristic of human colon tumors. Although the c-myc gene is overexpressed in approximately 70% of human colonic cancers, previous studies have not detected frequent gene amplification or rearrangement of c-myc in these tumors, although such amplification has been reported in chemically induced rodent colon cancer and quantitative analysis of gene copy number has shown the gene to be amplified at a low level in mucinous and poorly differentiated human colon carcinomas. Using rigorously controlled blot methodology, we have established that the c-myc gene, located at 8q21, exhibited amplification of 87% to 35-fold in 7 of 10 human colonic carcinoma cell lines. This was highly significant even at a low level of amplification in HT29 cells (P < 0.0001). Cytogenetic analysis by G-banding did not detect aneuploidy involving chromosome 8q, suggesting that the amplification for the c-myc gene on 8q was relatively specific, and this was consistent with a lack of amplification detected for the c-mos gene on 8q24, which was assayed similarly. The same methodology then revealed amplification of c-myc from 1.5-fold to 5-fold in 32% of tumors from 149 patients entered into a multi-institutional Phase III study of adjuvant therapy for colon cancer. c-myc status was not related to time to recurrence or death, but low levels of c-myc amplification identified a subset of patients who showed a statistically significant increase in disease-free survival, and a corresponding trend to longer overall survival, in response to adjuvant therapy with 5-fluorouracil plus levamisole. Presence of c-myc amplification was not related to incidence of p53 mutations.

MATERIALS AND METHODS

Cell Lines. Colonic carcinoma cell lines were grown, DNA was extracted, and quantitative analysis of c-myc gene copy number was assayed as described (Refs. 8 and 9; see below). Multiple preparations of DNA were analyzed for most of the cell lines (see “Results”), and each analysis was done in triplicate using multiple preparations of probes for the c-myc gene (pcMC41-Spp; Ref. 10) and for the standard β-globin gene (pBpors; Ref. 11).

Human Tissue. Written consent was obtained from all patients whose tissue was analyzed in this study. The investigations reported herein were approved by the Institutional Review Board of the Montefiore Medical Center in accordance with assurances filed with and approved by the United States Department of Health and Human Services.

Blocks of paraffin-embedded tissue from patients entered into EST2284, an intergroup trial of adjuvant therapy for Duke’s B2 and C colon cancer, were collected and coded by the Pathology Coordinating Office of the ECOG and then forwarded to the Albert Einstein Cancer Center. Blocks of tumor tissue were trimmed of excess paraffin and of histologically normal tissue. DNA was then extracted from 100–200 5-μm sections of each tumor block. Sections were incubated in TE (500 mM Tris, 20 mM EDTA, and 10 mM NaCl, pH 9.0) containing 20% SDS and 20 mg/ml proteinase K (Life Technologies, Inc., Gaithersburg, MD) at 48°C for a total of 72 h, with the further addition of another 20 mg/ml proteinase K at both 24 and 48 h. Samples were then extracted with equal volumes of phenol and chloroform:isoamyl alcohol (99:1). The organic phase was reextracted with TE. The two aqueous phases were then reextracted twice with chloroform:isoamyl alcohol, and DNA was precipitated by the addition of 0.1 volumes of 2.5 M sodium acetate, pH 5.2, and 2.5 volumes of ethanol. The final DNA pellets were resuspended in 10 mM Tris, pH 8.0. For analysis of relative gene copy number, DNA was bound to 3 The abbreviations used are: ECOG, Eastern Cooperative Oncology Group; 5FU, 5-fluorouracil; SSCP, single-strand conformation polymorphism; TS, thymidylate synthase.
different normal placentas each in quintuplicate. The range of values obtained for these 20 standards is shown in Fig. 2 and defines the normal range for c-myc/globin for normal tissue. As additional controls, triplicate spots of DNA from HT29 cells and from COLO320 cells (low and high c-myc amplification, respectively) were also included on each blot and are shown in the example given in Fig. 2.

