Overexpression of the c-myc Proto-oncogene in Colorectal Carcinoma Is Associated with a Reduced Mortality That Is Abrogated by Point Mutation of the p53 Tumor Suppressor Gene

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ABSTRACT

The survival of 119 colorectal cancer patients was analyzed in the light of the overexpression status of the c-myc proto-oncogene mRNA and the point mutation status of the p53 tumor suppressor gene in the primary adenocarcinoma. The presence of >3 fold overexpression of c-myc mRNA in the primary tumor was found to be associated with a better prognosis than patients who evinced no overexpression (P = 0.02, log rank analysis). Point mutation of the p53 tumor suppressor gene was found to be associated with a poorer patient prognosis (P = 0.007, log rank analysis). Endogenous levels of c-myc and point mutation of p53 both contributed independently toward a poorer patient prognosis in Cox regression modeling. The better prognosis seen in patients who overexpress c-myc was offset when c-myc overexpression was coupled with a point mutated p53 gene. These results suggest that in colorectal adenocarcinoma c-myc deregulation leads to increased apoptotic death, but that this response may be modulated by a more downstream event such as point mutation of the p53 gene.

INTRODUCTION

The c-myc proto-oncogene is the cellular homologue of the v-myc oncogene of avian myelocytomatosis virus (1) and a member of the myc family of genes (2). In a normal cell, the myc genes are believed to be involved in the regulation of cellular proliferation and differentiation (3, 4). Aberrant or deregulated myc expression has been associated with the pathogenesis of human tumors (3) and more recently the process of apoptosis (5).

In human colorectal adenocarcinomas, overexpression of the c-myc mRNA has been reported to occur in 60–80% of cases, and as yet no correlation has been found between c-myc mRNA overexpression and either patient survival or disease recurrence (6) or metastatic potential (7), although these studies have been based on relatively small cohort sizes. In a previous study of 100 colorectal adenocarcinomas, we noted no association between c-myc overexpression and patient age and sex or tumor stage and location (8).

The p53 tumor suppressor gene encodes a M, 53,000 phosphoprotein (9) that is believed to be able to both promote and repress cell proliferation (10), probably by binding to DNA in a sequence-specific manner and modulating gene expression (11). More recently, p53 has been found to play a role in mediating cell death (12).

Altering the p53 tumor suppressor gene are among the most common changes found in a wide range of neoplasia (13). Very often missense mutations are detected (14), often coupled with the allelic loss of the second allele of p53 (15). Most mutations occur in an approximately 600-nucleotide region of the p53 mRNA, corresponding roughly to exons 5–9 of the p53 gene (13).

In colorectal adenocarcinoma, point mutation of the p53 gene is found in approximately 50% of cases, although this rate is significantly associated with tumor stage and disease progression (16), and point mutation of the p53 gene has been found to be associated with a poorer patient prognosis (17, 18).

In this report, we have examined the relationship between c-myc deregulation and point mutation of the p53 tumor suppressor gene, and, more important, how these two changes influence the biological behavior (as assessed by patient survival) of colorectal adenocarcinomas.

MATERIALS AND METHODS

Patients. Patients and tumor collection were as described previously (8). Patient follow-up was taken as the time between surgery and last departmental contact (scheduled follow-up, telephone contact, or mail response) or patient death. Patients dying of causes unrelated to colorectal cancer were treated as censored events. Mean follow-up was 28 months (95% confidence interval, 25–31 months).

c-myc Overexpression. c-myc overexpression was determined relative to normal matched mucosa of the same patients using Northern blot analysis as described in detail elsewhere, and the cohort includes samples previously described (8). Briefly, total RNA was fractionated through formaldehyde agarose gels, transferred to solid matrix, and hybridized to random primed labeled cDNA probes for c-myc (pG-5′-c-myc; American Type Culture Collection, Rockville, MD) and β-actin (Clontech Laboratories, Palo Alto, CA). After hybridization, filters were exposed to Fuji-RX medical X-ray film. Autoradiographs were quantitated by scanning densitometry. Corrected c-myc signal in the tumor was compared to corrected c-myc signal in the primary donor.
Table I  Clinical status of patients

<table>
<thead>
<tr>
<th>No. of patients</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>67</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>63.34</td>
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<tr>
<td>Range</td>
<td>27–89</td>
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<tr>
<td>SD</td>
<td>13.29</td>
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<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>Dukes' A</td>
<td>31</td>
</tr>
<tr>
<td>Dukes' B</td>
<td>27</td>
</tr>
<tr>
<td>Dukes' C</td>
<td>29</td>
</tr>
<tr>
<td>Dukes' D</td>
<td>32</td>
</tr>
<tr>
<td>Tumor location</td>
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<tr>
<td>Rectal</td>
<td>51</td>
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<tr>
<td>Left colon</td>
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<tr>
<td>Right colon</td>
<td>20</td>
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<tr>
<td>Postoperative treatment</td>
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<td>87</td>
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<td>11</td>
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<tr>
<td>Radiotherapy only</td>
<td>8</td>
</tr>
<tr>
<td>Radiotherapy and chemotherapy</td>
<td>13</td>
</tr>
</tbody>
</table>

The mucosa by comparison to the β-actin control signal to obtain a number representing the level of overexpression in the tumor.

**p53 Point Mutation.** p53 point mutations were detected by single-stranded conformational polymorphisms (19) using the modification of Sakai and Tsuchida (19) as described in detail elsewhere (21, 22). Cohort includes samples described previously (16, 17).

**Statistical Analysis.** All data were analyzed using the SPSS computer program (SPSS Inc., Chicago IL). Kaplan-Meier plots (23) were analyzed using the log rank analysis. Cox regression models were constructed using the Forward-Wald method of entry, and entry into the model was conditional upon $P < 0.05$.

