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Genes of Laminin B1 Chain, α1 (IV) Chain of Type IV Collagen, and 72-kd Type IV Collagenase Are Mainly Expressed by the Stromal Cells of Lung Carcinomas

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In this study, we analyzed the expression of messenger (m)RNAs for laminin B1 chain, α1 (IV) chain of type IV collagen, and 72-kd type IV collagenase in 15 primary lung carcinomas and in two metastatic adenocarcinomas to the lung. The results show that the mRNA synthesis for these proteins mainly occurs in the stromal fibroblasts and endothelial cells. In a proportion of tumors, mRNAs for laminin B1 chain and 72-kd type IV collagenase could also be observed in carcinoma cells, but the amount of mRNAs was considerably lower in them than in the stromal cells. There were no convincing signals for the presence of the α1 (IV) chain of type IV collagen mRNA in any of the carcinoma cells. A simultaneous expression or lack of expression of signals for laminin B1 chain and 72-kd type IV collagenase mRNAs could be observed in carcinoma cells of 12 cases, suggesting that the activation of these two genes may be somehow connected. There was no association between the mRNA expression and the differentiation degree or the size of the tumors. The occurrence of the mRNA synthesis for the 72-kd type IV collagenase in stromal fibroblasts and endothelial cells indicates that the stromal cells of tumors have a more pronounced impact on the spread of the neoplastic disease than previously thought. The results further show that in their ability to synthesize these proteins the stromal cells of tumors resemble those of developing embryonic tissues. This resemblance is probably connected with the constant remodeling of extracellular matrix in response to the proliferative activity of carcinoma cells. (Am J Pathol 1993, 142:1622–1630)

Basement membranes (BMs) are found in epithelio-mesenchymal interphases where they separate these two types of tissue from each other.[1] They are composed of several proteins, including type IV collagen, laminin, entactin (nidogen), and proteoglycans.[2] In malignant growth, the BMs are more or less disrupted, making it possible for the neoplastic cells to invade the surrounding tissues.[3,4] The disruption of the BMs has been found, at least partly, to be due to various proteases and collagenases that digest the BM structures.[3,5–8]

Type IV collagen, which is the major structural component of BMs, is known to be resistant to the action of interstitial collagenases of types I, II, and III.[9,10] There are two related metalloproteinases, 72-kd and 92-kd type IV collagenases, which cleave native type IV collagen molecules at a single site in the helical region at about 25% of the distance from the amino-terminal end.[11–15] Activity of both of these enzymes has been shown to be increased in malignant transformation.[4,12,16]

So far there is only little knowledge about the cells which produce laminin, type IV collagen, and BM-degrading type IV collagenases. In our previous study, we have shown that the fibroblastic stromal cells of early human placenta are more important for laminin, type IV collagen, and 72-kd type IV collagenase production than has previously been though.[17,18] As to the 72-kd type IV collagenase, it has been shown mainly by *in vitro* studies that some tumor cells such as melanoma cells, as well as human mononuclear phagocytes and proliferating fibroblasts, secrete the enzyme.[19–21] Type IV collagenases with identical or closely related molecular weights

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to 72-kd and 92-kd have also been extracted from the plasma membranes of some carcinoma cells including small cell carcinoma of lung.[22]

The knowledge of type IV collagenases until now is mainly based on biochemical and immunohistochemical investigations. In this study, we investigated the expressions of the genes for the B1 chain of laminin, the α1 (IV) chain of type IV collagen, and the 72-kd type IV collagenase in 17 malignant lung tumors of various histological types using *in situ* hybridization and immunohistochemical methods, with special emphasis on the interrelated expressions of these genes by stromal fibroblasts, endothelial cells, and malignant tumor cells.

Materials and Methods

Seventeen lung carcinomas were collected from the files of the Department of Pathology, Oulu University Central Hospital, between the years 1982 and 1991. Some (nine cases) of the tumor material was obtained fresh from the operation, some (eight cases) was retrospectively collected from the files of the Department of Pathology.

Representative samples of the fresh tumor tissue (also containing the tumor margin) were fixed in 4% paraformaldehyde overnight and washed several times in 10% sucrose at 4 C, after which the tissue was stored in liquid nitrogen. A part of the fresh material was directly frozen and stored in liquid nitrogen. The rest of the tissues was fixed in 10% neutral formalin and embedded in paraffin for conventional light microscopy. The retrospectively collected material had been fixed in 10% formalin and embedded in paraffin.

The diagnosis of all the tumors was based on light microscopy with hematoxylin-eosin and periodic-acid-Schiff stainings, and in selected cases the silver nitrate method of Grimelius according to the criteria of the World Health Organization.[23] The material consisted of nine squamous cell carcinomas, three adenocarcinomas, three small cell lung carcinomas, and two metastatic adenocarcinomas to the lung (one uterine endometrial carcinoma and one breast carcinoma). The clinical data and the histological diagnoses with the degree of differentiation (i.e., the grade of the tumors) are all presented in Table 1.

