Human ductal adenocarcinomas of the pancreas express extracellular matrix proteins*

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Summary Pancreatic ductal adenocarcinomas are characterised by a dense connective tissue reaction. To test the hypothesis that stroma components are synthesised and produced by the tumour cells themselves, eight cell lines as well as six xenografted tumours from human ductal adenocarcinomas of the pancreas were examined for the expression of extracellular matrix proteins (ECM), using cDNA probes and antibodies to collagen types I, III and IV, vitronectin, fibronectin, undulin and laminin. All tumour cell lines (CAPAN-1, CAPAN-2, AsPC-1, BxPC-3, PANC-1, PaCa-2, PaCa-3, PaCa-44) and xenografted human pancreatic tumours expressed at least one of the examined ECM at the RNA (collagen type IV > laminin = fibronectin = vitronectin > collagen type III > undulin > collagen type I) or protein level (collagen type IV = collagen type III > vitronectin > laminin > collagen type I = fibronectin > undulin). In nude mouse tumours expression of laminin and collagen I was most pronounced in well-differentiated carcinomas. In a few tumours, collagen type III, vitronectin and undulin were expressed on the luminal side of the neoplastic glands, suggesting loss of normal polar differentiation. Incubation with fetal calf serum modulated ECM RNA levels to a varying extent in all but one cell line (AsPC-1). The results suggest that human pancreatic ductal adenocarcinomas cells are capable of synthesising and producing extracellular matrix proteins in vitro and in vivo, but that the extent and pattern of ECM expression differs between the various tumours and conditions tested.

Well-differentiated ductal adenocarcinomas of the pancreas show abundant dense stroma, usually intact basal membranes and regular laminin deposits, while these features are either inconspicuous or even absent in poorly differentiated ductal carcinomas of the pancreas (Klöppel & Fitzgerald, 1986; Klöppel et al., 1985). The factors which determine the stromal development in these pancreatic carcinomas are not known. Immunocytochemical, radioimmunological and biochemical in vivo and in vitro studies have indicated that tumours of epithelial origin, including pancreatic carcinoma, can produce extracellular matrix proteins (ECM), notably laminin (Haglund et al., 1984; 1989; Altshuler et al., 1981; Haberern-Blood et al., 1987; Mahlbacher et al., 1992) and that in cell cultures laminin appears to increase tumour cell polarity and exocytosis (Mollenhauer et al., 1987).

As these findings suggest a role for laminin and possibly also other ECM in pancreatic carcinoma differentiation, it is important to know whether the expression of ECM can be indeed traced back to the RNA level of the tumours and correlated to their morphology. Our study therefore addressed the following questions: are ductal pancreatic adenocarcinoma cells capable of producing different components of the extracellular matrix in vitro and in vivo?; is the expression influenced by the degree of tumour differentiation?; and can the expression of ECM be modulated by the tissue culture conditions?

Materials and methods

Tumour cell lines

Cell lines from human pancreatic ductal adenocarcinomas were either obtained from ATCC (Bethesda, MD, USA) (AsPC-1, BxPC-3, CAPAN-1, CAPAN-2, PANC-1) or donated by J. Mollenhauer (Rush University, Chicago, IL, USA) (PaCa-2, PaCa-3, PaCa-44). These cell lines have already been described in detail (Kyriazis et al., 1982; Haberern & Kupchik, 1985; Liewh et al., 1990; Mai et al., 1990; Haberern-Blood et al., 1987; Klöppel et al., 1985). The PaCa-2 and PaCa-3 cell lines were established from the corresponding nude mouse tumours (see below). The PaCa-2 cell line has been renamed PaTu-II in some publications (Mai et al., 1990). Cells were grown in the appropriate culture medium (RPMI and DMEM respectively) containing 10% fetal calf serum (FCS), supplemented with 1% penicillin and 1% streptomycin. Batches of the first expansion were frozen down in order to obtain large stocks for all further experiments. The purity of tumour cell lines with regard to contamination by fibroblasts was monitored by phase-contrast microscopy, haematoxylin and eosin (H&E) staining and immunofluorescence for cytokeratin (Trautmann et al., 1993; Rafiee et al., 1992).

For the serum deprivation studies, cells from confluent monolayers were seeded in new tissue culture flasks in medium containing 10% FCS. After 48 h, cells were washed twice with sterile phosphate-buffered saline (PBS) and then incubated for 2–5 days in serum-free medium, depending on the growth characteristics. RNA was then harvested from proliferating cultures (see below).

