Noncorrelation of the Expression of the c-myc Oncogene in Colorectal Carcinoma with Recurrence of Disease or Patient Survival

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ABSTRACT

Our previous work has shown that 26 of 38 cases (68.4%) of primary adenocarcinoma of the colon exhibited significantly elevated levels of c-myc RNA compared to normal colonic mucosa (M. D. Erisman, P. G. Rothberg, R. E. Diehl, C. C. Morse, J. M. Spandorfer, and S. M. Astrin. Mol. Cell. Biol., 5: 1969–1976, 1985; P. G. Rothberg, J. M. Spandorfer, M. D. Erisman, R. N. Staroscik, H. F. Sears, R. O. Petersen, and S. M. Astrin. Br. J. Cancer, 52: 629–632, 1985). In this study, we have compared those levels of expression to the clinical profiles of the affected individuals in an effort to define useful correlates, especially with regard to recurrence of disease and patient survival. Log-rank comparisons of recurrence curves for the entire patient population, for those patients with low levels of c-myc RNA in resected tumor tissue, and for those patients with significantly elevated levels of RNA show no statistically significant differences. Similarly, no statistically significant difference was observed between the high- and low-myc RNA groups with respect to their survival during the postoperative period of observation (median, 25 months). Consequently, the levels of c-myc gene expression observed in primary tumor tissue did not help to define those individuals at higher or lower risk for recurrence of disease and did not point to the likelihood of increased or decreased survival in individuals undergoing surgery for adenocarcinoma of the colon.

INTRODUCTION

The c-myc protooncogene is the cellular homologue of the viral transforming sequence in MC29 virus, which causes the acute development of a variety of cancers in inoculated birds. The human, murine, and avian c-myc loci have been cloned and sequenced, and expression of the RNA and protein products has been studied in some detail (1, 2). The gene codes for an approximately Mr 64,000 phosphoprotein localized in the nucleus of infected cells, which has been suggested to be involved in RNA processing (3), DNA synthesis (4), and the regulation of transcription (5). The genetically engineered expression of the c-myc gene in transgenic mice has been shown to result in the heritable predisposition to abnormal patterns of differentiation and frank neoplastic disease in selected tissues (6–9); in cell cultures, constitutively elevated expression of the gene has been shown to alter both the growth factor responsiveness of cells (10) and their tumorigenic potential (11).

The oncogenic capability of the cellular gene was first recognized in studies of avian leukemia virus-induced bursal lymphomas in chickens (12). In those tumors, integration of a defective provirus adjacent to the c-myc locus enhances c-myc gene expression and is an initiating cause of disease. Involvement of the c-myc gene in the development of murine plasmacytomas and human Burkitt's lymphomas has also been well established (13, 14). Both diseases involve reciprocal translocation of the gene with immunoglobulin sequences which alter normal regulation of its expression. Elevated expression of the c-myc locus due to amplification of the gene has been observed in a number of primary lung (15) and breast (16) cancers in which it may contribute to tumor progression. A significant number of primary acute leukemias (17) and colon carcinomas (18) have also been shown to express the gene at elevated levels in the absence of gross genetic change at the locus. These cases represent a class of tumors that are deregulated through mechanisms which remain to be elucidated.

In addition to recent efforts to define the etiological role of protooncogene expression in human cancer, there has been a growing effort to exploit what is presently known for the purpose of diagnosis, staging, and prediction of outcome in the clinical setting. The prospective use of protooncogene RNA expression patterns to refine the French-American-British categories of acute myelocytic leukemia into subtypes with improved prognostic significance has been proposed (19). The development of enzyme-linked immunosorbent assay (20) and radioimmunoassay (21) methods will allow for similar kinds of diagnostic evaluations at the protein level. Monoclonal antibodies directed against the c-myc protein have already been used in the retrospective, immunohistochemical evaluation of testicular (22), lymphoid (23), cervical (24), and colonic lesions (25) and in the radiolocalization of tumors in individuals with bronchogenic carcinoma (26). DNA probes have been used to show that amplification of the c-myc gene occurs more frequently in lung cancer patients treated with combination chemotherapy (15); we have presented evidence to suggest that expression of the c-myc gene may serve as one marker for the genetically distinct class of colon tumors defined by the inheritable predisposition known as familial polyposis coli (27).