Determination of relative c-myc copy number was done by hybridizing replicate blots to a c-myc probe (pMC41-Spp; Ref. 10) and a β-globin probe (pBPst; Ref. 11). Probes were labeled to high specific activity with [32P]dCTP and [32P]dATP by the random priming method. Following hybridization and washing of the blots as described previously (12), quantification of hybridization was done by cutting out individual spots and counting them in a liquid scintillation counter (LKB, Gaithersburg, MD). Identical results were obtained when spots were scanned and hybridization quantified using a Phosphorimagier (Molecular Dynamics, Sunnyvale, CA). Data were entered into an Excel spreadsheet (Microsoft Corp., Redmond, WA). Background (mean of 10 blank spots on the blot) was subtracted from each spot. Mean hybridization for the c-myc and β-globin probes was calculated for each sample, and the c-myc copy number was expressed as the ratio of c-myc to β-globin for each sample, relative to the mean of the same ratio for the 20 placental DNA samples.

SSCP analysis was used to screen for mutations in the p53 gene. Exons 5, 6, 7, and 8 of the p53 gene were amplified by PCR using primers from Novagen (Madison, WI). Amplification using [32P]dCTP was done over 40 cycles of 1 min at 95°C, 1 min at 60°C, and 2 min at 72°C, followed by a final incubation at 72°C for 5 min. The amplified products were fractionated on a mutation detection enhancement gel (FMC Bioproducts, Rockland, ME) at 8 W for 14–18 h, and products were visualized using a Molecular Dynamics Phosphorimagier. Aberrantly migrating bands were excised and eluted in TE, and an aliquot was used as template for a secondary PCR amplification using the same primers in the absence of radiolabeled nucleotide, with each cycle time decreased to 30 s. The final product was sequenced using the PCR sequencing kit from United States Biochemical Corp. (Cleveland, OH), using the 3' dNTP labeling step described by the manufacturer.

Data Analysis. Relative c-myc copy numbers in cell lines were compared to those in placenta with two-sample Student’s t tests, using individual groups and pooled variances. For the clinical data, survival and time to progression curves were estimated using the method of Kaplan and Meier (13). Survival was measured from the time of study entry until death, and data from patients still alive were censored as of the time of their last follow-up. Time to progression was measured from the time of study entry to the first documented time of relapse or disease progression. Data from patients alive with no evidence of disease were censored at the time of their last follow-up. Data from patients who died with no evidence of disease were also censored for the purpose of time to progression analyses. A log-rank test (14) was used to compare survival and time to progression between various patient groups.

RESULTS

c-myc Amplification in Human Colonic Carcinoma Cell Lines. Fig. 1A (open column) illustrates the mean ± SD of c-myc/globin hybridization for seven different placental DNAs, each assayed at least in triplicate. Quantitative dot blot hybridization demonstrated that 7 of 10 colonic carcinoma cell lines (filled columns) exhibited increases of c-myc relative to globin, ranging from less than 2-fold to 35-fold above the placenta value. As reported previously (15), COLO320 cells exhibited amplification of approximately 35-fold (Fig. 1A). In the other cell lines, the increase ranged from 6-fold for SW620 cells to 1.87-fold (87%) for HT29 cells. This 87% increase in c-myc/globin in HT29 cells (mean of 10 separate DNA preparations) was highly significant (P < 0.0001) and was consistent with a previous report of low-level c-myc amplification in HT29 cells (16). Three of the cell lines, DLD-1, LOVO, and RKO, did not show evidence for altered c-myc copy number (Fig. 1A). To confirm and extend these results, two approaches were taken. First, the data in Fig. 1A for the cell lines were compared to data generated by standard Southern blot analysis of the same DNA preparations digested with EcoRI. Because we have already published
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such data, the Southern blots are not shown, but, as we have demonstrated previously (8), there was excellent correlation between the results obtained by the two methods (Fig. 1B; \( P < 0.0001 \)). When the higher amplification of the COLO320 cells was eliminated from the analysis (Fig. 1B, inset), the correlation between data obtained from dot blots and Southern blots for levels at the lower end of the range (up to 6-fold increase) was again highly significant (\( P < 0.004 \)).

