Urokinase-type Plasminogen Activator in Colorectal Cancer: Relationship with Clinicopathological Features and Patient Outcome

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ABSTRACT

Urokinase-type plasminogen activator (u-PA) is a serine protease that has been implicated in cancer invasion and metastasis. We quantitated u-PA levels in normal colorectal mucosa, adenomatous polyps, and colorectal cancers and correlated these levels with clinicopathological features and patient survival. Detergent extracts were prepared from 133 colorectal cancers, 133 corresponding colorectal mucosal samples, and 15 synchronous adenomatous polyps. u-PA levels were determined using an ELISA, and a cancer:normal u-PA ratio was calculated for each case. u-PA levels were higher in cancers than in normal tissues, whereas adenomas had intermediate levels (P < 0.0001). u-PA levels were unrelated to clinical or pathological features. Survival was decreased in patients with a high cancer:normal u-PA ratio (P = 0.007). Multivariate survival analysis of patients undergoing curative surgery confirmed that the u-PA cancer:normal ratio was related to outcome (relative risk, 2.67; P = 0.02) and was independent of tumor stage (relative risk, 2.26; P = 0.03). Our study suggests that a high ratio of cancer to normal mucosal u-PA indicates an increased risk of colorectal cancer progression. Measurement of u-PA may provide useful prognostic information in patients undergoing curative surgery for colorectal cancer. The aggressive behavior of colorectal cancers with a high u-PA ratio suggests that the protease might be a suitable target for the development of therapeutic agents to prevent invasion and metastasis.

INTRODUCTION

The malignancy of a solid tumor is due to its ability to invade and metastasize. Cancer invasion is facilitated by disruption and remodeling of natural barriers such as basement membrane and extracellular matrix. Proteases play a crucial role in this process. Increased levels of proteases have been found in many cancers, including breast (5-7), gastric (8-10), ovarian (11, 12), prostatic (13-15), and colorectal (16-19).

u-PA has been shown to be a prognostic marker in a wide range of malignancies, especially breast cancer (20). We have previously shown using semiquantitative immunohistochemical techniques that patients with a similar stage of colorectal cancer, namely Dukes’ B, have different outcomes depending on the amount of u-PA detected in the epithelial component of the tumor (21). Ganesh et al. (18) found that the ratio of tumor u-PA to normal mucosal tissue-type PA was predictive of outcome in colorectal cancer. The present study quantitates u-PA levels in colorectal cancer, adjacent normal colorectal mucosa, and synchronous adenomatous polyps to examine the role of u-PA in colorectal cancer progression and patient survival.

PATIENTS AND METHODS

Patients and Study Design. One hundred thirty-three patients (median age, 70 years; range 30-84; 78 male, 55 female) undergoing colorectal cancer surgery in St Vincent’s Hospital between September 1991 and August 1994 were included in this study, which was approved by the hospital ethics committee. Clinical and pathological information was retrieved from the St. Vincent’s Hospital Colorectal Cancer Database. Cancers were staged by a modified Dukes’ system (22) that includes a stage D for those with residual tumor or distant metastases at operation or involvement of the resection margin by tumor on histological examination. By definition, patients who had stage A, B, or C cancers resected were deemed to have undergone curative resection. Patients were followed up until August 1995 or death; median follow-up was 26 months. The number of patients receiving adjuvant therapy (6 in all) was small. Inclusion of these patients did not alter the results of analysis.

Tissue Preparation. Colorectal cancer resection specimens were obtained at time of surgery. Tissue samples (mean weight, 300 mg) were taken from the primary cancer and from macroscopically normal mucosa at least 5 cm from the tumor margin. Samples were also taken from synchronous adenomas when these were present. Fat and muscle were removed prior to snap-freezing the tissues in liquid nitrogen, which were then stored at -70°C until further use. Samples were subsequently homogenized on ice in Tris-HCl (pH 7.4) containing 1% Triton X-100 using a Silverson homogenizer with 20-s bursts for 4

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The abbreviations used are: u-PA, urokinase-type plasminogen activator; PA, plasminogen activator; MMP, matrix metalloproteinase.
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Fig. 1 u-PA levels (horizontal lines, median; I, interquartile range) in normal colorectal cancer patients (Cuzick’s test for trend; P < 0.0001).

cycles. Samples were then centrifuged at 10,000 × g for 15 min at 4°C. The supernatant was removed and stored at −20°C.