In this report, we have examined the recurrence of disease and survival in our colon adenocarcinoma study group in order to determine whether or not elevated expression of the c-myc gene in primary tumor tissue is a prognostic indicator of clinical outcome.

MATERIALS AND METHODS

Sample Collection. Operations were performed on 38 individuals with adenocarcinoma of the colon at the American Oncologic Hospital of the Fox Chase Cancer Center and Jeanes Hospital in Philadelphia between June 1983 and October 1984. Colon tumor tissue and normal colonic mucosa (near the margins of the resection) were removed by the attending pathologist, frozen in liquid nitrogen, and stored at −70°C until analyzed.

Oncogene Analysis. Total cellular RNA and DNA were recovered concomitantly from each tumor/normal pair by centrifugation of guanidinium thiocyanate homogenates over a cesium chloride cushion as described in detail previously (17, 18). The level of c-myc gene expression in each tumor relative to its normal control was judged by evaluation of dot blots of total cellular RNA and Northern blots of polyadenylate-enriched mRNA using a probe for exon 3 of the human c-myc locus as described previously (18). Levels of RNA expression in tumor tissues relative to normal colonic mucosa are presented using the following grading system: −, not elevated; +, ±2-fold elevated; ++,
about 5-fold elevated; ++, about 10-fold elevated; and +++, >10-fold elevated (20-40-fold).

Statistical Methods. Recurrence and survival curves for this population were obtained from right-censored data using the product limit estimator (28, 29). Survival and recurrence data for various groups of patients defined by the level of c-myc gene expression in primary tumor tissue were compared for equality of survival or recurrence using the log rank test (28, 29). The impact of other clinical parameters (covariates) on the survival and recurrence of this population was assessed for equality of survival or recurrence using the Mann-Whitney test (31).

RESULTS

Clinical Profiles of Colon Adenocarcinoma Patients in the Study. As shown in Table 1, the sex, age, preoperative CEA levels, c-myc RNA levels, site of resection, Duke’s stage of disease (Astler-Coller modification), size of the primary lesion (intraluminal), number of positive nodes per number of nodes examined, degree of differentiation of the tumor, clinical status (months of follow-up), and recurrence of disease (months postresection) were cumulated for the purpose of this study. The first surgery in this group of 38 individuals occurred in June 1983 and the last in October 1984. Follow-up times range from 0 to 40 months, with a median follow-up time of 25 months. The clinical status of all 38 patients entered into the study was monitored successfully during the follow-up period.

Among the group, there were 22 males (ages 55 to 86 years, median, 68 years) and 16 females (ages 53 to 88 years, median, 71 years). Preoperative CEA levels ranged from 0.1 to 5690.0 ng/ml, with a median value of 2.5 ng/ml. The site distribution of tumors included 8 rectal, 5 rectosigmoid, 12 sigmoid, 1 transverse colon; 6 splenic flexure; S, sigmoid colon; RS, rectosigmoid colon; R, rectum.

The data presented for the patient population examined in this study represent primary clinical information from which all statistical comparisons of survival and recurrence of disease were generated for patient subgroups characterized by their differences in expression of the c-myc gene in primary tumor tissue.