Xenografted pancreatic ductal adenocarcinomas

Freshly isolated tumour tissue was transplanted subcutaneously on to nu/nu mice as described previously (Klöppel et al., 1985; Giovanna & Fogh, 1985). The PaCa-2, PaCa-3 and PaCa-39 tumours have been studied previously (Klöppel et al., 1985). Tumour histology was checked after the first passage and compared with that of the primary tumour (Klöppel et al., 1985). For further studies, tumours were grown for at least three passages, excised from the anaesthetised animals and immediately frozen in liquid nitrogen. The histological differentiation of the tumours was determined as previously described (Klöppel et al., 1985).

RNA extraction

Preparation of RNA from fresh-frozen tissue and cultured cells was carried out as described previously (Chirgwin et al., 1979). In brief, minced tissue and cells were disintegrated by ultrasound in the presence of a chaotropic agent (sodium...
dodecyl sulphate, SDS), mixed with guanidium thiocyanate (GTC), subjected to centrifugation, extracted with phenol/ chloroform, precipitated and finally assessed for quality and quantity by mini-gel electrophoresis and spectrophotometry (Maniatis et al., 1982).

**Slot-blot hybridisation and densitometry**

RNA slot-blot analysis was performed as described previously (Löhr & Oldstone, 1990). In brief, 25 μg RNA aliquots were denatured at 65°C for 15 min in 6 x SSC (1 x SSC = 0.15 M sodium chloride, 0.015 M trisodium citrate), 7.4% formaldehyde, then serially diluted in 15 x SSC. Samples were applied to nitrocellulose membranes using a 72 slot mini-fold blot apparatus. Nitrocellulose membranes were baked for 2 h at 80°C, prehybridised for 4 h at 37°C in 50% deionised formamide, 5 x SSC, 2.5 x Denhardt’s solution, 100 μg/ml boiled, sonicated salmon sperm DNA, and then hybridised for 24 h between 37°C and 42°C with the labelled probes (see below). After hybridisation, membranes were washed in 2 x SSC, 0.1% SDS, at 37°C for 30 min, in 0.1 x SSC, 0.1% SDS, at 55°C or 65°C for 30 min, then exposed against Kodak XAR-5 film at −70°C with Cronex lighting plus an intensifying screen. Following exposure to film, membranes were washed in 0.1 x SSC, 0.1% SDS, at 85°C for 2 h to remove cDNA hybrids. After re-exposure to film to ensure efficient loss of hybridisation signal, membranes were re-used in hybridisation experiments. Hybridisation experiments were performed at least twice with separate RNA preparations for all cell lines and ECM probes.

For densitometry of the RNA from pancreatic tumours transplanted to nude mice, films were scanned with an LKB Ultrascan XL laser densitometer (LKB/Pharmacia, Sweden). In order to maintain an internal standard for these experiments and as an additional control to ensure that mRNA levels were measured for equivalent amounts of total cellular RNA, levels of ECM RNA were related to the individual 28S ribosomal RNA levels (Lipkin et al., 1988; Bowles et al., 1986). For each sample, the ratio of the ECM hybridisation intensity to the ribosomal hybridisation signal was calculated. These ratios were used to compare the different gradings of each nude mouse tumour and controls.

Beside the calculation of relative densities, the hybridisation signal is rated in a semiquantitative grading. A very weak or absolutely no signal corresponding to densitometry readings ≤ 0.100 was rated 0; a weak signal corresponding to densitometry readings from 0.100 to 1.0 was rated 1; a signal corresponding to densitometry readings from 1.0 to 5.0 was rated 2; a signal corresponding to densitometry readings ≥ 5.0 was rated 3.

**Probes**

All cDNA probes were obtained as plasmids and transformed in competent bacteria (Escherichia coli, DH5α) (Maniatis et al., 1982). The probes encoded human specific sequences within the DNA of the examined ECM. According to sequence comparison analysis performed by the investigators these differ from the murine counterpart by 20–30%.

