Growth Potential of Human Colorectal Carcinomas in Nude Mice: Association with the Preoperative Serum Concentration of Carcinoembryonic Antigen in Patients

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ABSTRACT

A preoperative serum carcinoembryonic antigen (CEA) concentration greater than 5 ng/ml portends a poor prognosis for patients with colorectal carcinoma. The purpose of this study was to determine if the tumorigenicity of colorectal carcinomas in nude mice was associated with the preoperative serum CEA concentration. Neoplasms from 53 patients were either implanted as fragments or dissociated with collagenase and DNase, and 3 x 10^6 viable cells were injected into the flanks of BALB/c nude mice. The growth potential of tumors resected from patients with CEA levels exceeding 5 ng/ml was greater than that of tumors from patients with normal serum CEA: 26 of 33 carcinomas from patients with CEA > 5 ng/ml were tumorigenic in nude mice, whereas only 8 of 22 neoplasms from patients with normal serum CEA were tumorigenic in nude mice (P < 0.001). Primary colorectal cancers, not metastases, were the basis for the association between tumorigenicity and preoperative CEA. Tumorigenicity was also associated with stage of disease, since Dukes' A primary tumors and metastases were more tumorigenic than Dukes' A to C primary tumors. Growth in nude mice was not associated with other prognostic factors such as tumor site, mucin production, local invasion, or stage of histological differentiation. The tumorigenic capability of human colorectal carcinomas may be associated with the preoperative serum CEA concentration and may reflect an increased potential to develop clinical metastases.

INTRODUCTION

CEA is a serum marker for several neoplastic diseases, most importantly adenocarcinoma of the colon or rectum, whose function remains unknown nearly 2 decades after its original description (1). In general, patients with a serum CEA concentration higher than 5 ng/ml prior to surgery and whose disease is limited to bowel wall (Dukes' B) or regional lymph nodes (Dukes' C) have a shorter disease-free interval and a lower survival rate after potentially curative surgery than do similarly staged patients who have a normal serum CEA concentration (2, 3). Since CEA is metabolized by the liver, CEA in the portal venous blood draining a colon or rectal neoplasm will be cleared by the liver and it will not be detected in the systemic circulation. If the liver contains metastases that secrete CEA into the systemic circulation or its metabolic activity is compromised by disease, for example by chronic hepatitis, then the serum levels of CEA will rise.

Although patients with elevated serum CEA may have occult micrometastases, it is also possible that primary tumors from such patients are more aggressive than primary tumors from patients with a normal CEA. If this were the case, then tumors from patients with an elevated serum CEA should have a higher growth potential than neoplasms of patients with a normal level of serum CEA. This hypothesis was untestable until it was discovered that human neoplasms can be transplanted successfully into athymic nude mice (4), giving investigators the opportunity to assess the tumorigenicity of human neoplasia. Moreover, Giavazzi et al. (5) showed that nude mice can be used to ascertain the growth rates and metastatic potential of cells harvested from primary and metastatic human colorectal carcinomas. Thus, the nude mouse may be utilized to assess the growth potential of various human colorectal cancers.

The purpose of the present study was to test the hypothesis that neoplasms resected from patients with an elevated serum CEA concentration are more tumorigenic than tumors from patients with a normal serum CEA. Adenocarcinomas of the colon or rectum and their metastases were enzymatically dissociated and cells implanted into the flanks of nude mice. Tumorigenicity was defined as the progressive growth of a neoplasm within 6 months at the inoculation site that was successfully passaged into a second set of nude mice. Other prognostic factors were also investigated for their association with tumorigenicity in addition to CEA. Thus, this study extends our previous work by determining whether CEA is associated with the growth potential of human colorectal carcinoma implanted into nude mice.

MATERIALS AND METHODS

Animals. Six- to 8-week-old BALB/c nude mice were obtained from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research Facility. The mice were age and sex matched for each experiment and were housed in laminar flow cabinets under specific-pathogen-free conditions.