RNA Probes and in Situ *Hybridization*

A detailed description of the preparation of paraffin and cryostat sections for the in situ hybridization has been previously described.[17] A 906-bp *Pst*I fragment from HL 2 coding for the BII domain of the laminin B1 chain gene,[24] a 916-bp *Bam*HI–Hind III fragment from the HT 21 coding for the NC1 domain and part of the 3′ untranslated region of the α1 (IV) collagen gene,[25] and a 635-bp *Sca*I–Sac I fragment of the K-191 human 72-kd type IV collagenase complementary DNA clone[26] were cloned into the M13 polylinker site of pSP64 and pSP65 vectors (Promega, Madison, WI). For transcription, a riboprobe transcription kit (Promega) was used, and the transcripts were labeled with either [^{35}S]rCTP or [^{35}S]-rUTP, yielding specific activities of 3×10^6 cpm/40

Table 1. *Clinical Data*

Case No.	Age (yr)	Sex	Tumor size (cm)	Tumor type	Location
1	69	M	3.5	S,I	L,I
2	64	F	4.5	S,I	L,U
3	64	M	2.5	S,I	R,U
4	59	M	4	S,II	L,U
5	55	M	2	S,II	L,I
6	66	M	3	S,II	L,I
7	68	M	3	S,II	R,U
8	73	M	9	S,III	L,I
9	57	M	6	S,III	R,I
10	61	M	4	A,II	R,U
11	55	M	4.5	A,III	R,U
12	65	M	2	A,III	R,U
13	70	F	4	A*	R,I
14	59	M	1.5	A†	L,U
15	58	F	3.2	SM	R,I
16	68	M	4	SM	R,I
17	64	F	?	SM	L,?

A = adenocarcinoma; S = squamous cell carcinoma; SM = small cell carcinoma, I = grade I; II = grade II; III = grade III; R = right; L = left; C = central; U = upper; I = inferior; ? = data not available.

* Metastatic adenocarcinoma from the endometrium.

† Metastatic adenocarcinoma from the breast.

μl. All the solutions used with the RNA probes were treated with 0.1% diethylpyrocarbonate (Fluka, Buch, Switzerland).

In situ hybridization with anti-sense and sense RNA probes has been described previously by Autio-Harmainen et al.[17] The prehybridization steps included incubation in 0.2 mol/L HCl (20 minutes, room temperature [RT]) followed by a 5-minute wash in diethylpyrocarbonate-H_2O and proteinase K treatment (0.1 mg to 1 mg/ml, 15 to 30 minutes, 37 C). After the glycine treatment and washings, the proteolysis was stopped by immersing the sections in 4% paraformaldehyde in phosphate-buffered saline for 20 minutes. The sections were acetylated in 0.25% to 0.5% acetic anhydride in 0.1 mol/L triethanolamine for 10 minutes, washed, and after dehydration they were allowed to air-dry for 1 to 2 hours at RT before placing the hybridization mixture on them. The sections were first incubated overnight in the hybridization mixture that did not contain the radioactive probe, after which the radioactive hybridization mixture was placed on the sections. The mixture contained the radioactive RNA probe, 10 mmol/L dithiotreitol, 10 mmol/L Tris-HCl, 10 mmol/L $NaPO_4$, 5 mmol/L ethylenediaminetetraacetic acid, 0.3 mol/L NaCl, yeast transfer tRNA (1 mg/ml), deionized formamide 50% of the volume and dextran sulphate 10% of the volume, 0.02% (w/v) Ficoll, 0.02% (w/v) polyvinylpyrrolidone, and bovine serum albumin (0.2 mg/ml). Of the hybridization mixture, 40 μl (approximately 7×10^5 cpm/10 μl hybridization buffer) was placed on each section, after which the sections were covered with diethylpyrocarbonate-H_2O-washed coverslips. The hybridization took place at 50 C overnight. After hybridization, the coverslips were removed in a washing buffer containing all the other constituents of hybridization mixture except for dextran sulphate and transfer RNA. This took place at 60 C for 2×30 minutes. Slides were then washed in fresh buffer at 50 C for 1 to 4 hours, rinsed in 0.5 mol/L NaCl in 10 mmol/L Tris-HCl, 1 mmol/L ethylenediaminetetraacetic acid (TE) at 37 C for 15 minutes, and they were then incubated in 0.5 mol/L NaCl in TE containing 40 μg/ml RNAse A (Sigma Chemical Co., St. Louis, MO) at 37 C for 30 minutes. Washings proceeded followingly: 0.5 mol/L NaCl in TE (37 C, 15 minutes), 2× standard saline citrate (45 C, 2×15 minutes), 1× standard saline citrate (45 C, 2×15 minutes). After washings, the sections were dehydrated in graded series of ethanol containing 300 mmol/L ammonium acetate and air-dried at RT for 1 to 2 hours.

Autoradiography was performed by dipping the slides into Kodak NTB-3 nuclear track emulsion diluted 1:1 with 1% glycerol in H_2O. After exposing for 7 to 15 days, the slides were developed in Kodak D19 developer at RT for 5 minutes, rinsed in acetic acid, and fixed for 5 minutes at RT. The sections were counterstained with hematoxylin and eosin.

Immunohistochemistry

Antibodies against the 7S domain of type IV collagen and the P1 fragment of laminin were kind gifts from Drs. Leila Risteli, and Juha Risteli, Collagen Research Unit, Department of Medical Biochemistry, University of Oulu, Finland. The 7S domain of type IV collagen was purified from human kidney[27] and the fragment P1 of laminin from human placenta.[28] Antisera were raised in rabbits, and specific antibodies were prepared by immunoadsorption on the relevant antigen, coupled to Sepharose 4B, after cross-adsorption with other immobilized extracellular matrix proteins.