**u-PA Measurement.** u-PA antigen levels were measured using the Immubind u-PA ELISA kit (American Diagnostica, Greenwich, CT). This sandwich ELISA detects single-chain, high molecular weight, and receptor-bound u-PA in addition to u-PA that is complexed to type 1 and 2 PA inhibitors. Protein content was measured by the Coomassie Blue dye method (Bio-Rad, Hercules, CA). u-PA antigen was expressed as ng/mg of protein. Cancer: normal mucosa u-PA ratios were calculated for each case.

**Statistical Analysis.** Nonparametric data were assessed with the Mann-Whitney U test and Cuzick’s test for trend (23). Kaplan-Meier survival curves were constructed, with cancer-related death as the end point. Differences in survival between groups were compared using the log-rank test. The optimum cutoff value for the u-PA cancer:normal ratio was determined by the maximal log-rank test (24). Multivariate survival analyses were performed with the Cox proportional hazards model using the Statistical Package for the Social Sciences (SPSS, Chicago, IL). The assumptions of the proportional hazard model were checked by observing constant vertical differences over time between plots of the logarithm of the integrated hazard function for each variable (25). Ps less than 0.05 were considered significant in all analyses.

**RESULTS**

u-PA antigen levels ranged from 0.002 to 1.71 ng/mg of protein in normal mucosa, 0.125 to 0.89 ng/mg of protein in adenomatous polyps, and 0.08 to 2.53 ng/mg of protein in colorectal cancers. Fig. 1 shows median u-PA levels in all three tissue types. There was a stepwise increase in u-PA antigen levels from normal mucosa to adenoma to carcinoma (P < 0.0001). u-PA levels were higher in both colonic mucosa and cancers when compared with rectal mucosa and cancers (Table 1). However, the ratio of cancer to normal u-PA was almost identical in patients with colon cancer when compared to those with rectal cancer (Table 2). Absolute levels of u-PA in carcinomas were not associated with any clinico-pathological features or patient outcome.

Table 1 u-PA levels (ng/mg of protein) in rectal and colonic tissues

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Median (interquartile range)</th>
<th>u-PA cancer:normal ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa</td>
<td>Rectum (n = 63) 0.10 (0.07-0.17)</td>
<td>0.005 (0.30-1.02) 0.03</td>
</tr>
<tr>
<td></td>
<td>Colon (n = 70) 0.17 (0.09-0.26)</td>
<td>0.37 (0.27-0.69)</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test.

Table 2 Clinical and pathological features of 133 colorectal cancer patients stratified by u-PA cancer:normal ratio

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of patients</th>
<th>Median (interquartile range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;70 years</td>
<td>63</td>
<td>3.83 (2.18-6.04)</td>
<td>0.94*</td>
</tr>
<tr>
<td>≥70 years</td>
<td>70</td>
<td>3.59 (2.22-6.81)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>78</td>
<td>3.97 (2.34-6.81)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>55</td>
<td>3.53 (2.17-6.03)</td>
</tr>
<tr>
<td>Tumor site</td>
<td>Rectum</td>
<td>63</td>
<td>3.77 (2.30-6.21)</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>70</td>
<td>3.75 (2.07-6.57)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>&lt;5 cm</td>
<td>68</td>
<td>3.95 (2.19-6.43)</td>
</tr>
<tr>
<td></td>
<td>≥5 cm</td>
<td>65</td>
<td>3.74 (2.23-6.42)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>A</td>
<td>10</td>
<td>2.79 (1.53-5.39)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>56</td>
<td>3.72 (2.07-6.79)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>39</td>
<td>3.58 (2.18-6.55)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>28</td>
<td>4.31 (2.56-6.43)</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td>Well or moderately differentiated</td>
<td>117</td>
<td>3.61 (2.07-6.29)</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated</td>
<td>16</td>
<td>4.68 (3.47-7.27)</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test.