Patients. The 53 patients in this study were admitted to the surgical service of the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston and underwent resections of primary or metastatic colorectal adenocarcinomas. Informed consent was granted for this study in accordance with institutional and federal guidelines. Pathological staging was performed according to Astler and Coller (6). Representative samples of tumor and mucosa were reviewed by one investigator (K. C.) to determine nuclear grade, lymphatic, neural, or vascular invasion, extracellular mucin production or the presence of signet cells. There were 36 primary lesions: one Dukes' A, a lesion that is limited to mucosa alone; 3 Dukes' B1, a carcinoma that penetrated to muscularis propria without lymph node metastasis; 12 Dukes' B2, a carcinoma that penetrated through bowel wall but without lymph node metastasis; 2 Dukes' B3, a lesion that invaded contiguous organs without lymph node metastases; 8 Dukes' C2, a carcinoma that has penetrated the bowel wall and the regional lymph nodes; and 10 Dukes' D, a carcinoma that has metastasized to distant sites (Table 1). There were 24 metastases: 3 lymph node; 3 pelvic recurrences; one mesenteric nodule; 1 lung metastasis; and 16 hepatic metastases. Three patients had liver metastases removed along with their primary tumors and 4 patients had 2 or more metastases removed. The mean age of the patients was 55.2 years with a range of 31 to 77 years. Twenty-six patients were men. Two patients were Hispanic, 3 patients were black, one Asian, and the rest white.

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2 To whom requests for reprints should be addressed, at Department of Surgery, Box 106, University of Texas, M. D. Anderson Hospital, 1515 Holcombe Boulevard, Houston, TX 77030.

3 The abbreviation used is: CEA, carcinoembryonic antigen.
Preparation of Cell Suspensions. Specimens removed at surgery were immediately examined by a surgical pathologist, and samples were harvested and transported at 4°C within 10 min in minimal essential medium (Grand Island Biologic Co., Grand Island NY) containing 300 units/ml penicillin and 300 µg/ml streptomycin. Tissue dissociation was performed exactly as described previously (5) using collagenase type I and DNase type I (Sigma Chemical Company, St. Louis, MO). The viability of the dissociated cells was determined and the cells were immediately injected into nude mice.

Implantation Techniques. Fragments of tissue (1-mm cubes) were implanted by trocar in the flanks of groups of one to 11 nude mice in the middorsoverentral line approximately 1 cm cephalad to the inguinal crease. The number of mice per group was dictated by the number of cells after dissociation or the amount of tumor. Each mouse received only one tumor inoculation. S.c. inoculations were performed by injecting 3 × 10^6 viable dissociated cells through a 27-gauge needle into the subcutis in the same site as the trocar implant. I.m. injections were made by injecting a similar number of dissociated viable cells into the middle of the quadriceps femoris. Implantation sites were inspected twice a week. When nodules were approximately 1 cm in diameter, they were resected under aseptic conditions and aliquoted. One aliquot was dissociated and reimplanted while the other aliquot was submitted for histopathology. Tumorigenic nodules were successfully transplanted and appeared histologically to be similar to the original tumor. Tumors considered non-tumorigenic if a progressively growing nodule did not appear at the inoculation site within 6 months.

Histopathological Examination. Specimens used in our studies were classified by routine histopathological examination in the Pathology Department, University of Texas M. D. Anderson Hospital. Xenografts of tumor tissue were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 4-µm intervals, and stained with hematoxylin and eosin.

Statistics. The effect of prognostic variables upon the growth potential of human colorectal carcinoma xenografts in nude mice was tested by using 2×2 and 2×K contingency tables with χ^2 analysis as stated in the text. In addition, Fisher's Exact Test was used when observed frequencies were less than 5 occurrences/cell. Significance was set at the 5% level.