The avidin-biotin complex method[29] was used on sections cut from formalin-fixed, paraffin-embedded specimens. The sections were treated with 0.4% pepsin (Merck, Darmstadt, Germany) in 0.01 mol/L HCl to enhance the availability of antigenic determinants.[30] Before antigen-antibody reaction, the endogenous peroxidase was inactivated with 0.1% hydrogen peroxide in methanol. For control stainings, phosphate-buffered saline and normal rabbit serum were used instead of the primary antibody.

The monoclonal antibody against the human type IV collagenase (72 kd) was purchased from Molecular Oncology Inc. (Gaithersburg, Maryland, Cat #4001), and its preparation has been described previously.[18] For immunohistochemical stainings, 4-μ, paraffin-embedded sections were stained using avidin and biotinylated horseradish peroxidase complex (Dakopatts, Copenhagen, Denmark). Before antigen-antibody reaction, the endogenous peroxidase was inactivated with 0.1% hydrogen peroxide in methanol. The primary antibody was used with a dilution of 1:25. For control stainings, phosphate-buffered saline and normal rabbit serum were used instead of the primary antibody.

Analysis of the Results of the in Situ *Hybridization*

Signals for laminin B1 chain, $\alpha 1$ (IV) chain of type IV collagen, and 72-kd type IV collagenase mRNA were analyzed separately in carcinoma cells, stromal fibroblasts, and endothelial cells by reviewing

the slides in general and counting grains of 50 cells of each cell type randomly in every tumor. The number of grains with the sense probes were subtracted from those of the corresponding anti-sense probes. The number of grains in each cell with the sense probes were in the range of 10.3 to 12.2. The proportion of positive cells in tumor tissue was quantitated as follows: –:none of the cells positive (0%), + = occasional cells positive (<5%), ++ = moderate amount of cells positive (5 to 50%), +++ = most of the cells positive (50 to 90%), ++++ = nearly all or all cells positive (>90%). Number of grains was also evaluated in lung macrophages, alveolar epithelial cells, bronchial epithelial cells, and lymphocytes. Bright-field images were used to analyze the morphological details, whereas the intensity of signals was better visualized in the dark field images.

Results

The results on the expression of laminin B1 chain, α1 (IV) chain of type IV collagen, and 72-kd type IV collagenase mRNA in carcinoma cells, stromal fibroblasts, and endothelial cells in the lung carcinomas are shown in Tables 2 and 3.

Laminin B1 Chain, α1 (IV) Chain of Type IV Collagen and 72-kd Type IV Collagenase mRNA in Carcinoma Cells

Eight tumors had no detectable laminin B1 chain mRNA in carcinoma cells. In nine tumors, such signals could be found. Four of these (three squamous cell carcinomas and the metastatic endometrial adenocarcinoma) contained a moderate number of carcinoma cells giving positive signals for the laminin B1 chain mRNA, whereas in the rest of the tumors (three squamous cell carcinomas, one adenocarcinoma, and the metastatic breast adenocarcinoma) only occasionally could such carcinoma cells be found. There were no carcinoma cells that were convincingly positive for the α1 (IV) chain of type IV collagen mRNA in any of the cases.

Low level of signals for the 72-kd type IV collagenase mRNA was detected in carcinoma cells of seven cases, whereas ten tumors remained negative in this respect. Only in one squamous cell carcinoma was the number of positive cells estimated to be moderate, whereas in all the other positive cases there were only occasional tumor cells which contained mRNA for the enzyme.

In comparing the expressions of the different mRNAs with each other, an association could be detected with respect to the laminin B1 chain and the 72-kd type IV collagenase mRNAs. Simultaneous presence of their mRNAs was observed in tumor cells of six lung carcinomas. Conversely, there were six carcinomas in which the expression of both types of mRNA was negative. This association is statistically significant with the P value of 0.007, according to Fisher's exact probability test. Corresponding statistically significant associations could not be obtained with respect to the differentiation degree of the tumor, the tumor size, or the age of the patients. The tumor cells of small cell carcinomas lacked the expression of mRNAs for all the investigated proteins.

Table 2. *Average* Number of Grains per Cell with Laminin, Type IV Collagen, and 72-kd Type IV Collagenase mRNA Probes in Tumor Cells, Fibroblasts, and Endothelial Cells*

	Laminin	Type IV collagen	Type IV collagenase
Tumor cells	4.4 ± 2.4	0.8 ± 2.9	9.9 ± 0.3
Fibroblasts	16.0 ± 4.2	12.4 ± 2.4	31.9 ± 5.3
Endothelial cells	9.7 ± 5.6	12.8 ± 7.2	17.0 ± 3.1

* The grain counts of sense probes have been substracted from those of the corresponding anti-sense probes.

Laminin B1 Chain, α1 (IV) Chain of Type IV Collagen and 72-kd Type IV Collagenase mRNA in Fibroblasts and Endothelial Cells in Tumor Tissue

Stromal fibroblasts and endothelial cells gave positive signals for laminin B1 chain, α1 (IV) chain of type IV collagen, and 72-kd type IV collagenase mRNA (Figures 1 to 5) in all the 17 cases of the material. The signals for all the three mRNAs were always stronger in fibroblasts and endothelial cells than in carcinoma cells (see Table 3). The signals for the 72-kd type IV collagenase and for the α1 (IV) chain of type IV collagen mRNAs were stronger than those for the laminin B1 chain mRNA (see Table 3). Cells surrounding the tumor cell islands gave stronger signals for laminin, type IV collagen, and 72-kd type IV collagenase mRNA than those which were located more distantly in the stroma.