The collagen plasmids were a gift from E. Vworio [collagen types I (pHALCL1U) (Mäkela et al., 1988) and III (pHFS3) (Sandberg & Vuroio, 1987)] and D. Prockop, Jefferson, Philadelphia, PA, USA (type IV, HT21; Phialajani et al., 1985). The probe for fibronectin was donated by R.O. Hynes, MTT, Cambridge, MA, USA (Schwarzbauer et al., 1983), van Dyk and Smith, Scripps Clinic, La Jolla, CA, USA (Seifert et al., 1991), undulin by D. Schuppan, Freie Universität, Berlin, Germany (Just et al., 1991) and laminin (B-chain) by J. Uitto, Jefferson University, Philadelphia, PA, USA (Olsen et al., 1989). Control probes included oste to the 28S ribosomal RNA (S138, a gift from P. Southern, University of Minnesota, MN, USA; Lipkin et al., 1988) and one to the ribosomal protein S6 (J. Kruppa, Hamburg, Germany; Heinzle et al., 1988). Probes were prepared by the random hexoprimr method with 32P (Feinberg & Vogelstein, 1983), yielding specific activities in the range of 5 x 106 c.p.m. μl−1.

For each hybridisation, 1–2 x 106 c.p.m. probe per ml of hybridisation mix was used.

**Immunofluorescence**

Cultured pancreatic tumour cells and cultured human foreskin fibroblasts as well as 5 μm thick cryostat sections of xenografted human pancreatic carcinomas, and of normal pancreatic tissue obtained from eight organ donors (Trautmann et al., 1993) were fixed in acetone at −20°C for 10 min and immunostained by indirect immunofluorescence. The primary antibodies were against procollagen type I (carboxy-terminal, monoclonal mouse IgG; Chemicon, Tecomula, CA, USA), collagen type III (polyclonal rabbit IgG; Pasteur Diagnostika, Lyon, France), collagen type IV and laminin (polyclonal rabbit-IgG; Heyl Chemie, Berlin, Germany), fibronectin (cell-binding fragment, monoclonal mouse IgG; Boehringer, Mannheim, Germany), vitronectin (polyc
c

**Immunocytochemistry**

Cryostat sections of the xenografted pancreatic carcinomas were immunostained by the avidin–biotin–peroxidase method (Dakopatts) as described previously (Löhr & Oldstone, 1990). The primary antibodies different from those listed above were against collagen type I (polyclonal rabbit antiserum; Chemicon) and fibrinectin (polyclonal rabbit IgG; Dakopatts). As a secondary antibody, biotinylated goat anti-rabbit IgG (Dakopatts) was employed at a 1:20 dilution.

Specificities and optimal working dilutions of antibodies for both immunofluorescence and immunocytochemistry were tested in tissue sections for normal pancreas and pancreatic carcinomas. Staining patterns of ECM on this normal control pancreas were identical to those reported previously (Uscanga et al., 1984). Controls included incubations with rabbit and mouse gamma-globulins (6 and 8 μg of protein per ml; Jackson Immunoresearch Lab., West Grove, PA, USA) instead of the primary antibody as well as incubations with the secondary antibody only. All polyclonal antibodies were preabsorbed with the corresponding rodent ECM according to the specifications of the manufacturers. Slides were viewed and photographed with a Carl Zeiss ‘Axiopt’ microscope. Staining of the tissue was graded semiquanti
tatively as follows: no staining was rated as 0 [if there were very few cells positive (focus), this was noted separately]; weak staining intensity of all cells was rated 1, moderate staining 2 and strong staining 3.

**Electron microscopy**

Cell lines in culture were fixed in 2.5% glutaraldehyde and 0.1% cacodylate buffer at pH 7.4, followed by fixation with 1% osmium tetroxide in the same buffer, and then pelleted, dehydrated and embedded in Spurr’s resin (Löhr et al., 1989). For assessment of the ultrastructural differentiation of the tumour cell lines, a modification of the grading system described by Kern et al. (1986) was used. Experiments were performed repeatedly on different samples and different passages of tumour cell lines.
Results

Grade of differentiation

Ultrastructural analysis revealed that the CAPAN-1 cell line showed the characteristics of grade 1 tumour, the CAPAN-2, BxPC-3, AsPC-1, PANC-1 cell lines those of grade 2 and the PaCa-2, PaCa-3 and PaCa-44 cell lines those of grade 3. The nude mouse tumours, KLE, PANK and PaCa-39, were graded 1, SCHU graded 1–2 and PaCa-2 and PaCa-3 graded 3. In brief, grade 1 tumours were characterised by regularly shaped nuclei, junctional complexes, numerous microvilli on the cell surfaces and abundant apical mucin granules. Grade 2 tumours showed some irregularity of the nuclei, polytopic distribution of microvilli, few mucin granules and vesicles filled with membrane material. In grade 3 tumours, the cells had polymorphous nuclei and abortive microvilli, but no desmosomes or mucin granules. Contaminating fibroblasts could not be detected in any of the cell lines.