Second, cytogenetic analysis was carried out on six of these colonic carcinoma cell lines. This revealed multiple, heterogeneous, chromosomal abnormalities among the lines, but of the three lines examined with low-level amplification of the c-myc gene (HT-29, SW480, and SW620), each contained two copies of chromosome 8 that appeared normal by G-banding (data not shown). The COLO320 cell line, containing high amplification, is known to exhibit a homogeneously staining region at the site of amplification (15). In addition, for seven cell lines, we reassayed c-myc by quantitative hybridization and, in parallel, assayed for amplification of c-mos, which is located at q24 on chromosome 8 near c-myc, which is at q21 (Fig. 1C). The c-myc/globin data for these cell lines are very similar to that shown in Fig. 1A: there is no amplification in DLD-1 and LOVO; there is low-level amplification for HT29, SW480, SW620, and SKCO1, at the same relative levels as shown in Fig. 1A; and there is high amplification for COLO cells. Most important, it is clear that there is no evidence for increased copy number of c-mos relative to globin. This is in agreement with data we have previously published on DNA from primary colon tumors (8). This is important for two reasons: first, it demonstrates, along with the cytogenetic data, that it is highly unlikely that the increased c-myc copy number is due to aneuploidy for chromosome 8; and second, it rules out the possibility that the increase in c-myc/globin ratio is due to a deletion of the globin gene used as a hybridization standard, rather than an increase in c-myc.

To summarize the data for the cell lines, both low-level and higher-level c-myc amplification (HT29 and COLO320 cells, respectively) are revealed by the dot blot methodology and are highly statistically significant, even at the low level for HT29 cells, consistent with the relative levels of c-myc amplification for both of these cell lines, which have been reported independently from other groups (15, 16); these relative increases are corroborated by Southern blots; and cytogenetic analysis and the lack of c-mos amplification, also on chromosome 8, make it highly unlikely that the amplification is due to aneuploidy for chromosome 8, consistent with our previous findings (8).

**c-myc Amplification in Human Colonic Carcinomas.** The questions to be addressed were: first, whether increased c-myc copy number was a characteristic of primary colon tumors, as it is for the majority of colonic carcinoma cell lines; and second, whether the increase in human colonic carcinomas is predictive of outcome or response to adjuvant chemotherapy. To approach the latter question in a rigorous way, we used the methodology described above with DNA extracted from paraffin-embedded tumor tissue of patients entered on EST2284. It is necessary to use dot blot analysis rather than Southern blots, because DNA from paraffin-embedded tissue is not of high molecular weight. However, the data in Fig. 1B and data previously published (8) establish that the same results are obtained by both dot blot and Southern blot analyses. The samples are from a subset of patients entered by the ECOG into a randomized multi-institutional intergroup trial of adjuvant chemotherapy for colon cancer, with ECOG, the North Central Cancer Treatment Group, and the Southwest Oncology Group participating. This trial confirmed the survival benefit for Duke's B2 and C patients receiving levamisole plus 5FU compared with those treated with surgery alone or levamisole alone (17).

Fig. 2 shows a typical experiment in which DNA samples from EST2284 were analyzed. The normal range of c-myc/globin in 20 placentals samples is illustrated by the shaded area. Of the 20 DNA samples from patient tissue analyzed on this blot, each sample analyzed in triplicate, four samples (88-1, 89a, 92b, and 103b) were clearly elevated from 2- to 4-fold, comparable to the levels determined for the colonic carcinoma cell lines. Four others (i.e., 96g, 103a, 104a, and 104b) were elevated at lower levels, but above the range determined by analysis of the 20 placentals samples.

The same analyses were performed, blinded from the clinical data, on DNA isolated from 459 paraffin-embedded biopsy specimens from EST2284 representing 194 patients. This sample represents a subset of patients entered into the larger Phase III intergroup trial; specifically, only ECOG patients and those whose tissue was available for analysis. Amounts of DNA adequate to yield significant hybridization for analysis was obtained from 386 (84%) of these samples. Of these 386 samples, 276 were tumor tissue, and they represented tissue from 149 patients. Seventy-three of these 149 patients have progressed, and a total of 65 of the 149 patients have died. Fig. 3, a and b, shows time to progression and overall survival by treatment, respectively. The treatment effect on time to progression (\( P = 0.07 \)) and a corresponding trend with respect to overall survival are comparable in this unselected subset to what has been reported for the entire data set derived from this Phase III study (17), although the \( P \) values reported here are larger due to the reduced sample size.