Table 2 shows the relationship between u-PA cancer:normal ratio and clinico-pathological features. u-PA cancer:normal ratio was not significantly associated with any of the clinico-pathological features studied. Patients with poorly differentiated tumors tended to have higher cancer:normal u-PA ratios than those with well or moderately differentiated cancers (P = 0.07).

Fig. 2 shows patient outcome stratified by tumor stage and cancer:normal u-PA ratio. Survival was closely associated with tumor stage (log-rank test; P < 0.0001), but it was also related to the cancer:normal u-PA ratio (P = 0.007). Substage analysis (Fig. 3) showed that the u-PA cancer:normal ratio was predictive of outcome for patients undergoing curative surgery (Stage A, B, and C disease; P = 0.007). A Cox regression analysis, which included cancer:normal u-PA ratio and all clinical and pathological variables, confirmed that u-PA cancer:normal ratio was related to outcome independent of tumor stage in patients undergoing curative surgery (Table 3).

**DISCUSSION**

This study was designed to correlate levels of one member of the PA family, namely u-PA, with clinico-pathological fea-
Fig. 2 Survival of 133 colorectal cancer patients stratified by tumor stage (left) and cancer: normal u-PA ratio (right).

Fig. 3 Survival of 133 colorectal cancer patients stratified by cancer: normal u-PA ratio. Top, stages A, B, and C; bottom, stage D.

Table 3 Final regression model showing independent prognostic features in 105 patients undergoing curative surgery

<table>
<thead>
<tr>
<th>Feature</th>
<th>Relative risk</th>
<th>SE (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor stage</td>
<td>0.816</td>
<td>0.278</td>
<td>2.26 (1.08-4.72)</td>
</tr>
<tr>
<td>High cancer: normal u-PA ratio</td>
<td>0.981</td>
<td>0.375</td>
<td>2.67 (1.19-5.97)</td>
</tr>
</tbody>
</table>

use of different standards and antibodies in the respective ELISAs.

This study is the first to show a relationship between cancer: normal ratio of u-PA and patient survival, independent of cancer stage. This suggests that any observed changes in u-PA levels in colorectal cancer provide prognostic information independent of conventional pathological staging. Therefore, we postulate that u-PA cancer: normal ratio is an indicator of the biological aggressiveness of colorectal carcinomas.

Ganesh et al. (18), in a study measuring various components of the PA system in colorectal cancers and normal adjacent mucosa, reported that the ratio of cancer u-PA level to normal mucosal tissue-type PA was correlated with patient survival. In contrast, our study reveals that measurement of u-PA alone yields useful prognostic information. The use of a cancer-normal ratio allows each patient to act as his or her own internal control. In addition, the ratio takes account of the significant regional variations that we demonstrated between the level of u-PA in both normal and malignant colonic and rectal tissues.

At present, it is difficult to predict accurately the prognosis of patients with stage B and C cancers (33). A variable independent of tumor stage but predictive of patient outcome would be of considerable benefit in identifying those patients at greatest risk of tumor-related death. Patients with stage C cancer and a low u-PA cancer: normal ratio tended to have a better outcome than patients with stage B cancer who had a high ratio (data not shown). This observation highlights the utility of u-PA cancer: normal ratio in predicting survival of patients independent of tumor stage. Prospective studies should be performed using standardized extraction and analytical methods in conjunction with a previously determined optimized cutoff value for u-PA ratio.

In addition to being a prognostic marker, u-PA may also be a potential target for antimetastatic therapies. In model systems, both inhibition of u-PA activity (34) and prevention
of u-PA binding to its receptor (35) have been shown to limit the formation of metastasis. It is of interest that inhibitors of MMPs inhibit cancer growth in experimental models (36, 37) and have recently entered clinical trials for treatment of selected cancers. Combined inhibition of both MMPs and u-PA may be effective in preventing development of metastases in colorectal cancer.

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