Association between High Levels of Ornithine Decarboxylase Activity and Favorable Prognosis in Human Colorectal Carcinoma

Nagahide Matsubara, Oili A. Hietala, Susan K. Gilmour, Keuk Y. Yum, Samuel Litwin, Perry Watts, Edward J. Brennan, and Thomas G. O'Brien

Abstract

Several studies have documented increased expression of ornithine decarboxylase (ODC) in neoplastic colorectal tissue versus normal-appearing colonic mucosa. The present study was undertaken to determine whether there is an association between the degree of overexpression of ODC in colorectal carcinomas and survival in a series of 74 patients. A high level of tumor ODC expression was found to be significantly associated with greater survival in our patient series. Patients with tumor ODC activities greater than the median and especially in the highest quartile experienced a more favorable outcome than those patients with ODC values below the median or in the lowest quartile \((P = 0.03\) and \(0.02\), respectively). The presence of a GTP-activatable isoform of ODC was also significantly associated with a favorable prognosis but only in tumors of the right colon \((P = 0.01)\). There was no association found between ODC activity and tumor grade, tumor size, or patient age, sex, or race.

Our results demonstrate that high levels of ODC expression (and presence of a GTP-activatable isoform for right-sided colon tumors) predict a favorable prognosis in human colorectal carcinoma. Knowledge of a patient’s ODC status at the time of surgery may be useful in decisions regarding adjuvant therapy. Understanding the mechanism(s) involved should lead to new therapeutic approaches for advanced colorectal carcinoma.

Introduction

Expression of the enzyme ODC is frequently elevated in many human neoplasms versus appropriate control tissues. In the large bowel, for example, several groups of investigators have reported elevated ODC activity in colorectal carcinoma compared to uninvolved adjacent mucosa. There are also reports of significant increases in this enzyme activity in premalignant adenomas compared to normal-appearing tissue. As an important regulatory enzyme in the polyamine biosynthetic pathway, increased activity of ODC in premalignant and malignant colonic lesions is thought to reflect the increased proliferative activity ongoing in these tissues. The importance of elevated ODC activity and polyamines in early stages of tumor development has also been suggested from work in experimental rodent models of carcinogenesis.

In all published studies of ODC levels in colorectal tumors, there is a large interindividual variation in the magnitude of the increased enzyme activity in the patient’s tumor. While some of this variation may be due to intrinsic sampling problems, it is possible that differences in tumor ODC levels may reflect different biological properties of the tumors since the variance is typically much smaller for adjacent mucosal ODC activities. Such biological differences could have a significant role in determining long-term patient outcome.

Despite the large body of literature on ODC activity and polyamine levels in human colorectal carcinoma, there have been no studies investigating the relationship between the degree of elevation of ODC activity and subsequent patient outcome. In this study, we report that patients whose primary tumors have high levels of ODC at the time of resection live significantly longer than those patients with low tumor ODC activities. In addition, the presence of a novel isoform of ODC is also predictive of favorable outcome in patients with tumors located in the proximal colon. These results suggest a role for ODC and polyamines in the late stages of colon tumor development, including metastasis, that is distinct from the effects of ODC overexpression early in neoplastic development.

Materials and Methods

Patients. From 1987 to 1990, samples of colorectal carcinoma and adjacent mucosa were obtained from patients undergoing surgical resections as primary therapy for their disease. All except one patient (from the Hospital of the University of Pennsylvania) was from the Lankenau Hospital. The patients were required to have undergone a routine bowel-cleansing procedure preoperatively, and their tumors had to be large enough (generally, >2.5 cm in the smallest dimension) to provide sufficient tissue for biochemical analysis without compromising pathological diagnoses. For the purposes of this study, patients with Dukes’ D tumors were excluded since our objec-
consistent with our previous work (12); consistent with our previous work, a given tumor
0.1 mCi determinations were conducted in the presence and absence of 
are the mean specific activity of all tumor samples. Enzyme 
tein, where 1 unit 
scribed previously (6). Results are expressed as units/mg pro-
distances (6-20 samples of adjacent mucosa were also collected at different 
(0.2-1.0 g each) were removed from different non-necrotic 
cluded and there were no Dukes’ A tumors in our series. Patients with modified Dukes’ B1, B2, C1, and C2 tumors were 
handled similarly. Tissues were later removed from liquid N2 for storage at 
80°C until enzymatic analyses, usually within 2 months.