We could not detect any convincing signals for laminin B1 chain, α1 (IV) chain of type IV collagen, or 72-kd type IV collagenase mRNA in normal alveolar macrophages, bronchial epithelial cells, lymphocytes, or alveolar epithelial cells. We found no notable differences in the grain counts between

Table 3. *Results of in* Situ *Hybridization*

	Laminin B1 chain			α1 (IV) chain of type IV collagen			72-kd type IV collagenase		
	T	F	E	T	F	E	T	F	E
					Squamous cell carcinomas				
1	+	++	++	–	–	++	+	++++	+++
2	++	++	+	–	+++	+++	++	++++	++++
3	–	++	+++	–	++	++++	–	++++	+++
4	–	+	++	–	+	++	–	++	++
5	–	++	++	–	–	++	+	++++	++++
6	+	+	*	–	++	++	+	+	*
7	++	++	+++	–	+	++	–	++	++
8	++	++	+++	–	++	++	+	+++	+++
9	+	–	*	–	++	++	–	–	*
					Adenocarcinomas				
10	–	–	+	–	++	+++	–	++	++
11	+	+++	++	–	++	++	+	++	++
12	–	++	++	–	–	*	–	++	++
					Metastatic adenocarcinomas				
13	++	++	++	–	+	+++	+	++	++
14	+	++	++	–	+	+	–	++	++
					Small cell carcinomas				
15	–	+	+	–	+	++	–	++	++
16	–	–	+	–	–	+++	–	–	+
17	–	+	*	–	++++	*	–	–	*

T = tumor cells; F = fibroblasts; E = endothelial cells.

Scoring of *in situ* hybridization findings: –, none of the cells positive (0%); +, occasional cells positive (<5%); ++, moderate amount of cells positive (5 to 50%); +++, most of the cells positive (50 to 90%); ++++, nearly all or all cells positive (>90%); *, indeterminate.

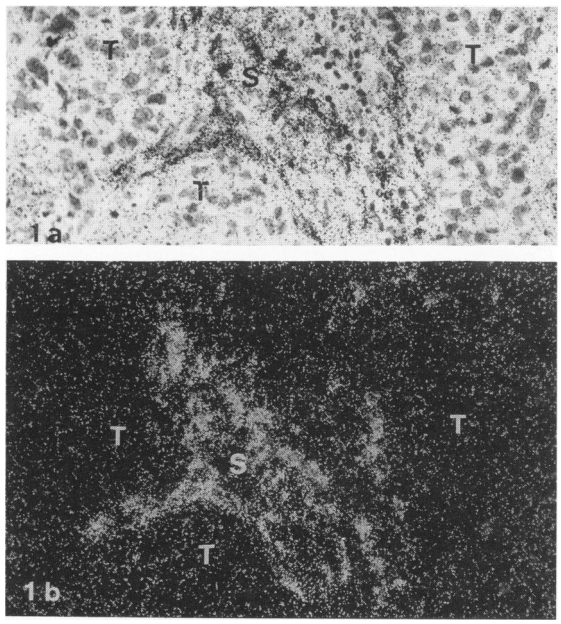

Figure 1. *In a squamous cell carcinoma (case 3), signals for the α1 (IV) chain mRNA of type IV collagen can be seen in the stromal cells (S), whereas the tumor cells (T) are negative. Bright-field* (a) *and dark-field* (b) *images of the same area* (in situ *hybridization,* ×*265).*

formalin-fixed older tumor samples or paraformaldehyde-fixed fresh tumor samples. No notable differences were found in the grain counts in different tumor areas, e.g., the marginal areas versus the central areas with any of the probes.

Immunohistochemistry

Thirteen cases were stained with the antibodies to laminin and type IV collagen. The antibodies stained the BMs of capillaries and poorly organized, interrupted BMs around the carcinoma cell islands. Four of the tumors expressed intracytoplasmic positivity for laminin in carcinoma cells, whereas there was no intracytoplasmic staining for type IV collagen in any of the cases. All the tumors containing intracytoplasmic laminin immunoreactivity also contained signals for laminin B1 chain mRNA in the *in situ* hybridization. There were two cases, however, in which no intracytoplasmic immunoreactivity was found, even though tumor cells expressed mRNA for the B1 chain of laminin. In line with the *in situ* hybridization results, no intracytoplasmic immunoreactivity was found for type IV collagen in any of the 13 cases stained.