RESULTS

Effect of Implantation Technique upon Growth Potential. The effect of implantation upon tumorigenicity was studied in 20 carcinomas that were injected by more than one route of inoculation into the flanks of nude mice. Similar results were obtained with 18 of these comparisons. In one case s.c. fragments grew and dissociated tumor cells inoculated i.m. did not. In another case, tumor cells inoculated into muscle, but not fragments implanted s.c., were tumorigenic. In addition, the number of mice included in each group did not significantly influence the growth potential of colorectal carcinoma xenografts: 17 of 29 inoculations were tumorigenic when only one or 2 mice were given injections compared to 22 of 31 inoculations into groups of 3 or more mice; χ^2 = 1.004; P = not significant (Tables 1 and 2). Thus, the growth potential of human colorectal carcinoma xenografts depended more on the intrinsic growth potential of the neoplasm than on the route of implantation or number of nude mice inoculated. As a result, a neoplasm was tumorigenic if it produced a progressively growing tumor at the implantation site in at least one of the nude mice given an injection.

Growth Potential of Primary and Metastatic Neoplasms. Metastases were slightly but not significantly more tumorigenic than primary tumors: 16 of 24 metastases (67%) were tumorigenic compared to 23 of 36 primary neoplasms (64%) (Tables 1 and 2). However, if a tumorigenic metastasis is defined as the growth of at least one metastasis when multiple metastases...
from the same patient are tested for tumorigenicity, then metastases from 14 of 17 patients (82%) were tumorigenic (metastases are still not significantly more tumorigenic on a per patient basis than are primary tumors). Interestingly, the grow potential of multiple neoplasms harvested from the same patient were often different. Primary tumors and metastases from two patients grew while the metastasis, but not the primary tumor, from a third patient was tumorigenic. Further, the growth potential of multiple metastases harvested from the same patient were heterogeneous: in 2 patients both metastases grew, in a third patient one metastasis was tumorigenic while a second was nontumorigenic, and in a fourth patient two metastases grew while a third metastasis did not.

Analysis of Prognostic Factors and Tumorigenicity. Most prognostic factors were not associated with the growth potential of colorectal carcinoma xenografts. Moderately to well differentiated tumors were as tumorigenic as poorly differentiated primary neoplasms (59 and 64%, respectively) (Table 3). The growth potential of tumors that produced either extracellular or intracellular (signet cell carcinomas) mucin was not greater than that of tumors that did not produce mucin (63 and 68%, respectively). Neither age, site of primary neoplasm, nor growth pattern in the patient correlated with tumorigenicity in nude mice. Primary neoplasms that invaded either blood vessels, lymphatics, or nerves (68% grew) were not significantly more tumorigenic than noninvasive tumors (45% grew). However, tumorigenicity was associated with the stage of disease: metastases (82% tumorigenic) and Dukes’ D primary tumors (90% tumorigenic) were more tumorigenic than Dukes’ A to C carcinomas (54% grew) by a 2 X 3 contingency table analysis. Stronger association was observed between serum CEA concentration and the concentration of CEA in the sera of patients prior to operation and tumorigenicity (Table 3). These results suggest that the growth potential of colorectal carcinomas is associated with advanced disease and the serum CEA concentration in the patient.

Association of Tumorigenicity with Serum CEA Concentration. The association between postoperative serum CEA concentration and growth potential of human colorectal carcinomas in nude mice was analyzed further. The serum concentration of CEA was associated with the growth potential of primary neoplasms. Fourteen of 16 primary tumors resected from patients with a serum CEA greater than 5 ng/ml were tumorigenic compared to only 5 of 16 primary neoplasms from patients with normal levels of serum CEA. The association between CEA and tumorigenicity was evident even in the Dukes’ A to C primary cancers that did not have clinically detectable metastases. The tumorigenicity of metastases was not associated with CEA when either individual lesions or patients were analyzed. Results for individual metastases are presented in Table 4. When the data are analyzed by patients, there still is no association between CEA and the tumorigenicity of metastases. Thus, CEA is associated with the growth potential of primary but not metastatic human colorectal carcinomas in nude mice.