To explore the effects of c-myc amplification on time to progression, we divided the patients into two groups defined by no amplification (<1.5) or amplification (≥1.5). This categorized 32% of the patients as exhibiting elevated copy number. Kaplan-Meier survival curves were constructed for overall survival and time to relapse for the two groups. These curves suggest that there was no overall association between c-myc copy number and either time to relapse or survival (Fig. 3, c and d).

To address the possibility that c-myc has an interactive effect with treatment, we repeated these analyses by treatment groups. Among those patients without detectable amplification of c-myc in their tumors, there is no difference in either time to progression or survival for any treatment group (Fig. 3, e and f). However, among the 32% of...
patients with ≥1.5-fold amplification of c-myc in their tumors, there was a better outcome in the levamisole plus 5FU group (n = 12) compared to surgery alone (n = 25) or surgery plus treatment with levamisole (n = 11) in terms of time to progression (P < 0.05; Fig. 3g) and a corresponding trend toward longer survival (P < 0.09; Fig. 3h). The improved outcome with 5FU plus levamisole treatment in the c-myc-amplified group is pronounced, comparable to the improved outcome seen in the larger intergroup trial (Ref. 17; see “Discussion”).

**Relationship between c-myc Amplification and p53 Mutation.** Deletion and mutation of p53 alleles on 17p is a frequent event in colonic tumors (18–20). p53 may function as a checkpoint control to monitor whether the genome has been correctly replicated before permitting cell cycle progression (21–23). Thus, one hypothesis is that increase in gene amplification would be more frequent in tumors in which p53 is inactivated by mutation (23, 24). We therefore determined the frequency of mutations in exons 5, 6, 7, and 8 of the p53 gene, the location of the hotspots for mutations in human colonic tumors (20), in 11 tumor samples from patients on EST2284 which had levels of c-myc amplification >3.5, and in 15 samples in which no increase was detected. p53 mutations were assayed by a combination of SSCP and sequence analysis. Fig. 4A shows the SSCP analysis of exon 5 of seven tumor DNA samples and a sample of placental DNA. Two of the tumor DNAs (97 and 164) produce abnormally migrating bands characteristic of p53 mutations. All such abnormally migrating bands were excised and sequenced as described in “Materials and Methods.” Fig. 4B is an example of the portion of the sequence from the placental DNA sample and the abnormal band in sample 97. The arrows illustrate a G residue in the wild-type sequence has been altered to a T, thus confirming the presence of a mutation. Table 1 summarizes the data from these analyses and illustrates that there is no difference in the frequency of p53 mutations in relation to c-myc amplification.

**DISCUSSION**

Although amplification of the c-myc gene was linked to its overexpression in a chemically induced rodent colon tumor (3), frequent overexpression of the gene in human colonic tumors was reported in the absence of amplification (4). In this study, we have established, by objective quantitative analysis, that increased c-myc gene copy number is in fact a common event in human colonic carcinoma cell lines and primary colonic carcinomas. It is important to note that in both
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primary colon tumor but who have at least one regional lymph node with metastatic disease (26). However, the need for prognostic markers is acute in this setting to spare those patients for whom it is unnecessary from the toxicity of adjuvant therapy and to target the most aggressive therapy to those patients who have the highest likelihood of recurrence and progression. We therefore investigated the prognostic value of c-myc amplification in a Phase III study of 5FU-based adjuvant therapy using tissue from patients accrued by the ECOG. The data demonstrate that those patients whose primary tumors exhibited c-myc values of >1.5 relative to placenta had a significantly longer disease-free survival and a trend to longer overall survival, in response to 5FU plus levamisole, than did those patients with no amplification in the resected tumor. Thus, a subset of approximately one-third of the patients was identified in this Phase III trial as having the highest probability of improved outcome with adjuvant therapy. The data show no overall relationship between presence of c-myc amplification and time to progression or survival (Fig. 3, c and d). However, when the complication of the treatment effect (Fig. 3, g and h) was eliminated by considering the clinical data only for the observation arm in relationship to c-myc as a continuous variable, there was a trend to poorer overall outcome with increasing c-myc copy number, although this was not statistically significant (data not shown).