Twelve of the tumors were analyzed for the immunohistochemical expression of the 72-kd type IV collagenase. In 11 cases, positive immunohistochemistry was observed in carcinoma cells (Figure 6). Eight cases gave parallel results by immunohistochemistry and *in situ* hybridization, whereas in four cases, the carcinoma cells were positive by immunohistochemistry but did not express detectable amounts of 72-kd type IV collagenase mRNA. Fibroblasts and endothelial cells showed strong staining

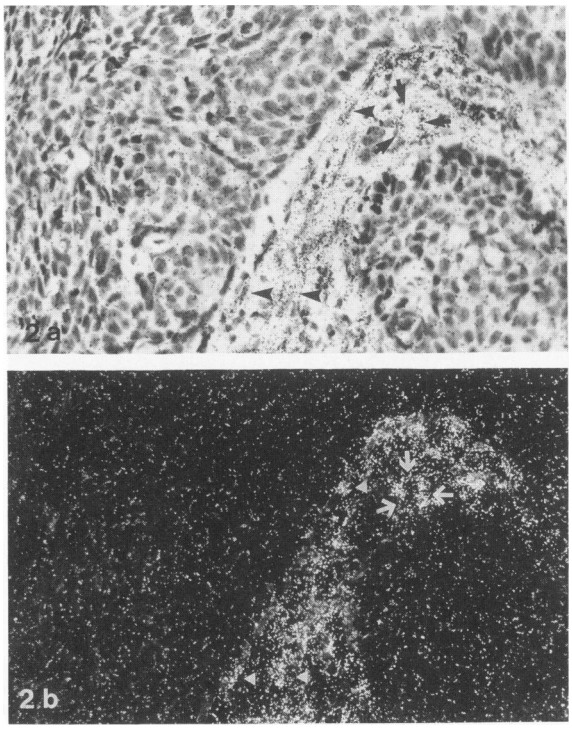

Figure 2. *Hybridization signals for the 72-kd type IV collagenase mRNA is present in the stromal fibroblasts (arrowheads) and endothelial cells (arrows) in the same tumor as in Figure 1. Tumor cells (T) are again negative. Bright-* (a) *and dark-field* (b) *(4) images of the same area* (in situ *hybridization,* ×265).

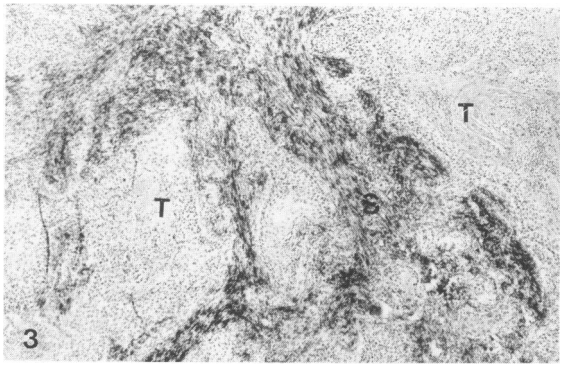

Figure 3. *In another case of a squamous cell carcinoma (case 1), strong signals for the 72-kd type IV collagenase can be seen in the stromal cells (S), whereas the tumor cells (T) are negative* (in situ *hybridization,* ×105).

for the antibody to the 72-kd type IV collagenase in all the cases.

Discussion

In this study we used *in situ* hybridization for the analysis of the expression of the B1 chain of laminin, the α1 (IV) chain of type IV collagen, and the 72-kd type IV collagenase mRNAs in 15 primary lung carcinomas that represented different histological types of World Health Organization classification and two metastatic adenocarcinomas to the lung. The results showed that mRNAs for all three molecules can be found mainly in stromal fibroblasts and in endothelial cells. In a portion of cases, however, the tumor cells also synthesized laminin B1 chain and 72-kd type IV collagenase mRNAs, the amount of signals remaining, however, considerably lower in them than in the surrounding fibroblasts or endothelial cells. Synthesis of the α1 (IV) chain of type IV collagen could not be observed in tumor cells at all.

Stromal cells seem to be the most important source of laminin, type IV collagen, as well as 72-kd type IV collagenase in lung carcinomas. This result emphasizes the primitive nature of tumor stroma, and the findings correspond well with those we have previously obtained from developing human placenta where the villous stromal cells also form an important site for the production of both BM proteins and their degrading enzymes.[17,18] Carcinoma cells themselves that have been traditionally thought to be mainly responsible of the BM formation around the tumor islands are in fact quite inactive to express the synthesis of the BM proteins.

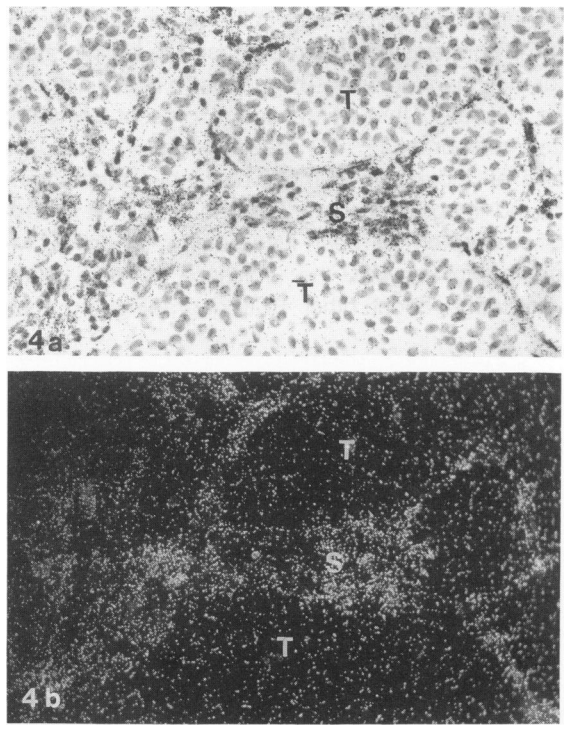

Figure 4. *In the metastatic breast carcinoma to lung (case 14), the stromal cells (S) express the mRNA for laminin B1 chain. Bright-* (a) *and dark-field* (b) *images of the same area* (in situ *hybridization,* ×265).