RNA levels of ECM in tumour cell lines and nude mouse tumours

RNA hybridisation signals of the pancreatic tumour cell lines to ECM probes are shown in Figure 1a and the semiquantitative evaluation in Table I. For this analysis, all cell lines were evaluated under culture conditions with FCS-containing medium. All tumour cell lines showed RNA levels for collagen type IV, 7/8 for laminin, 6/8 for fibronectin, 5/8 for vitronectin and collagen type I, 3/8 for collagen type III and 2/8 for undulin (Figure 1a, Table I). In some instances, only

![Figure 1](image-url)
trace RNA amounts of questionable significance could be detected (Figure 1a, Table I). The relative densitometry readings of the tumour cell RNA are shown in Table IIa. There was no correlation between tumour grade and ECM expression patterns.

Of the nude mouse tumours, 5/6 expressed collagen type III and all type IV collagen, fibronectin, vitronectin, laminin and undulin, but none collagen type I (Figure 1b, Table III). The relative densitometry readings of ECM of the nude mouse tumour RNA are listed in Table IIb. Grade I and 2 tumours expressed higher levels of fibronectin and collagen type III than grade 3 tumours. Collagen type III, fibronectin, undulin and vitronectin were all present at higher levels in the grade I tumours compared with the normal pancreas (Table IIb).

### ECM expression

Tables I and III summarise the staining results of the cultured tumour cells and the nude mouse tumours. Positive staining was demonstrated for several ECM in all cell lines or nude mouse tumours. Seven of eight cell lines were positive for collagen type IV and 4/8 for laminin; all were positive for collagen type III, 6/8 for vitronectin, 2/8 for procollagen type I (Figure 2), but all were negative for undulin and fibronectin. Of the six tumours transplanted into nude mice, all tumours were positive for type IV collagen and 4/6 for laminin; all stained positive for type III collagen, fibronectin and vitronectin, 4/6 were positive for collagen type I and 3/6 were positive for undulin (Table III). Staining for laminin and collagen type I was related to the tumour grade (Table III, Figure 3). In addition to the expression of ECM on the basal side of the tumour cells, collagen type III, vitronectin and undulin were expressed on the luminal/apical side of the cells in some of the tumours (Figure 4). Staining of normal control pancreas with all antibodies revealed linear deposits of basement and interstitial ECM components, as has been reported (data not shown) (Usanga et al., 1984).

### Modulation of ECM expression by FCS

Incubation of tumour cells with and without FCS (Table IV, Figure 1a) revealed different effects on RNA expression in the various cell lines. Most ECM RNAs were up-regulated in the BxPC-3 cell line, but down-regulated in CAPAN-1 cells. AsPC-1 cells were unaffected by FCS deprivation. Some ECM RNAs were selectively inducible or up-regulated by incubation with FCS, e.g. collagen type I and type III in PaCa-44; fibronectin in BxPC-3; undulin in BxPC-3 and PaCa-44; as well as laminin in CAPAN-2 and PANC-1 (Table IV, Figure 1a). Profound down-regulation by incubation with FCS was observed for collagen type I in PaCa-2, and vitronectin and undulin in CAPAN-1 and PaCa-2, as well as the laminin in CAPAN-1. Collagen type IV was expressed at equal levels under both conditions (Table IV).

| Table I | RNA and protein levels of extracellular matrix proteins in human pancreatic adenocarcinoma cell lines |
| --- | --- |
| **Cell line** | **Grade** | **Collagen I RNA Protein** | **Collagen II RNA Protein** | **Collagen III RNA Protein** | **Fibronectin RNA Protein** | **Vitronectin RNA Protein** | **Undulin RNA Protein** | **Laminin RNA Protein** |
| CAPAN-1 | 1 | 0 | 0 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| CAPAN-2 | 2 | 1 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| AsPC-1 | 2 | 0 | 1-2 | 0 | 1-2 | 2 | 1 | 0 | 1 | 2 | 0 |
| BxPC-3 | 2 | 3 | 0 | 3-1-2 | 2 | 1 | 2 | 0 | 3 | 2 | 2 |
| PaCa-1 | 2 | 3 | 0 | 0-1-2 | 2 | 1 | 2 | 0 | 2 | 1 | 0 |
| PaCa-2 | 3 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 2 |
| PaCa-3 | 0 | 0 | 0 | 2 | 1 | 2 | 0 | 0 | 1 | 0 | 0 |
| PaCa-44 | 2 | 3 | 0 | 1-2 | 2 | 1 | 0 | 0 | 1 | 1 | 2 |
| Fibroblasts | Normal | 2 | 2-3 | 3 | 1-2 | 2 | 0 | 0 | 0 | 0 |

