Expression of a \( M, 32,000 \) Laminin-binding Protein Messenger RNA in Human Colon Carcinoma Correlates with Disease Progression

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ABSTRACT

Cell surface receptors for laminin may play an important role in tumor migration and metastasis. To evaluate laminin receptor/laminin-binding protein expression in human colon carcinoma, surgical specimens of primary colon cancers and liver metastases were examined by blot hybridization of total RNA with a complementary DNA clone which encodes a \( M, 32,000 \) human laminin-binding protein. The mRNA level of the laminin-binding protein was higher in primary colon carcinoma than in adjacent normal colonic epithelium in 20 of 21 cases. In all 6 cases of colon cancer liver metastases, the laminin-binding protein mRNA level was more than 3-fold greater in tumor than in adjacent normal liver tissue. The tumor/normal ratio of this laminin-binding protein mRNA expression in primary colon cancer has significant correlation with Dukes' classification \( (P < 0.001) \). Our results suggest that mRNA expression of the laminin-binding protein may be a marker of human colorectal cancer progression and biological aggressiveness.

INTRODUCTION

Metastasis is the major cause of morbidity and death for cancer patients. In spite of recent advances in early diagnosis, surgical resection of cancer, and adjuvant therapies, the majority of cancer deaths are still attributed to metastases which are resistant to various kinds of therapies. The tumor cells must interact with extracellular matrix components during the process of invasion and metastasis. For epithelial tumor cell migration, interaction with a series of basement membranes which separate epithelium from connective tissue and connective tissue from vasculature might be a most important process. Laminin \( (M = 800,000 \) glycoprotein), one of the major components of basement membranes, functions in the attachment, spreading, and migration of cells \( (1-9) \). Laminin is also implicated in cell growth, differentiation, mitogenesis, neurite outgrowth, and binding to other extracellular matrix components \( (9-15) \).

Many of these cell surface interactions may be mediated through the receptors for laminin \( (8, 16-23) \). Increased expression of these laminin receptors in various cancers has been reported \( (17, 20, 24-26) \). The interaction between laminin and its cell surface receptors may play a key role in the mechanism of tumor invasion and metastasis \( (18-20, 27) \). Laminin receptor can be shown to play a role in hematogenous metastases. In animal models, tumor cells selected for their ability to attach to laminin produced 10-fold more metastases following i.v. injection \( (4, 16) \). A differential expression of laminin and laminin-like substance has been demonstrated in high and low metastatic cell lines \( (18, 28-30) \).

Recently, we constructed a plasmid cDNA\(^3\) library from the poorly differentiated human colon carcinoma cell line Clone A. From this library we identified a cDNA clone encoding a human laminin-binding protein \( (26) \). In this investigation, we evaluated the mRNA expression of laminin-binding protein in human colon carcinoma. Surgical specimens of colon carcinoma and adjacent normal colonic epithelium were probed by blot hybridization of total RNA with the laminin-binding protein cDNA clone. Our results indicated a significant increase in the laminin-binding protein mRNA expression in colon tumors, as compared to normal colonic epithelium. These results have been correlated with tumor invasiveness and metastasis.

MATERIALS AND METHODS

Identification of cDNA Clone. A cDNA library was constructed in plasmid pBR322, using polyadenylated mRNA isolated from a poorly differentiated human colon carcinoma cell line, Clone A \( (26) \). A total of 1500 colonies were screened by hybridization with cDNAs generated from mRNA of Clone A or of a well differentiated human colon carcinoma cell line, CX-1. Ten cDNA clones were selected because they hybridized to a greater degree with cDNAs from Clone A than with those from CX-1. Molecular cloning and complete sequencing revealed that one clone, 8-2V \( (26) \), contained a 350-nucleotide stretch whose sequence was identical to the partial cDNA sequence of a human laminin receptor reported by Wewer et al. \( (20) \).

Plasmid DNA was purified and digested with PstI. After electrophoresis, the cDNA insert was eluted with Li/Dx filter syringes (Xydez, Gaithersburg, MD), extracted with phenol/chloroform, concentrated with guanidium isothiocyanate and ultracentrifuged through a cesium chloride solution at 32,000 rpm for 20 h. The concentration of RNA in Tris/EDTA buffer was measured at a wavelength of 260 nm, using a DU-70 spectrophotometer (Beckman, Fullerton, CA). Equal amounts \( (15 \) \( \mu \)g) of total cellular RNA extracted from surgical specimens were loaded onto the lanes of 1.0% agarose/formaldehyde gels and electrophoresed overnight. RNA in the gels was transferred to GeneScreenPlus filters (DuPont, Boston, MA) \( (32) \).