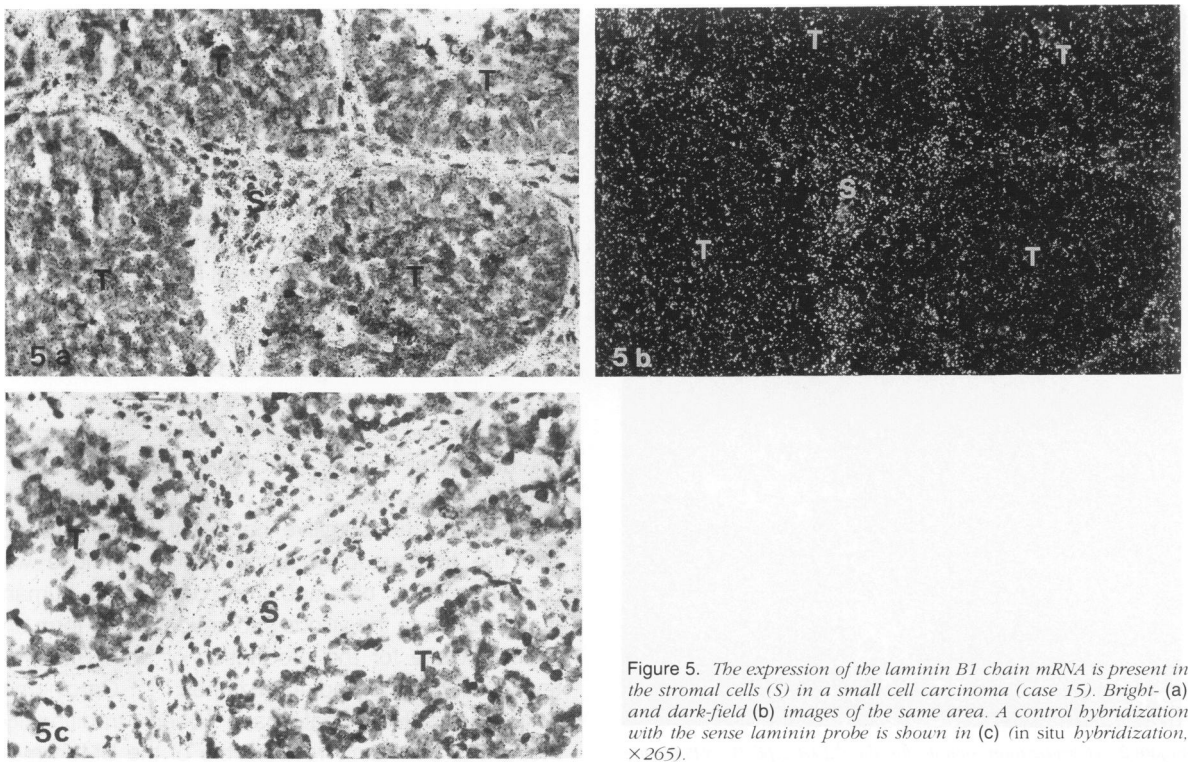

Figure 5. *The expression of the laminin B1 chain mRNA is present in the stromal cells (S) in a small cell carcinoma (case 15). Bright-* (a) *and dark-field* (b) *images of the same area. A control hybridization with the sense laminin probe is shown in* (c) (in situ *hybridization,* ×265).

Thus, there must be more complex mechanisms in the BM deposition and degradation than has been usually thought.

Adhesion of tumor cells to the extracellular matrix (ECM) proteins has been proposed to be important for malignant growth.[3] This adhesion is explained to be mediated through receptors present on tumor cell membranes.[31–33] Specific laminin receptors and an increasing number of various membrane-bound integrins mediate the adhesion.[33,34] A specific receptor has been also purified for one ECM-degrading enzyme, the urokinase.[35] There is no knowledge about other ECM-degrading enzymes that would function through membrane-bound receptors. Our findings of a strong mRNA signal for the 72-kd type IV collagenase in tumor stroma in relation to the low or absent signal in carcinoma cells added by the immunohistochemical presence of the enzyme in them would suggest that the function of type IV collagenase might also be mediated through a receptorlike mechanism, similarly to the activation of plasminogen by urokinase. A receptor-linked type IV collagenase could also explain the occurrence of membrane-bound type IV collagenases that have been shown to be present on some carcinoma cells, including the NCI-H82 small cell lung carcinoma cells.[22]

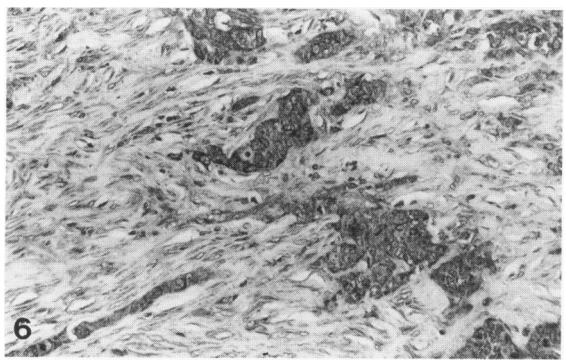

Figure 6. *Immunohistochemical demonstration of 72-kd type IV collagenase in carcinoma cells and stromal fibroblasts in a poorly differentiated squamous cell carcinoma (immunoperoxidase stain* ×265).