*Trace amounts of RNA (see Figure 1a). Single cells moderately positive. Single cells weakly positive. Note: all cell lines were cultivated in the presence of FCS.

| Table II | Relative hybridisation densities (medians) of extracellular matrix RNA levels (S138) in pancreatic carcinoma cell lines (incubated with FCS) and nude mouse tumours grouped by grading |
| --- | --- |
| **a Cell lines** | **Probe** | **Grade 1–2** | **Grade 3** | **Fibroblasts** |
|  | (n = 4) | (n = 4) | (n = 1) |
| Col I | 1.26 | 0.77 | 1.91 |
| Col III | 0.53 | 0.81 | 28.98 |
| Col IV | 2.22 | 1.68 | 2.19 |
| FN | 0.62 | 0.38 | 12.81 |
| LAM | 0.43 | 1.28 | 0.54 |
| UN | 0.13 | 0.46 | 12.75 |
| VN | 0.26 | 0.27 | 0.00 |

| **b Nude mouse tumours** | **Probe** | **Grade 1–2** | **Grade 3** | **Pancreas** | **Fibroblasts** |
|  | (n = 4) | (n = 2) | (n = 3) | (n = 1) |
| Col I | 1.27 | 1.26 | 0.35 | 3.66 |
| Col III | 2.33 | 0.21 | 0.46 | 9.56 |
| Col IV | 0.70 | 0.58 | 2.32 | 3.80 |
| FN | 1.16 | 0.49 | 0.25 | 2.19 |
| LAM | 0.99 | 1.41 | 2.16 | 0.46 |
| UN | 1.19 | 1.47 | 0.13 | 1.19 |
| VN | 2.38 | 2.13 | 0.81 | 1.35 |

| Table III | RNA and protein levels of extracellular matrix proteins in human pancreatic tumours transplanted to nude mice |
| --- | --- |
| **Tumour** | **Grade** | **Collagen I RNA Protein** | **Collagen II RNA Protein** | **Collagen III RNA Protein** | **Fibronectin RNA Protein** | **Vitronectin RNA Protein** | **Undulin RNA Protein** | **Laminin RNA Protein** |
| KLE | 1 | 0 | 1 | 2 | 3 | 2 | 2 | 3 |
| PANK | 1 | 0 | 3 | 2 | 2 | 2 | 2 | 2 |
| PaCa-39 | 1 | 0 | 2-3 | 1 | 3 | 2-3 | 2 | 3 |
| SCHU | 1-2 | 0 | 2-3 | 1 | 2-3 | 2 | 3 | 2 |
| PaCa-2 | 3 | 0 | 0 | 1 | 1 | 2 | 1 | 2 |
| PaCa-3 | 3 | 0 | 0 | 0 | 0 | 1 | 2 | 1 |
| Fibroblasts | Normal | 2 | ND | 3 | ND | 3 | ND | 1 |
| Normal pancreas | 0 | 3 | 1 | 3 | 2 | 3 | 1 | 1 |

*Trace amounts of RNA (see Figure 1b). ND, not done.
Abundant production of ECM is one of the morphological features of ductal adenocarcinomas of the pancreas. In this study, we demonstrated that pancreatic carcinomas cells are capable of synthesising and producing various ECM both in vitro and in vivo and that this ECM expression can also be modulated by presence or absence of fetal calf serum. Although all pancreatic carcinoma cell lines synthesised various ECM components, the ability to express the various ECM mRNAs and proteins differed between the individual tumour cell lines and nude mouse tumours.