**RESULTS**

One hundred nineteen primary, single colorectal adenocarcinomas were examined for both overexpression of the c-myc mRNA and point mutation of the p53 tumor suppressor gene. A clinical summary of the patients is shown in Table I. Overexpression of the c-myc mRNA was determined using Northern blot analysis, and was defined as the occurrence of mRNA levels in the primary tumor at levels greater than 3-fold than that found in the matched mucosa of the same patient. Point mutation analysis of the p53 gene was undertaken by single-stranded conformational polymorphisms (19) using the modification of Sakai and Tsuchida (20), which allows a single-step screening of the region known to contain 98% of all point mutations of the p53 gene (14).

Overexpression of the c-myc mRNA (Fig. 1) was found in 60% (72/119) of cases, a value comparable to that of our previous study (8). Point mutation of the p53 gene was detected in 57% (68/119) of cases, again a value comparable to that of our previous studies (16, 17, 21, 22). We note no association between c-myc overexpression and the presence of p53 point mutations (Table 2).

Patient survival was analyzed in light of c-myc overexpression status with a Kaplan-Meier plot (Fig. 2). We note that overexpression of the c-myc mRNA is significantly associated with a better prognosis than the absence of overexpression ($P = 0.02$, log rank analysis).

The same patients were then analyzed in light of the presence or absence of point mutations of the p53 gene, again using a Kaplan-Meier plot (Fig. 2). Consistent with the reports by ourselves (17) and others (18), we find a significant association between point mutation of the p53 gene and a poorer patient prognosis ($P = 0.007$, log rank analysis).

We next addressed the question of the interaction between these two variables. This was first addressed using Cox regression modeling. Variables available for inclusion into the model included patient age and sex, tumor location, the presence or absence of greater than 3-fold c-myc overexpression, the numerical level of c-myc overexpression, as well as the presence or absence of p53 point mutations. Entry into the model was via the Forward-Wald method and was conditional upon $P < 0.05$. The final model included p53 point mutation ($P = 0.0072$) and lack of c-myc overexpression ($P = 0.02$, log rank analysis).
of c-myc overexpression \( (P = 0.02) \). Overall significance of the
model: \( \chi^2 = 12.3, P = 0.0021, 2 \) df.

A second analysis was undertaken by stratifying the
Kaplan-Meier plots. Statistical analysis was undertaken both
within the strata and by pooling over the strata (Figs. 3 and 4; Table 3).

A final Kaplan-Meier analysis was undertaken by ordering
the two variables into four groups, namely, myc endogenous
level/p53 point mutation negative, myc endogenous level/p53
point mutation positive, myc overexpressed/p53 point mutation
negative, and myc overexpressed/p53 positive (Fig. 5). Log rank
analysis of this survival plot shows that the four groups do not
have equivalent survival functions \( (P = 0.019) \). As can be seen,
the best prognostic group is the myc-overexpressing/p53 negative
cohort. The beneficial effect of c-myc overexpression is
removed if the tumor also contains a p53 point mutation. This is
seen clearly in Figs. 3 and 5, whereby c-myc-overexpressing
tumors show a large difference between samples with and
without p53 point mutations \( (P = 0.011) \).

DISCUSSION

Over the last few years, our understanding of neoplastic
growth has undergone some revision. Whereas it was previously
believed that neoplastic growth occurred as a result of aberrations
in the control of cell proliferation, it is becoming increas-
ingly apparent that neoplastic growth also results from aberrations
in the cells' own induced or programmed cell death pathways (5, 24). In confirmation of this, it has been noted that
rates of cell proliferation in neoplasia are comparable to those
found in normal surrounding tissues (25).

The c-myc proto-oncogene has been characterized as a
proto-oncogene which promotes cell proliferation (3, 4). How-
ever, in our analysis of 119 colorectal adenocarcinomas, we find
that overexpression of the c-myc mRNA is associated with a
more favorable prognosis. At first this would seem contradic-
tory. However, more recent investigations have shown that
deregulated c-myc expression plays a role in cellular apoptosis
(5). Indeed, transfection of the c-myc gene into rat fibroblasts is
associated with high levels of proliferation and apoptosis, in
contrast to ras transformation which only produces high levels
of cell proliferation (26). In further confirmation of this, some
tumors with deregulated myc expression have been character-
ized as relatively slow growing (27). Hence, it is possible that
the better prognosis in deregulated myc tumors is due to high
rates of cellular apoptosis. Currently, it is believed that dereg-
The p53 tumor suppressor gene is also known to be involved in some apoptotic pathways (12). The involvement of p53 in cell death is believed to be primarily limited to what is termed “induced” apoptosis, in which apoptosis occurs as a result of damage to the cells DNA. More recently, it is becoming apparent that a tumor’s response to antitumor therapies that act through the action of induction of apoptosis, such as chemotherapy and radiotherapy, is dependent on the mutation status of the p53 tumor suppressor gene (17, 28).

Most surprisingly, we have shown here that the improved prognosis that myc deregulation apparently offers is dependent on the p53 status of the tumor. This observation supports the work of Hermeking and Eick (29) who have shown in quiescent mouse fibroblasts that activation of c-myc leads to the induction of apoptosis and cell cycle reentry only in cells with functional p53. In p53 null cells, activation of c-myc induced cell cycle reentry, but not apoptosis. The interaction presented here between c-myc deregulation and p53 point mutation is a novel one for the cellular control of proliferation and apoptosis in colorectal adenocarcinoma and has implications for the progression of the disease.

ACKNOWLEDGMENTS

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REFERENCES

Overexpression of the c-myc proto-oncogene in colorectal carcinoma is associated with a reduced mortality that is abrogated by point mutation of the p53 tumor suppressor gene.

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