Table 1 Clinical profiles of the patients entered into the study

| Case | Sex | Age (yr) | CEAa | c-mycb | Site | Stagec | Size | Nodes | Differentiationd | Status | Recure
|------|-----|---------|------|--------|------|--------|------|-------|-----------------|--------|--------
| 1    | F   | 64      | 3.7  | ++     | R    | A      | NA   | 0/0   | W               | NED    | 32
| 2    | M   | 55      | 6.9  | ++     | R    | B1     | 43.2 | 0/6   | W               | NED    | 33
| 3    | M   | 69      | 17.0 | ++     | C    | D (LVR) | 243  | 0/12  | M               | EXP    | 37
| 4    | M   | 63      | 2.5  | R      | B1   | 8.0    | 0/0  | W     | NED             | 37
| 5    | M   | 62      | 2.5  | -      | AC   | C2     | 112.5| 4/14  | M               | NED    | 40
| 6    | M   | 77      | 2.2  | -      | R    | B2     | 1.3  | 0/5   | M               | AWD 9  |
| 7    | F   | 79      | 2.5  | +++    | SF   | D (OM) | 144.0| 0/20  | M               | NED    | 36
| 8    | M   | 69      | 2.5  | +++    | S    | B2     | 30.0 | 0/4   | W               | NED    | 36
| 9    | F   | 66      | 2.5  | +++    | S    | B1     | 5.3  | 0/0   | M               | NED    | 34
| 10   | F   | 70      | NA   | +++    | T    | D (LVR) | 7.5  | 7/10  | M               | EXP    | 2
| 11   | M   | 68      | 1.7  | ++     | S    | B1     | 12.0 | 0/12  | M               | NED    | 31
| 12   | F   | 53      | 0.8  | +      | C    | B1     | 29.3 | 0/9   | M               | NED    | 25
| 13   | F   | 80      | 0.3  | +      | HF   | D (LVR) | 16.0 | 1/27  | M               | EXP    | 13
| 14   | F   | 95      | 2.8  | +      | HF   | B1     | 28.9 | 0/2   | M               | NED    | 26
| 15   | F   | 79      | 3.9  | ++     | R    | B2     | 24.0 | 0/3   | M               | AWD    | 37
| 16   | M   | 61      | 56.5 | S      | B2   | 196.0 | 0/5  | M     | NED             | 25
| 17   | M   | 68      | 2.5  | ++     | AC   | C1     | 7.9  | 3/12  | M               | NED    | 25
| 18   | F   | 71      | 217.8| +      | C    | C1     | 34.1 | 1/6   | M               | EXP    | 5
| 19   | M   | 59      | 5690.0| ++++   | D    | (LVR) | 98.0 | 7/11  | W               | EXP    | 9
| 20   | F   | 77      | 2.5  | +      | RS   | D (LVR) | 35.0 | 1/1   | M               | EXP    | 10
| 21   | M   | 65      | 2.5  | ++++   | R    | B2     | 31.5 | 0/5   | M               | NED    | 32
| 22   | M   | 74      | 287.0| ++     | S    | B2     | 3.8  | 0/0   | M/P             | NED    | 23
| 23   | M   | 70      | 5.5  | ++++   | S    | C2     | 24.5 | 9/10  | M               | EXP    | 7
| 24   | M   | 78      | 4.8  | ++++   | RS   | B2     | 96.0 | 0/0   | M               | NED    | 22
| 25   | M   | 86      | 6.6  | +      | S    | B1     | 20.0 | 0/1   | M               | EXP    | 12
| 26   | M   | 58      | 2.5  | +++    | S    | B1     | 1.2  | 0/1   | M               | AWD    | 35
| 27   | M   | 64      | 2.5  | +      | S    | B1     | 112.0| 0/4   | M               | AWD    | 34
| 28   | M   | 81      | 2.5  | +      | C    | D (LVR) | 9.0  | 3/8   | M               | EXP    | 3
| 29   | F   | 65      | 1.5  | +      | R    | C2     | 14.0 | 10/10 | W/M             | EXP    | 12
| 30   | M   | 78      | 2.5  | +++    | C    | B1     | 2.7  | 0/7   | M               | NED    | 35
| 31   | F   | 68      | 14.1 | ++++   | RS   | D (LVR) | 48.8 | 0/20  | M               | EXP    | 19
| 32   | M   | 58      | 1.6  | ++++   | R    | B2     | 36.0 | 0/4   | M               | EXP    | 23
| 33   | M   | 80      | 2.9  | ++++   | RS   | B2     | 28.0 | 0/6   | M               | EXP    | 0
| 34   | M   | 59      | 9410.0| +     | S    | D (LVR) | 22.5 | 6/5   | M               | EXP    | 2
| 35   | F   | 71      | 2.5  | -      | AC   | C2     | 8.0  | 1/4   | M/P             | EXP    | 16
| 36   | F   | 88      | 12.2 | +      | HF   | B1     | 176.8| 0/11  | M/P              | NED    | 24
| 37   | M   | 84      | 29.6 | +      | S    | B2     | 5.3  | 0/4   | P               | NED    | 32
| 38   | F   | 69      | 2.5  | +      | RS   | B1     | 3.6  | 0/3   | M               | EXP    | 25