The ECM detection system used in this study was specifically constructed to recognise human ECM. Thus, the cDNA probes were specific for human ECM under stringent hybridisation and post-hybridisation conditions, as used in our experiments, and the antibodies were specifically directed against human ECM since no cross-reactivity with mouse ECM was detected and most of them were pre-absorbed with a mixture of rodent ECM by the manufacturers. Furthermore, on immunocytochemistry, the staining was localised to the tumour cells. This was particularly easy to appreciate for those ECM which were expressed at the interface of tumour cells and on their luminal side where fibroblasts were absent. In tissue culture, cells of apparent epithelial origin, as demonstrated by their cytokeratin positivity, showed a membranous or cytoplasmic staining for ECM which again argued in favour of ECM production by the tumour cells themselves. On phase-contrast and electron microscopy, haematoxylin and eosin staining and immunofluorescence for cytokeratin all cells of the pancreatic tumour cell lines proved to be of epithelial origin (Rafiee et al., 1992). Fibroblasts could not be observed. We conclude, therefore, that the ECM detected in pancreatic tumour cell lines and xenografted nude mouse tumours are derived from the tumour cells and not from contaminating or associated fibroblasts.
EXTRACELLULAR MATRIX IN PANCREATIC CARCINOMA

Figure 4 Immunocytochemical staining for collagen type III a (bar 40 μm), vitronectin b (bar 40 μm) and undulin c (bar 25 μm) on the luminal/apical side of tumour cells (arrows) in addition to the basement membrane side.

Whether in our system the host fibroblasts incorporated in the xenografted tumours also produced ECM is unclear, because neither our probes nor antibodies were specifically directed against murine ECM. However, two other studies with human lung, liver and colon carcinoma cell lines xenografted to nude mice demonstrated that host stromal cells as well as epithelial cells contribute jointly to the ECM production (Cleutjens et al., 1990; Damjanov et al., 1985).

Immunocytochemical and radioimmunological studies suggest that laminin and hyaluronic acid are produced by pancreatic carcinoma cells (Haglund et al., 1984). However, no interstitial matrix proteins such as collagen type III were detected (Alitalo et al., 1981; Haberern & Kupchik, 1985) or investigated (Cleutjens et al., 1990). Our study confirms the laminin production by pancreatic carcinoma cells in vivo and in vitro and in addition showed that the tumour cells are able to produce and synthesise other ECM which so far have not been detected or investigated.

Of the interstitial ECM, many tumour cell lines and nude mouse tumours expressed collagen type III and vitronectin, at either the RNA or protein level, while only few cell lines and tumours transcribed and produced undulin and fibronectin. The fact that in an earlier study fibronectin could not be detected in AsPC-1, PANC-1, CAPAN-1 and BxPC-3 cells by the immunoperoxidase technique may be because of the

| Table IV | RNA levels of extracellular matrix proteins in human pancreatic adenocarcinoma cell lines cultured in the absence (−FCS) or presence (+FCS) of fetal calf serum |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Grade    | Collagen I | Collagen II | Collagen III | Fibronectin | Undulin | Laminin |  |  |
| 1        |            |            |             |            |         |         |  |  |
| 2        | →          | ←          | ←           | →           | ←       | ←       |  |  |
| 3        | →          | ←          | ←           | →           | ←       | ←       |  |  |
| 4        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 5        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 6        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 7        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 8        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 9        | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 10       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 11       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 12       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 13       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 14       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 15       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 16       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 17       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 18       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 19       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 20       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 21       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 22       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 23       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 24       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 25       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 26       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 27       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 28       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 29       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 30       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 31       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 32       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 33       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 34       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 35       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 36       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 37       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 38       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 39       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |
| 40       | ↓          | ↑          | ↑           | ↓           | ↑       | ↑       |  |  |

Increased expression of ECM proteins in the presence of FCS compared to the absence of FCS. The arrows indicate the direction of regulation. 1. Down-regulation by incubation with FCS. 4. Up-regulation by incubation with FCS. 4. No change in expression by FCS. For other abbreviations see Table 1 and text.
use of antibody poorly characterised at that time (Haberern & Kupchik, 1985).

Of the basement membrane components, collagen type IV showed expression of mRNA and protein in almost all cell lines and nude mouse tumours. In contrast, only a few cell lines and tumours expressed the other basement membrane constituent laminin, as has been shown before (Haberern & Kupchik, 1985). This suggests that the synthesis and expression of two components of basal membrane ECM, laminin and collagen type IV, are regulated independently. Major disturbances in the regulation of the production of basement membrane components in tumour cells may explain the common finding of a reduction or absence of basement membranes in carcinomas in vivo (Haglund et al., 1984; Liotta et al., 1984; Kern et al., 1987). Alternatively, the defective basal membrane composition could be due to a disturbed release of laminin and collagen type IV from the tumour cells as a response to the host stroma (Kallioninen et al., 1984) and/or a selective extracellular proteolysis (Haberern & Kupchik, 1985; Barsky et al., 1983).