Contrary to *in situ* hybridization findings, immunohistochemistry showed the presence of 72-kd type IV collagenase in carcinoma cells. This finding is compatible with two recent investigations, in which similar immunohistochemical reactivity was reported in a majority of colon[36] and invasive breast carcinomas.[37] Even though we could find evidence of 72-kd type IV collagenase synthesis in carcinoma cells of about half of the cases, the main sources of the enzyme in lung tumors, however, are stromal fibroblasts or endothelial cells. The discrepant results obtained by *in situ* hybridization and immunohistochemistry could again be explained by assuming that the neoplastic cells contain receptors that bind

the 72-kd type IV collagenase. Another alternative is that there is a difference in the rate of production of 72-kd type IV collagenase between carcinoma and stromal cells and that carcinoma cells could be able to store the enzyme in the cytoplasm on the basis of a different secretory pathway these cells might use.[38]

Synthesis of 72-kd type IV collagenase mRNA in stromal fibroblasts and in endothelial cells was evident in every lung tumor studied, but the amount of signals was most pronounced in squamous cell carcinomas. This finding may simply be explained by the methodological factors, or it may be a real finding that differentiates squamous cell carcinomas from other types of lung tumors. It is known that the synthesis of 72-kd type IV collagenase is induced by transforming growth factor-β.[39] It has also been shown that non-small cell lung carcinoma cell lines are able to synthesize transforming growth factor-β.[40] Perhaps the higher level of 72-kd type IV collagenase in the stroma of squamous cell carcinomas reflects the higher synthetic capacity of this growth factor by tumor cells of squamous carcinoma.

In comparing the synthesis of the three investigated types of mRNA molecules with each other, we noted an interesting association between the expressions for laminin B1 chain and 72-kd type IV collagenase mRNAs by the tumor cells. Whenever the tumor cells expressed laminin, they also expressed type IV collagenase mRNAs and vice versa. This may suggest that the synthesis of these two proteins is linked together. Perhaps the activation of laminin B1 chain and 72-kd type IV collagenase genes are somehow interrelated.

In summary, the main finding of this study was to locate the synthesis of mRNAs for laminin B1 chain, α1 (IV) chain of type IV collagen, and 72-kd type IV collagenase in the stromal cells of lung carcinomas. This finding emphasizes the importance of tumor stroma for malignant growth and spread. The synthesis of BM proteins and the degrading 72-kd type IV collagenase by the same cells indicate a simultaneous activation of the genes that encode these proteins. This is probably related to the fact that the neoplastic tissue requires a constant remodeling of ECM in response to the proliferative activity of the carcinoma cells and to the activity of various growth factors involved in the process.

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References

1. Martinez-Hernandez A, Amenta PS: The basement membrane in pathology. Lab Invest 1983, 48:656–677
2. Timpl R: Structure and biological activity of basement membrane proteins. Eur J Biochem 1989, 180:487–502
3. Liotta LA: Tumor invasion and metastases: role of the extracellular matrix. Cancer Res 1986, 46:1–7
4. Tryggvason K, Höyhtyä M, Salo T: Proteolytic degradation of extracellular matrix in tumor invasion. Biochem Biophys Acta 1987, 907:191–217
5. Barsky H, Siegal GP, Janiotta F, Liotta LA: Loss of basement membrane components by invasive tumors but not by their benign counterparts. Lab Invest 1983, 49:140–147
6. Bosman FT, Havenith M, Cleutjens JPM: Basement membranes in cancer. Ultrastruct Pathol 1985, 8:291–301
7. Salo T, Liotta LA, Keski-Oja J, Turpeenniemi-Hujanen T, Tryggvason K: Secretion of basement membrane collagen degrading enzyme and plasminogen activator by transformed cells—role in metastasis. Int J Cancer 1982, 30:669–673
8. Shields SE, Ogilvie DJ, McKinnell RG, Tarin D: Degradation of basement membrane collagens by metalloproteases released by human, murine and amphibian tumors. J Pathol 1984, 143:193–197
9. Liotta LA, Abe S, Robey PG, Martin GR: Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. Proc Natl Acad Sci USA 1979, 76:2268–2272
10. Sage H, Woodbury RG, Bornstein P: Structural studies on human type IV collagen. J Biol Chem 1979, 254: 9893–9900
11. Fessler L, Duncan K, Fessler JH, Salo T, Tryggvason K: Characterization of the procollagen IV cleavage products produced by a specific tumor collagenase. J Biol Chem 1984, 259:9783–9789
12. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature 1980, 284:67–68
13. Murphy G, Ward R, Hembry RM, Reynolds JJ, Kühn K, Tryggvason K: Characterization of gelatinase from pig polymorphonuclear leucocytes. Biochem J 1989, 258: 463–472
14. Salo T, Liotta LA, Tryggvason K: Purification and characterization of a murine basement membrane collagen degrading enzyme secreted by metastatic tumor cells. J Biol Chem 1983, 258:3058–3063
15. Wilhelm SM, Collier IE, Marmer BL, Eisen AZ, Grant GA, Goldberg GL: SV-40-transformed human lung fibroblasts secrete a 92-kDa type IV collagenase which