a Preoperative CEA values are reported in ng/ml. NA, not available.
b c-myc mRNA levels in primary tumor tissue are reported relative to levels in normal colonic mucosa from the same patient as: -, no increase; +, 2-fold increase; ++, about 5-fold increase; +++, about 10-fold increase; +++++, >10-fold increase.
c Stage is reported using the Dukes’ classification (Astler-Coller modification) with site of perioperative metastatic disease indicated for D-stage cases (LVR, liver; OM, omentum).
d Tumor volumes reported in cm³ are the product of length, width, and depth measurements taken during postoperative gross pathological examination.

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c-myc IN COLON CARCINOMA: RELATIONSHIP TO RECURRENCE AND SURVIVAL

Stratification of cases via the Dukes' classification revealed the following distribution of stage of lesion: 1 (2.6%) A; 8 (21.1%) B1; 14 (36.8%) B2; 1 (2.6%) C1; 5 (13.2%) C2; and 9 (23.7%) D. These data reflect a disproportionately small number of C1 stage tumors and slightly higher numbers of B2 and B1 stage cases than anticipated. The significance of this unexpected staging pattern is unclear. However, it does not represent a conscious bias in the case selection process, since tissue was collected for evaluation with no prior knowledge of clinical profile.

The tumor volumes ranged from 1.2 to 243 cm³ (median, 24.5 cm³), and histopathological grading of tumors revealed the following distribution of cases: 4 (10.5%) well differentiated; 1 (2.6%) well-to-moderately differentiated; 28 (73.7%) moderately differentiated; 4 (10.5%) moderately-to-poorly differentiated; and 1 (2.6%) poorly differentiated. Six recurrences (16%) were documented among the 38 individuals examined. The survival data revealed 16 individuals (42.1%) who expired, 4 (10.5%) individuals alive with disease, and 18 (47.4%) individuals alive with no evidence of disease in the postoperative period of observation (range, 0 to 40 months; median, 25 months).

Expression of the c-myc Gene in Primary Colon Tumors. Expression of the c-myc gene in each tumor was judged relative to normal colonic mucosa from the same patient (18, 27). The levels of c-myc gene mRNA in these tumors in the cases studied have been determined to be: not elevated in 4 (—; 10.5%); ≤2-fold elevated in 8 (+; 21.1%); about 5-fold elevated in 15 (++; 39.5%); about 10-fold elevated in 7 (+++; 18.4%); and >10-fold elevated in 4 (++++; 10.5%). Twenty-six of the 38 tumors (68.4%) exhibited elevated levels of c-myc transcripts which were at least 5-fold greater than normal tissue. RNA levels ranged up to a 40-fold increase in the most extreme case examined. Twelve (31.6%) of the cases exhibited no increase in expression or had expression levels ≤2-fold elevated, which we regard (within error) as not being significantly different from the 38 samples of normal mucosa examined.