In several cell lines (e.g. CAPAN-1, CAPAN-2) there was a discrepancy between the expression of some ECM (e.g. collagen type III and vitronectin) at the RNA and protein level. In case of a distinct RNA transcription but no or little protein production, the most plausible explanation is a slow-down of the post-translational synthesis of this matrix protein, as demonstrated under cultured conditions. Alternatively, the ECM may have been subjected to tumour-derived collagenase activities, as described previously (Hooft, 1983; Liotta et al., 1984; Zetter, 1990). However, there is so far no ready explanation for the reverse situation in which there was protein expression without relevant RNA expression. The possibility that such a constellation was due to rapid RNA degradation can be largely excluded by the finding that a concomitantly performed hybridisation with ribosomal RNA (S138) revealed good signals in all instances. A high RNA turnover rate of distinct ECM mRNA could be another possibility.

The ECM expression pattern in the nude mouse tumours revealed a correlation with the tumour grade for fibronectin (RNA level), collagen type I and laminin (protein level). For laminin such a correlation has already been described (Haglund et al., 1984; Haberern-Blood et al., 1987). No clear correlation of expression levels of differentiation was found in the tumour cell lines. This may be due to the difference between the in vitro and in vivo situation and could suggest that a stromal factor plays a role in cellular differentiation (Hooft, 1983; Hall et al., 1990).

The luminal (apical) expression of collagen type III in pancreatic tumour cells is a somewhat unexpected finding. However, beside mucins naturally expressed on the apical side of secretory epithelia (Haglund et al., 1986; Båtge et al., 1986), other non-mucinous proteins such as receptors for transferrin and epidermal growth factor (EGF) have also been found to be expressed on the luminal side of pancreatic tumour cells in vivo (native carcinoma and nude mouse model) (Klöppel, 1989; Chen et al., 1990).

Direct comparison between the in vitro and in vivo expression of ECM was possible for PaCa-2 and PaCa-3 (Klöppel et al., 1985; Mai et al., 1990). Although we found identical expression patterns for most ECM there were a few exceptions. The xenografts of PaCa-2 and PaCa-3 failed to express undulin and laminin, whereas other growth factors and collagen type IV and fibronectin at the protein level which was not observed in vitro. This could indicate that host factors, particularly the availability of external fibroblasts, may stimulate the production of certain ECM in vivo (Cleutjens et al., 1990; Damjanov et al., 1985).

In a first attempt to investigate whether the expression of ECM is constitutive or can be modulated principally by means of growth-promoting factors, tumour cell lines were cultured with and without fetal calf serum (FCS). In one cell line, BxPC-3, RNA expression of all ECM tested, except collagen type IV, was up-regulated by incubation with FCS. In contrast, RNA expression of all ECM was found to be decreased in the CAPAN-1 cell line when incubated with FCS. In some other cell lines, ECM RNAs (particularly for collagen type III) were partly up-regulated or down-regulated. As FCS containing various growth factors and cytokines such as EGF, transforming growth factor α, transferrin and interleukins, any of these may have had an effect on ECM production in the tumour cell lines. Further experiments are necessary to clarify the role of growth factors in the regulation of ECM (Longnecker et al., 1989; Korc, 1991; Lemoine & Hall, 1990; Kalthoff et al., 1991).

In summary, we have demonstrated that cell lines and xenografted tumours derived from human pancreatic ductal adenocarcinomas are capable of synthesising and processing a variety of ECM. To date, it is unclear whether this expression of ECM by tumour cells only serves to produce the tumour stroma, or may also exert an effect on tumour cell differentiation and, possibly, tumour cell growth. Recent data on transmembrane connections between ECM and intracellular filaments suggesting a role for ECM in transducing signals for regulation of differentiation genes via cell membrane receptors (integrins) lend support to the hypothesis of the coexistence of a structural and a humoral signal pathway (Singer, 1979; Hay, 1983; Bissell et al., 1982; Alberts et al., 1989).

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