Table 3 Univariate analysis of effect of prognostic variables upon tumorigenicity of human colorectal carcinoma xenografts in nude mice

<table>
<thead>
<tr>
<th>Stage of disease (60)*</th>
<th>CEA (5)</th>
<th>Invasion (30)</th>
<th>Mucin production (41)</th>
<th>Grade 3 (52)</th>
</tr>
</thead>
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<tr>
<td>x²</td>
<td>8.71</td>
<td>9.99</td>
<td>1.53</td>
<td>0.10</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
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* Numbers in parentheses, numbers of xenografts evaluable for each prognostic variable.

DISCUSSION

The tumorigenicity of primary human colorectal carcinoma xenografts in nude mice is associated with preoperative serum CEA concentration. This report provides a biological basis for the clinical observations (2, 3) that patients with Dukes’ B and C colorectal carcinomas have a poor prognosis when their serum CEA is greater than 5 ng/ml prior to operation. Neoplastic growth depends upon decreased response to growth inhibitors, increased utilization of growth factors, or evasion of deleterious host responses. Since increased levels of CEA are associated with the tumorigenicity of primary neoplasms, CEA may either support the growth of primary colorectal carcinomas or inhibit host resistance to neoplasms. There is some evidence that CEA is an immunosuppressant. When lymphocytes from patients with colorectal carcinoma are incubated with CEA, a factor is secreted that inhibits immune responses (7). Although athymic nude mice lack mature T-cells and are unlikely to utilize such a suppressor mechanism, CEA may have an inhibitory effect upon other host effector cells.

We are not aware of reports that CEA is a growth factor for colorectal carcinoma. Indirect evidence suggests that CEA expression is associated with the early events of colorectal carcinogenesis. Whitehead and Skinner (8) repeatedly biopsied a villous adenoma in a patient who refused operation and performed sequential immunohistochemical studies over several years. CEA expression coincided with the development of an invasive adenocarcinoma. Similarly, Greaves et al. (9) reported that CEA expression was augmented in polyps that contained either severe dysplasia or early invasive carcinoma compared to benign adenomatous polyps. However, the amount of tissue-extractable CEA is similar for tumorigenic and nontumorigenic neoplasms in Western transfers of extracts of primary and metastatic colorectal carcinomas. Thus, we could not demonstrate increased levels of CEA in extracts of tumorigenic neoplasms and as a result do not have evidence to support the role of CEA as a growth factor.

Tumorigenicity in nude mice did not correlate with such standard prognostic factors as mucin production, cellular differentiation, or invasion of blood vessels, lymphatics, or nerves. These factors have an adverse effect upon the survival of patients with colorectal carcinoma (10–15). However, the major determinant of survival is the development of metastases. These
prognostic factors may be associated with the metastatic potential of human colorectal cancers rather than their tumorigenicity in nude mice. While tumorigenicity is a necessary precondition for metastasis (16–17), tumorigenicity in the flanks of nude mice by itself may not be associated with the propensity of a neoplasm to produce metastasis in the patient. As a result, tumorigenicity of colorectal carcinoma xenografts may not be associated with standard prognostic variables.

In contrast, tumorigenicity of colorectal carcinomas was associated with stage of disease. Since serum CEA is more likely to be elevated in patients with advanced disease, tumorigenicity may be a characteristic of neoplasms that have progressed to a state capable of producing metastases. Thus, both CEA production and tumorigenicity may be markers of an advanced state of neoplastic progression. If this is true, then tumorigenicity may aid in detecting those neoplasms among the “early” Dukes’ A to C lesions that have the potential to develop clinical metastases.

In summary, tumorigenicity of primary human colorectal carcinoma xenografts correlated with the preoperative serum CEA concentration in the patients. Thus, although patients with an elevated serum CEA may be more likely to have occult metastases that secrete CEA at the time of surgical resection, primary neoplasms from these patients have a higher growth potential than neoplasms from patients with a normal level of serum CEA.

REFERENCES


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