ODC Analyses. These were performed exactly as des-
cribed previously (6). Results are expressed as units/mg protein, where 1 unit = 1 nmol CO2 produced per h. All samples 
taken from each tumor were assayed, and the results presented 
are the mean specific activity of all tumor samples. Enzyme 
determinations were conducted in the presence and absence of 
0.1 mM GTP in order to detect the presence of a novel isoform of 
ODC (12); consistent with our previous work, a given tumor is 
considered positive for the GTP-activatable isoform of ODC 
if at least one tumor ODC sample was stimulated 20% or more 
by the nucleotide (6).

Statistical Analyses. We applied the Cox proportional 
hazards regression to analysis of survival with covariates 
and Prognosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (36.5)</td>
</tr>
<tr>
<td>Female</td>
<td>47 (63.5)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>60 (81.1)</td>
</tr>
<tr>
<td>Black</td>
<td>13 (17.6)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>5 (6.8)</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>14 (18.9)</td>
</tr>
<tr>
<td>Descending</td>
<td>11 (14.9)</td>
</tr>
<tr>
<td>Transverse</td>
<td>5 (6.8)</td>
</tr>
<tr>
<td>Ascending</td>
<td>20 (27.0)</td>
</tr>
<tr>
<td>Cecum</td>
<td>19 (25.7)</td>
</tr>
<tr>
<td>Dukes’ stage</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>23 (31.1)</td>
</tr>
<tr>
<td>B2</td>
<td>27 (36.5)</td>
</tr>
<tr>
<td>C1</td>
<td>6 (8.1)</td>
</tr>
<tr>
<td>C2</td>
<td>18 (24.3)</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>Age† (yr)</td>
<td>71.6 ± 11.0</td>
</tr>
<tr>
<td>ODC activity (units/mg protein)</td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>1.78 ± 0.53</td>
</tr>
<tr>
<td>Mucosa</td>
<td>0.22 ± 0.32</td>
</tr>
</tbody>
</table>

† At time of surgery.

The relationship between tumor ODC activity and patient 
survival was assessed in two ways. First, as shown in Fig. 3, 
patients whose tumor ODC activity was above the median value 
for all patients had significantly longer survival than those with 
tumor ODC values below the median. Survival after 6 years in 
the high ODC group was 73.7% versus 46.8% in the low ODC

RESULTS

The ODC activities and some clinicopathological features 
of the first 60% of our patient series has previously been 
published (6). To summarize these data, colorectal tumor ODC 
activities were significantly higher (>5-fold) than adjacent 
mucosal ODC activities. In addition, 33% of the tumor samples and 
7.5% of the adjacent mucosa contained a novel GTP-activat-
able isoform of ODC first described in mouse and human skin 
tumors (12, 14). The purpose of the present study was to 
determine whether there was any association between the abso-
lute level of tumor ODC activity or the presence of a GTP-
activatable isoform with prognosis. To address this question, 
patients with modified Dukes’ B1, B2, C1, and C2 tumors were 
followed for survival. Patients with Dukes’ D tumors were 
excluded and there were no Dukes’ A tumors in our series. 
Consistent with our previous results using a subset of this 
patient population, as well as the work of others (3-6), the mean 
ODC activity in the tumor samples was substantially higher than 
mucosal ODC values (1.78 units/mg protein versus 0.22 
units/mg protein). As shown in Fig. 1, there was no obvious 
relationship between Dukes’ stage of the tumor and tumor ODC 
activity. Similarly, there was no apparent relationship between 
the differentiation status of the tumors in our series and ODC 
activity (Fig. 2).