The categorization of patients with amplified c-myc in the resected tumor based on a c-myc level ≥1.5 compared to placenta was done blinded from the clinical data. This cutoff is not arbitrary: first, it represents an increase of one gene copy, and determination of loss of an allele by quantitative analyses such as that used here have been reported frequently in the literature (e.g., Refs. 27 and 28); second, the methodology was clearly established to be sufficiently rigorous to identify changes in gene copy number in the HT29 cell line of a similar magnitude, which has also been reported by another laboratory (16).

There are three additional important considerations in interpreting the clinical results that help to place the correlation of improved outcome with c-myc amplification in the proper context. First, statistical significance for increased disease-free survival in response to 5FU plus levamisole adjuvant therapy for colon cancer has been shown to be effective in improving overall survival (17, 25) and is now recommended for the approximately 50,000 Dukes’ C patients (stage III) in the United States who have had surgical removal of the primary colon tumor but who have at least one regional lymph node with metastatic disease (26). However, the need for prognostic markers is acute in this setting to spare those patients for whom it is unnecessary from the toxicity of adjuvant therapy and to target the most aggressive therapy to those patients who have the highest likelihood of recurrence and progression. We therefore investigated the prognostic value of c-myc amplification in a Phase III study of 5FU-based adjuvant therapy using tissue from patients accrued by the ECOG. The data demonstrate that those patients whose primary tumors exhibited c-myc values of >1.5 relative to placenta had a significantly longer disease-free survival and a trend to longer overall survival, in response to 5FU plus levamisole, than did those patients with no amplification in the resected tumor. Thus, a subset of approximately one-third of the patients was identified in this Phase III trial as having the highest probability of improved outcome with adjuvant therapy. The data show no overall relationship between presence of c-myc amplification and time to progression or survival (Fig. 3, c and d). However, when the complication of the treatment effect (Fig. 3, g and h) was eliminated by considering the clinical data only for the observation arm in relationship to c-myc as a continuous variable, there was a trend to poorer overall outcome with increasing c-myc copy number, although this was not statistically significant (data not shown).

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**Table 1. Presence of p53 mutations in colon tumors that do and do not have c-myc amplification**

<table>
<thead>
<tr>
<th>Exon 5</th>
<th>Exon 6</th>
<th>Exon 7</th>
<th>Exon 8</th>
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<tr>
<td>myc amplification &gt;3.5</td>
<td></td>
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<tr>
<td>24</td>
<td>mut +</td>
<td></td>
<td></td>
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<tr>
<td>91K</td>
<td>mut +</td>
<td></td>
<td></td>
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<tr>
<td>97-8</td>
<td>mut +</td>
<td></td>
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</tr>
<tr>
<td>105A</td>
<td>mut +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>129B</td>
<td>mut +</td>
<td></td>
<td></td>
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<tr>
<td>150-2</td>
<td>mut +</td>
<td></td>
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</tr>
<tr>
<td>164-2</td>
<td>mut +</td>
<td></td>
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<tr>
<td>165-2D</td>
<td>mut +</td>
<td></td>
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<tr>
<td>443</td>
<td>mut +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>454-2B</td>
<td>mut +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>491</td>
<td>mut +</td>
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| No myc amplification | | | |
|----------------------|--------|--------|
| 106C                 | mut +  | mut +  |
| 106C                 | mut +  | mut +  |
| 172A2                | mut +  | mut +  |
| 231                  | mut +  | mut +  |
| 356                  | mut +  | mut +  |
| 364                  | mut +  | mut +  |
| 457-5                | mut +  | mut +  |
| 460-1                | mut +  | mut +  |
| 462                  | mut +  | mut +  |
| 463-4                | mut +  | mut +  |
| 464-6                | mut +  | mut +  |
| 519                  | mut +  | mut +  |
| 520-1                | mut +  | mut +  |
| 521-B1               | mut +  | mut +  |
| 534I                 | mut +  | mut +  |