The cDNA insert was \( ^{32}P \) labeled by nick translation and was used to hybridize blots on the filters. Specific activities of the DNA probes were about \( 2-5 \times 10^6 \) cpm/\( \mu \)g of DNA. After overnight hybridization at 42\( ^\circ \)C, followed by washing, the filters were exposed to X-ray films (Kodak X-OMAT AR) at \(-70^\circ \)C. Hybridization signals on the filters were about 2-5 \( \times 10^6 \) cpm/\( \mu \)g of DNA. After overnight hybridization at 42\( ^\circ \)C, followed by washing, the filters were exposed to X-ray films (Kodak X-OMAT AR) at \(-70^\circ \)C.
LAMININ-BINDING PROTEIN mRNA EXPRESSION

CASE 1

1.2kb

TC NC TC NC TC NC TC NC TC NC TC NC

DUKES' D C C B B

Fig. 1. Northern blot analysis of RNA from primary colon carcinoma (TC) and adjacent normal colonic epithelium (NC) with 32P-labeled cDNA encoding the laminin-binding protein.

Clinicopathological Information. Age, sex, tumor location, histology, tumor size, and pathological data relating to depth of invasion, lymph node metastasis, and liver metastasis were obtained from the hospital records for the patients. The study cohort consisted of 8 males and 13 females. The mean age of the patients was 65 years (range, 48 to 82 years). The distribution of primary colon tumors was as follows: cecum, 6; ascending colon, 3; descending colon, 3; sigmoid colon, 6; and rectum, 3. Histologically, 19 tumors were classified as moderately differentiated adenocarcinomas. There was 1 well differentiated tumor and 1 poorly differentiated tumor. The Dukes' staging of the primary tumors was: Dukes' A (tumor invading into but not through the bowel wall, without lymph node involvement), 6; Dukes' B (tumor invading through the bowel wall, without lymph node involvement), 6; Dukes' C (with involvement of regional lymph nodes), 7; and Dukes' D (with distant metastasis), 2.

Statistical Analysis. The tumor/normal ratios of mRNA expression in primary colon cancers were correlated with Dukes' staging, and the degrees of significance were calculated by the Kruskal-Wallis H test.

RESULTS

RNA Blot Hybridization. The cDNA clone which encodes a human laminin-binding protein hybridized with a 1.2-kilobase mRNA, and the level of this mRNA expression was higher in primary colon carcinoma than in adjacent normal colonic epithelium in 20 of 21 patients (Fig. 1). Semiquantitative analysis of the laminin-binding protein expression in these matched pairs of tissues revealed over 150% expression of this mRNA in primary colon carcinoma, when compared to adjacent normal mucosa, in 71% of patients. In 24% of patients, the level of the laminin-binding protein expression in tumors was between 100 and 150%, when compared to adjacent normal tissue (Table 1).

Expression of this mRNA was also greater in colon cancer metastases to liver than in adjacent normal liver tissue for all 6 patients studied. The level of expression in metastatic tumors was 3-fold to 10-fold higher than that in normal liver tissue (Fig. 2 and Table 1).

Correlation of the Laminin-binding Protein mRNA Expression with Clinical and Histological Criteria. There was no correlation of the laminin-binding protein mRNA expression with age, sex, tumor location, or tumor size. Since 19 of 21 tumors were moderately differentiated adenocarcinomas, no valid conclusion regarding tumor differentiation and the laminin-binding protein mRNA expression could be made. The classification of the patients according to the Dukes staging of primary tumor correlated with laminin-binding protein mRNA expression, as shown in Table 2 and Fig. 1. Despite the small sample size used in this study, there was a statistically significant trend towards higher expression of the laminin-binding protein in tumors of advanced Dukes' stage (P < 0.001).

DISCUSSION

One class of receptors, laminin receptors and laminin-binding proteins, are under investigation for their role in tumor invasion and metastasis. Several proteins have been reported to be laminin receptors; the M, 110,000 and 180,000 proteins are involved in neurite formation (33) and the M, 67,000 laminin receptor has an important role in cell attachment and migration (17, 20-25). There may also be a cell adhesion receptor for laminin in the integrin family of adhesion receptors, which are thought to link the cell cytoskeleton to the extracellular environment (34-39). While the question of whether our 1.2-kilobase mRNA encodes this M, 67,000 laminin receptor awaits further investigation, previous communication from our laboratory and other recent investigations showed that the cDNA sequence encoding the M, 32,000 protein contained all the partial sequence of the M, 67,000 laminin receptor reported (26, 40-42). Further, some antibodies raised to the M, 32,000
contain high affinity cell surface binding sites, so-called laminin expression of the laminin-binding protein, at the mRNA level, of differentiation in human colon carcinoma cell lines. Poorly of human melanoma A2058 cells with endogenous laminin, 42). While uncertainties remain as to the exact functional receptors, for laminin (17, 20, 24, 25). The anti-A/r 67,000 protein were shown to react with the M, 67,000 protein by-staging of the primary tumors. Furthermore, liver metastases in freshly obtained primary human colon cancer tissues. More importantly, this expression was shown to correlate with Dukes' progression and biological aggressiveness. In the current investigation, we have demonstrated a significantly high level of mRNA expression of the laminin-binding protein. These data imply that this laminin-binding protein mRNA may be expressed more in invasive tumors or in tumors which metastasize to lymph nodes or to liver. This study of fresh human tissue samples reaffirms the animal data and the work carried out on human cell lines and suggests an important role of laminin receptors/laminin-binding proteins in tumor invasion and metastasis from a more clinical point of view.