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tumor ODC values below the median. Survival after 6 years in 
the high ODC group was 73.7% versus 46.8% in the low ODC
Fig. 1 Scatterplot of tumor ODC activity versus modified Dukes' stage. The Astler-Coller modification (39) of the original Dukes' criteria was used. $n$, number of patients with each stage tumor. Bars on the data points, mean ± SE of ODC activity.

Fig. 2 Scatterplot of tumor ODC activity versus tumor grade. Patients with preponderantly mucin-secreting tumors ($n = 4$) were excluded from this analysis. Mod, moderate.

group ($P = 0.03$). Second, we asked whether there was any association between the presence of a GTP-activatable isoform of ODC and survival. When all patients were included, there was a trend toward longer survival of those patients with a GTP-activatable tumor ODC, but this association was not significant ($P = 0.10$). However, when only patients with tumors of the proximal colon were analyzed, there was a highly significant increased probability of survival of patients whose tumors
expressed a GTP-activatable ODC (Fig. 4). We have previously reported that tumors which express this isoform are predominantly located in the proximal colon, especially the cecum (6). There was no significant difference in the survival of patients with right-sided versus left-sided tumors in our patient series (data not shown), therefore the presence of this ODC isoform in a patient’s tumor appears to be a strong independent predictor of enhanced survival.

Other variables such as patient age, race, sex, tumor size, or location were not associated with survival in our patient series (Table 2). Univariate analysis did, as expected, demonstrate a significant association of tumor stage and grade with survival. Stepwise regression analysis with covariates (Cox regression) of survival produced significant results only for Dukes’ stage (P < 0.001) and for the ODC level using several different models (Table 3). Similar results were obtained whether tumor sites were
grouped or not. ODC activity was also the sole significant covariate ($P = 0.044$) when patients were stratified by Dukes' stage (B or C). Our sample size of stage B patients was not large enough to show a statistically significant effect of ODC activity (above versus below the median) on survival for this group only. Wilcoxon's two-sample tests failed to show significant differences in the ODC level as a function of sex, race, tumor-sidedness category, or the presence of a GTP-activatable ODC isoform.

**DISCUSSION**

Despite numerous reports which have documented elevated expression of ODC in colorectal carcinoma (3–6), we are unaware of any previous studies that have attempted to determine whether differences in tumor ODC levels predicted long-term outcomes, such as tumor recurrence or survival. In fact, there are very few studies for any human tumor type that have assessed the prognostic significance of elevated ODC expression, and these have typically involved small numbers of patients and short follow-up times (15, 16). Because of the necessity of sufficient levels of polyamines for growth and functioning of normal cells, it is frequently assumed that overexpression of ODC or other forms of altered polyamine metabolism may be involved in the loss of growth control characteristic of tumor cells. While this assumption may in fact be true, since in many experimental models changes in polyamine metabolism occur early in the carcinogenic process (17, 18), an entirely different question relates to the role of polyamine metabolism in very late stages of carcinogenesis. Namely, does overexpression of ODC, or other disturbances in polyamine metabolism, have any influence on the final stages of tumor development which culminate in disseminated cancer? While this issue could conceivably be addressed in experimental models of metastasis, we have chosen to study this question in humans, given the availability of a patient series in which we had already measured tumor ODC activities and for which outcome data could be obtained retrospectively. The results of this study clearly indicate that high tumor ODC activity at the time of surgery predicts favorable long-term survival compared to patients with low tumor ODC levels.