Fig. 4. p53 mutations in tumor tissue. DNA preparations from a normal placenta (Pla1) and tumor tissues were analyzed for mutations in exon 5 (shown) and in exons 6, 7, and 8 (not shown) by SSCP (A). The abnormally migrating band (box) in tumor sample 97 was excised and sequenced to confirm the presence of a mutation in the p53 gene relative to the wild-type sequence in placental tissue (B, arrows). A similar sequence analysis confirmed the presence of a mutation from G to T in the aberrant band for sample 164 (A, box). The residual G residue present in the tumor sample is most likely due to heterogeneity of cells in the tumor from which the DNA was isolated.
5FU plus levamisole was obtained specifically for the group exhibiting c-myc amplification (P < 0.05), and a trend that approached significance (P < 0.09) was seen for overall survival. The magnitude of the improved outcome with 5FU-based treatment in the patients with c-myc amplification is quite large, comparable to the differences observed in the original intergroup trial, but the P values are larger only because of reduced sample size, reflecting the availability of tissue. In this regard, Fig. 3, a and b, presents analyses of our patient subset without regard to c-myc status, as in the intergroup trial. The same improved outcome was seen for the 5FU plus levamisole-treated patients as in the original trial, but, reflecting the smaller number of patients, the statistical significance was at a lower level, almost identical to the significance levels we obtained for improved treatment outcome in the c-myc group. Second, the study population was from a controlled clinical trial in a carefully defined group of patients who received standardized treatment and evaluation. Third, measurements of c-myc were done blindly with respect to individual patient characteristics, treatment, or outcome, eliminating the possibility of investigator bias. Hence, systematic error is avoided. Thus, we believe it likely that analyses in additional clinical trials will show a stronger association of c-myc amplification with treatment outcome. Larger numbers of patients will be necessary to determine the cutoff for relative c-myc copy number which is most accurate in discriminating among patients with different clinical outcomes and may permit analysis stratified by stage (e.g., B2 versus C), which may be an important variable. Further studies, especially prospective studies, are therefore critical to determine the utility of evaluation of c-myc status in clinical management of surgically resected colon cancer patients.

The reason that c-myc amplification may predispose patients to better treatment response is unknown. An intriguing hypothesis is raised by the report that a complex exists between TS and c-myc mRNA (29). This may influence rates of c-myc mRNA translation, but it could also lower cellular levels of TS. TS is a critical target enzyme of 5FU, and tumors with the lowest TS mRNA expression had increased sensitivity to 5FU (30). This, however, may be complicated by the fact that TS translation is autoregulated by binding of TS protein to TS mRNA (31), and the final levels of TS may be a function of relative binding to TS and c-myc mRNA, which could also be influenced by the interaction of TS with 5-fluoro-dUMP, a metabolite of 5FU.

The mechanisms that bring about the low-level amplification are unknown, but they apparently do not involve mutations in the p53 gene, because there was no difference in the incidence of p53 mutations in tumors that do and do not exhibit low-level amplification. In addition, p53 mutation is generally a late event in colon tumorigenesis, and in the flat mucosa of one patient, we have found low-level amplification specific to an area of atypia, a very early lesion (data not shown). This is consistent with the findings of others that overexpression of c-myc in human colon tumors may be an early event in disease development, linked to mutations in the APC gene (32, 33), which are present in almost all colonic tumors, including the very earliest benign adenomas (34).

Finally, although the c-myc gene is of obvious interest in terms of tumor initiation and progression, we have suggested that low-level amplification of many loci is likely to be common in colonic tumors (8). Since our initial report on low-level c-myc amplification, a number of investigations have made it clear that sensitive methodology reveals a large number of subtly amplified loci in a variety of tumor types that were not detectable by conventional hybridization analysis (35–41). Thus, frequent and heterogeneous low-level amplification of a large number of loci may characterize colon tumors, as do widespread deletions throughout the genome (42), and together, such extensive structural alterations may in part underlie the large number of alterations in gene expression seen in colon tumors (43–47). Although well-studied loci, such as APC, MCC, p53, DCC, and c-myc, may be the sine qua non of colonic cancer, the great heterogeneity in alterations of gene structure and function in colon tumors may require more complex multiparametric analysis of larger numbers of interacting loci to reveal patterns of alterations that are predictive of outcome in this clinically heterogeneous disease (43, 45–47).

REFERENCES


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