Our current efforts to develop monoclonal antibodies against the laminin receptors/laminin-binding proteins may further elucidate this concept by allowing immunohistochemical analysis of archival specimens from patients with defined outcome. Retrospective analysis of the laminin-binding protein expression in these tumors and correlation with the patterns of spread from the 5-year follow-up data of these patients will be carried out to provide further data concerning the role of laminin receptors in the biology of tumor metastasis. In addition to serving as a marker for biological aggressiveness, laminin receptors may provide a target for novel therapeutic strategies. Treating cells with the receptor-binding fragment of laminin(C1 fragment) markedly inhibits or abolishes lung metastases from hematogenously introduced tumor cells (4, 18). The YIGSR pentapeptide elutes the M, 67,000 laminin receptor from a laminin affinity column and also inhibits lung metastases from this mouse tumor and also inhibits lung metastases from this mouse tumor.

Table 2 Tumor/normal ratio of the laminin-binding protein mRNA expression in patients with primary colon carcinoma: correlation with Dukes' classification

<table>
<thead>
<tr>
<th>Tumor/normal ratio</th>
<th>Dukes' classification</th>
<th>Total*</th>
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<tbody>
<tr>
<td>&lt;1.0</td>
<td>A 1 0 0 0</td>
<td>1</td>
</tr>
<tr>
<td>&gt;1.0 and &lt;1.5</td>
<td>B 4 1 0 0</td>
<td>5</td>
</tr>
<tr>
<td>&gt;1.5</td>
<td>C 1 5 7 2</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>D 6 6 2</td>
<td>21</td>
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* P < 0.001.

protein were shown to react with the M, 67,000 protein by Western blotting, suggesting that the two proteins may be immunologically related or the products of related genes (40–42). While uncertainties remain as to the exact functional interplay between these two proteins, their relationship has led to them being referred to together as the “67/32-kD laminin receptor” (40).

Using antibodies against the M, 67,000 laminin receptor, it has been shown that many types of normal and malignant cells contain high affinity cell surface binding sites, so-called laminin receptors, for laminin (17, 20, 24, 25). The anti-M, 67,000 laminin receptor antiserum also blocks the surface interaction of human melanoma A2058 cells with endogenous laminin, resulting in the inhibition of laminin-mediated cell attachment (21). Previous work from our laboratory has analyzed laminin synthesis and attachment to a laminin substratum as a function of differentiation in human colon carcinoma cell lines. Poorly differentiated cell lines showed increased adhesion to a laminin substratum, which could be blocked by YIGSR (Tyr–Ile–Gly–Ser–Arg), a synthetic laminin pentapeptide from the B1 chain (23, 43). Our studies, as well as others, postulate a role for the laminin receptor in colon cancer progression.

Wewer et al. (20) showed that the level of the M, 67,000 laminin receptor mRNA varies in different cell types and correlates with the number of laminin receptors determined by Scatchard analysis of laminin receptor binding assays. These data suggest that the ability to bind to laminin may be controlled by the amount of laminin receptor mRNA available for translation. It is conceivable, therefore, that the laminin-binding protein mRNA expression may be a useful marker for tumor progression and biological aggressiveness. In the current investigation, we have demonstrated a significantly high level of expression of the laminin-binding protein, at the mRNA level, in freshly obtained primary human colon cancer tissues. More importantly, this expression was shown to correlate with Dukes' staging of the primary tumors. Furthermore, liver metastases of colon cancer expressed a high level of mRNA for this laminin-binding protein. These data imply that this laminin-binding protein mRNA may be expressed more in invasive tumors or in tumors which metastasize to lymph nodes or to liver. This study of fresh human tissue samples reaffirms the animal data and the work carried out on human cell lines and suggests an important role of laminin receptors/laminin-binding proteins in tumor invasion and metastasis from a more clinical point of view.

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