**Table 2** Univariate analysis of clinical and pathological variables and survival in patients with colorectal carcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>$x^2$ (log rank)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODC activity</td>
<td>4.64</td>
<td>0.03</td>
</tr>
<tr>
<td>Age</td>
<td>0.29</td>
<td>0.59</td>
</tr>
<tr>
<td>Race</td>
<td>2.37</td>
<td>0.12</td>
</tr>
<tr>
<td>Sex</td>
<td>0.35</td>
<td>0.56</td>
</tr>
<tr>
<td>Tumor size</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td>Anatomical location</td>
<td>1.06</td>
<td>0.30</td>
</tr>
<tr>
<td>Dukes' stage</td>
<td>4.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>6.29</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*a Comparisons of the indicated variable with survival data were made as follows: ODC activity, greater or less than the median (0.69 units/mg protein); age, greater or less than the median (73.5 years); race, black versus white; sex, male versus female; tumor size, above and below median (5 cm); anatomical location, right versus left colon; Dukes' stage, Dukes' B versus Dukes' C; and tumor grade, well and moderately differentiated versus moderately poor and poorly differentiated.

**Table 3** Hazard ratios for ODC and Dukes' stage as prognostic factors in multiple regression models

<table>
<thead>
<tr>
<th>Model (variables* used)</th>
<th>Category</th>
<th>Hazard ratio</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (age, race, sex, tumor site, Dukes' stage, ODC, GTP)</td>
<td>Stage: B vs. C</td>
<td>2.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II (age, race, sex, sidedness, Dukes' stage, ODC, GTP)</td>
<td>Stage: $B_1$, $B_2$, $C_1$, $C_2$</td>
<td>8.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>III (age, race, sex, side, tumor grade, tumor size, ODC, GTP stratified by Dukes' stage: B or C)</td>
<td>ODC</td>
<td>0.59</td>
<td>0.044</td>
</tr>
</tbody>
</table>

*a Only ratios for variables significantly associated with survival are shown.

Another significant association, at least in proximal colon tumors, was the presence of a GTP-activatable isoform of ODC and a favorable prognosis. While the association between this ODC isoform and prognosis is statistically significant only for right-sided tumors in our series, it seems likely that this association would prove to be significant for left-sided tumors as well if a larger sample size was studied. The exact biochemical nature, and physiological significance, of the GTP-activatable isoform of ODC is unknown. This isoform was first detected in mouse epidermal tumors (12) and subsequently found to be expressed in human skin (14), colorectal (6), and gastric carcinomas (19). Among the properties that distinguish it from typical mammalian ODC are its greater stability, both in vivo and in heat-inactivation studies in vitro (12). We speculate that this isoform of ODC, if present, is physiologically more potent in polyamine synthesis than the typical ODC isoform due to its constitutive activation by intracellular GTP and its reduced turnover rate.

Inspection of our ODC data (Fig. 1) did not suggest an association of ODC activity with tumor histopathology, as classified by standard criteria (Dukes' stage, Astler-Coller modification). Porter et al. (5) also reported no association between ODC activity and Dukes' stage in another large study. Multiple stepwise regression analysis of survival data using all possible covariates (Cox proportional hazards model) indicated that the tumor ODC level was a significant prognostic factor ($P = 0.032$ or 0.040, depending on the model used) in addition to Dukes' stage ($P < 0.001$). The ODC level was also the sole significant covariate ($P = 0.044$) when patients were stratified by Dukes' stage (B or C). Thus, the level of tumor ODC activity is associated with favorable outcome independently of classical tumor histopathological criteria. From our analyses of survival data and the results presented in Fig. 1, we conclude that within a group of patients with a similar Dukes' stage tumor, those with a higher tumor ODC activity tend to have a more favorable outcome.
Our results have several implications for both basic mechanisms of carcinogenesis and clinical management of colorectal cancer. Regarding the former, it was at first unexpected to find an association between high levels of tumor ODC activity and greater probability of long-term survival. Experimental work in animal models has demonstrated that many tumor-promoting compounds are effective inducers of ODC; conversely, selective inhibitors of the enzyme, such as difluoromethylornithine, are effective chemopreventive agents when administered during the tumor promotion phase of carcinogenesis (9, 10, 21). Recently, overexpression of ODC by molecular genetic techniques in untransformed cells in culture resulted in induction of the transformed phenotype directly (22, 23) or greatly increased the efficiency of transformation by an oncogenic ras gene (24). Finally, transgenic mice with aberrant expression of a human ODC transgene are more susceptible to induction of benign skin lesions than control littermates (25). Thus, a positive role for ODC overexpression in the early stages of tumor development seems likely. However, there are very few studies which directly address the role of overexpression of ODC in late stages of tumor progression such as metastasis. Systemic difluoromethylornithine administration has been reported to inhibit metastasis in a murine melanoma (B16) model (26), but whether the relevant target for the drug was the tumor itself or host tissues could not be determined from that study design. Our results raise the possibility (which could be tested experimentally) that overexpression of ODC and presumably up-regulation of cellular polyamine levels can suppress the metastatic potential of colorectal carcinoma cells, thereby prolonging survival.