As reported previously (27), we have found no statistically significant correlation of c-myc expression in these tumors with the sex, age, preoperative CEA level, Duke's stage of disease, or degree of differentiation; however, there is a meaningful correlation with anatomical site of the lesion among the 26 tumors with moderately to significantly elevated levels of expression (++ to ++++; ≥5-fold increase). Twenty-two of these elevated cases were resected from the left colon (85%) and only 4 (15%) from the right side. The distribution of such myc-positive cases is very similar to the site distribution of primary lesions observed in genetically predisposed individuals diagnosed as having familial polyposis coli. Conversely, the distribution of myc-negative cases (5 left, 42%; 7 right, 58%) is very similar to the distribution pattern of primary colon lesions observed in familial non-polyposis coli patients. We have suggested that the differential expression of the c-myc gene may serve as one marker for the sporadic occurrence of these two genetically distinct forms of colorectal cancer on that basis (27).

We further suggest that the genetic predisposition observed in familial polyposis coli may involve a gene necessary for proper regulation of the c-myc locus.

Relationship between c-myc Gene Expression and Recurrence of Disease and Survival. Curves for patient survival and time to tumor recurrence were estimated using standard methods (Kaplan-Meier product limit). These curves were determined for all patients as a single population, and also for patients grouped according to levels of c-myc transcript found in the primary lesion. Survival and recurrence curves for the entire patient population, for those patients with less than a 2-fold increase in expression (−, +; non-elevated group), and for those cases with at least a 5-fold increase in expression (+++, ++++; elevated group) are shown in Fig. 1. The results of log-rank comparison of survival and recurrence curves for the three patient groups described above under the condition that patients without observed recurrence at the time of death were regarded as nonrecurrent (death ≠ recurrence) and time to recurrence curves for the three patient groups described above under the condition that patients who died were regarded as recurrent at the time of death (death = recurrence). The results of statistical comparisons of survival and recurrence using log-rank tests consistently failed to show any significant differences among the groups analyzed. As shown in Table 2, comparison of survival curves (Fig. 1A) for the non-elevated and elevated expression groups showed no significant
c-myc in Colon Carcinoma: Relationship to Recurrence and Survival

Survival and time to recurrence curves generated for patient subgroups characterized by the level of c-myc gene expression in primary tumor tissue were compared using log-rank tests as described in the text. The hypothesis tested in each case was that the survival or recurrence distributions being compared were identical. For both survival and recurrence comparisons, the groups of patients compared are shown along with the results of each comparison. In the case of recurrence, comparisons were done under two sets of conditions as defined in the footnotes.

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### Footnotes

* c-myc RNA levels are reported using the −, + grading system defined in Table 1 and in the text. The number of patients in each of the groups compared is indicated in brackets. All group survival and recurrence comparisons are simultaneous comparisons of the five individual classes of patients observed: −[4] +[8], ++[15], +++[7], and ++++[4].

### DISCUSSION

Our previous work has shown that a majority (about 70%) of primary adenocarcinomas of the colon exhibit significantly elevated levels of c-myc gene transcripts (≥5-fold elevated compared to normal mucosa) without rearrangement or amplification of the gene (18). These data have now been confirmed by other groups (32, 33) and are further supported by a detailed study of both protein and RNA levels in colon carcinoma cell lines that we have recently completed (34). In the context of well established etiological relationships between abnormal c-myc gene expression and several neoplastic diseases (1, 2), it is not unreasonable to suspect that altered expression of the gene may also contribute to the initiation or progression of cancer in the colon. We have sought correlates of expression of the gene in tumors with patient medical profiles which would be of value in the clinical setting (27).

In this paper, we summarize our findings regarding the relationship between levels of expression of the gene in primary level group. These smaller lesions consistently outnumber the larger lesions, and the latter occur with about equal frequency in each of the four groups which exhibit them. The tumor volumes in groups defined by the level of c-myc expression were compared using the Mann-Whitney test. Volumes in the ++++ group were found to be significantly greater than those in the + group (P = 0.048; two-tailed test). However, there are only four tumors in the ++++ group, and the volume of just one of these lesions was particularly large. Consequently, this result is probably spurious. When the 8 large tumors in this sample of 38 cases were removed from consideration and survival among the various c-myc expression-level groups was compared again, no significant results were obtained for the low volume subset (data not shown). The high volume subset was too small for meaningful analysis.