is identical to that secreted by normal human macrophages. J Biol Chem 1989, 264:17213–17221
16. Collier IE, Wilhelm SC, Eisen AZ, Marmer BL, Grant GA, Seltzer JL, Kronberger A, He C, Bauer EA, Goldberg GI: *H-ras* oncogene-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. J Biol Chem 1988, 263:6579–6587
17. Autio-Harmainen H, Sandberg M, Pihlajaniemi T, Vuorio E: Synthesis of laminin and type IV collagen by trophoblastic cells and fibroblastic stromal cells in the early human placenta. Lab Invest 1991, 64:483–491
18. Autio-Harmainen H, Hurskainen T, Niskasaari K, Höyhtyä M, Tryggvason K: Simultaneous expression of 72 kDa type IV collagenase and type IV collagen alpha1 (IV) chain genes by cells of early human placenta and gestational endometrium. Lab Invest 1992 (accepted)
19. Garbisa S, Ballin M, Daga-Gordini D, Fastelli G, Naturale M, Negro A, Semenzato G, Liotta LA: Transient expression of type IV collagenolytic metalloproteinase by human mononuclear phagocytes. J Biol Chem 1986, 261:2369–2375
20. Höyhtyä M, Hujanen E, Turpeenniemi-Hujanen T, Thorgeirsson U, Liotta LA, Tryggvason K: Modulation of type IV collagenase activity and invasive behaviour of metastatic tumor melanoma (A-2058) cells in vitro by monoclonal antibodies to type IV collagenase. Int J Cancer 1990, 46:282–286
21. Salo T, Turpeenniemi-Hujanen T, Tryggvason K: Tumor promoting phorbol esters and cell proliferation stimulates secretion of basement membrane (type IV) collagen degrading metalloproteinase by human fibroblasts. J Biol Chem 1985, 260:8526–8531
22. Zucker S, Wieman J, Lysik RM, Imhof B, Nagase H, Ramamurthy N, Liotta LA, Golub LM: Gelatin-degrading type IV collagenase isolated from human small cell lung cancer. Invasion Metastasis 1989, 9:167–181
23. World Health Organization. Histological Typing of Lung Tumors, 2nd ed. International classification of tumors, No 1. Geneva, World Health Organization, 1981
24. Pikkarainen T, Eddy R, Fukushima Y, Byers M, Shows T, Pihlajaniemi T, Saraste M, Tryggvason K: Human laminin B1 chain. A multidomain protein with gene (LamB1) locus in the g22 region of chromosome 7. J Biol Chem 1987, 262:10454–10462
25. Pihlajaniemi T, Tryggvason K, Myers JC, Kurkinen M, Lebo R, Cheung MC, Procop DJ, Boyd CD: cDNA clones coding for the Pro-alpha1 (IV) chain of human type IV procollagen reveal an unusual homology of amino acid sequences in two halves of the carboxy-terminal domain. J Biol Chem 1985, 260:7681–7687
26. Huhtala P, Chow LT, Tryggvason K: Structure of the human type IV collagenase gene. J Biol Chem 1990, 265:11077–11082
27. Risteli J, Bächinger HP, Engel J, Furthmayr H, Timpl R: 7-S collagen: characterization of an unusual basement membrane structure. Eur J Biochem 1980, 108: 239–250
28. Risteli L, Timpl R: Isolation and characterization of pepsin fragments of laminin from human placenta and renal basement membranes. Biochem J 1981, 193: 749–755
29. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 1981, 29:577–580
30. Ekblom P, Miettinen M, Rapola J, Foidart JM: Demonstration of laminin, a basement membrane glycoprotein in routine processed formalin fixed human tissues. Histochemistry 1982, 75:301–307
31. Malinoff HL, Wicha MS: Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J Cell Biol 1983, 96:1475–1479
32. Rao CN, Barsky SH, Terranova VP, Liotta LA: Isolation of a tumor cell laminin receptor. Biochem Biophys Res Comm 1983, 111:804–808
33. Terranova VP, Rao CN, Kalebic T, Margulies IM, Liotta LA: Laminin receptor on human breast carcinoma cells. Proc Natl Acad Sci USA 1983, 80:444–448
34. Ruoslahti E: Integrins. J Clin Invest 1990, 87:1–5
35. Ellis V, Behrendt M, Dano K: Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. J Biol Chem 1991, 266:12752–12758
36. Levy AT, Cioce V, Sobel ME, Garbisa S, Grigioni WF, Liotta LA, Stetler-Stevenson W: Increased expression of the Mr 70,000 type IV collagenase in human colonic adenocarcinoma. Cancer Res 1991, 51:439–444
37. Monteagudo C, Merino MJ, San-Juan J, Liotta LA, Stetler-Stevenson WG: Immunohistochemical distribution of type IV collagenase in normal, benign, and malignant breast tissue. Am J Pathol 1990, 136:585–592
38. Rivas RJ, Moore H-P: Spatial segregation of the regulated and constitutive secretory pathways. J Cell Biol 1989, 109:51–60
39. Salo T, Lyons JG, Rahemtulla F, Birkedal-Hansen H, Larjava H: Transforming growth factor β1 up-regulates type IV collagenase expression in cultured human keratinocytes. J Biol Chem 1991, 266:11436–11441
40. Bergh JCS: Gene amplification in human lung cancer. The *myc* family genes and other proto-oncogenes and growth factor genes. Am Rev Respir Dis 1990, 142: S20–26