Metastasis is a complex biological process presumably involving the interplay and aberrant regulation of numerous gene products. ODC may be one of several candidate genes, including DCC (27), E-cadherin (28), and nm23 (29) [although the specific roles of the latter two genes in colorectal carcinoma has been questioned (30, 31)], that can negatively regulate metastasis. However, ODC apparently does not function as a prototypical suppressor gene whose loss of function results in enhanced metastasis: rather, up-regulation of ODC expression may suppress development of metastatic potential, perhaps via the effect of elevated cellular polyamine levels on the expression of specific effector genes involved in metastasis. However, one important caveat of our results is the lack of information on actual polyamine levels in the tumors used in this study. Undoubtedly, many other factors in addition to ODC, such as intracellular ornithine concentration, polyamine uptake from luminal contents, and the levels of other enzymes, such as S-adenosylmethionine decarboxylase, contribute to the polyamine levels in colorectal tumors.

On the basis of previous work in colorectal carcinoma (6) and other tumor types (14, 32), the expression of ODC at the cellular level is likely to be heterogeneous: some individual cells and/or regions of the tumor may express much more ODC than other cells or regions. If high levels of ODC expression are incompatible with expression of a metastatic phenotype, then one would predict that in patients with both a primary adenocarcinoma of the colon and a metastatic lesion, the latter would have lower ODC activity than the primary tumor. This prediction appears to be true, based on the results of Herrera and Petrelli (33), who observed a significant reduction (>60%) in the ODC activities of liver metastases versus the primary tumors from which they originated. We infer from these results that within human colorectal carcinomas, cells with metastatic potential will have lower ODC expression than other tumor cells.

Our results could have an impact on the management of colorectal cancer in at least two areas. First, knowledge of a patient’s tumor ODC activity at the time of surgery may provide additional prognostic information that could be used to choose the most appropriate adjuvant therapy for that individual. Patients with low tumor ODC activities might be treated more aggressively than otherwise similar patients with high tumor ODC activities. Second, the approach of using inhibitors of polyamine biosynthesis (34) or unnatural polyamine analogues (35) as chemotherapeutic agents may need to be critically re-evaluated. Polyamine metabolism has always been a logical target for chemotherapy of hyperproliferative diseases, including cancer, and there have been positive results in experimental models (36, 37). The end points typically chosen for these studies, however, have been primary tumor growth in animal model systems or growth of tumor-derived cell lines in vitro. There have also been promising results using inhibitors of ODC, especially difluoromethylornithine, as chemopreventive agents in animal models of carcinogenesis (10, 11, 21). Despite these often impressive results in experimental models, the efficacy of polyamine-directed chemotherapy in humans has not been convincingly demonstrated. Our data showing that high tumor ODC expression is associated with favorable prognosis suggests that in humans at least, attempts to reduce tumor ODC or polyamine levels could be counterproductive: while such treatment may reduce the growth of the primary tumor as it does in animal model systems, the potential for metastasis may be enhanced. Although this idea is speculative, it is nevertheless testable in human tumor cell lines capable of metastasis in immune-deficient mice (38). Namely, can deliberate overexpression of ODC by suitable molecular techniques alter cellular polyamine levels in these cells and reduce metastatic potential? The results of such studies should determine whether strategies to deliberately up-regulate polyamine synthesis in cancer patients in order to improve long-term survival is a novel therapeutic approach worth considering.

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