Consequently, in the group of 38 colon cases evaluated over the 2-year (median) period of postoperative follow-up, the levels of c-myc gene expression determined did not define individuals at higher or lower risk for recurrence of disease and did not point to the likelihood of increased or decreased survival in a statistically significant way. These data suggest that measurement of c-myc gene expression levels in primary colon tumors is of no apparent prognostic significance in evaluating recurrence and survival after surgery for adenocarcinoma of the colon.
tumors and recurrence of disease and survival in the group of 38 cases which we have studied. These data indicate that the survival of individuals with elevated levels of c-myc gene expression in primary tumor tissue is not significantly different from that of individuals whose tumors did not express the gene at abnormally high levels. Absence of any statistically significant correlation of expression with the recurrence of disease was also established; the two findings taken together strongly suggest that no obvious clinical insight or prognostic value regarding patient outcome can be derived from measurement of c-myc RNA levels in primary tumors of the colon. A retrospective, immunohistochemical study of p64 c-myc expression in 42 colon cases reported recently (25) suggested that no correlation of expression with depth of invasion or 5-year survival was observed, which is in agreement with the results we present here.

The absence of potentially valuable correlation of c-myc gene expression in primary tumors with clinical profiles is perhaps disappointing, but not unusual, in ongoing efforts to establish a role for measurement of oncogene expression in clinical diagnosis. The wide variety of diseases known generically as cancer are diverse in etiology and phenotype and will undoubtedly continue to confound attempts to categorize them clinically using new molecular markers. Amplification of the c-myc gene in small cell carcinoma of the lung occurs more frequently in patients treated with combination chemotherapy whose cancers recur and is associated with more limited survival (15); while amplification of the gene in breast cancers is associated with patients older than 50 years of age, it is not correlated with estrogen and progesterone receptor status, stage of disease, or axillary lymph node involvement [survival was not reported (16)]. Quantitation of p64 c-myc levels in cervical carcinomas has revealed no correlation of protein level with grade or stage of disease, age of the patient, or prognosis (24); similar studies in human seminomas have indicated increased levels of staining for c-myc protein in tumor tissue, but no correlation of those increased levels with stage of disease or preoperative serum human chorionic gonadotropin levels (22). Taken collectively, these studies point out the absence of obvious relationships between c-myc gene expression and clinical parameters of diagnostic significance in a variety of tumor types and suggest that the exploitation of c-myc gene expression as a unique molecular marker may not be possible.

Similar attempts to correlate clinical information with changes in the structure and expression of the c-ras oncogene in human colon carcinoma have also been reported (35, 36). Tumor-specific point mutations in codon 12 of the Ki-ras gene occur with high frequency in primary adenocarcinomas. These mutations also occur in adenomas and may be regarded as a premalignant change, but the frequency of their appearance in primary tumors is not correlated with the age, sex, race, site of the lesion, degree of differentiation, or Dukes' stage of the tumor (36). Several other studies (37–42) involving the immunohistochemical evaluation of p21 levels in normal and neoplastic colon tissues express considerable disagreement in their conclusions and raise additional questions regarding the utility of p21 measurements in the clinical setting.

The aberrant expression of protooncogenes in colon carcinoma may not provide correlations with recurrence of disease and survival for reasons related to the distinction between tumor initiation and progression. In many colon carcinomas, deregulation of c-myc gene expression undoubtedly constitutes just one of a series of premalignant genetic changes, perhaps including point mutation of a ras locus, which are necessary for the development of a fully transformed phenotype. While these early changes may be sufficient for initiation of disease and may be required for maintenance of the neoplastic state, recurrence of disease and survival are probably linked more directly to other phenotypic changes that occur during tumor progression which affect the aggressive, invasive, and metastatic properties of the lesion. Consequently, clinical diagnostic measurement of oncogene expression in primary tumor tissue may tell us more about the etiology of individual cases than the prognosis for their successful resolution.

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