# Follicle-forming cat thyroid cell lines synthesizing extracellular matrix and basal membrane components: a new tool for the study of thyroidal morphogenesis

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## Abstract

Interactions between follicular epithelial cells and extracellular matrix (ECM) are supposed to play an important role in the development and maintenance of thyroid tissue architecture. In the present study we have therefore investigated the synthesis of ECM components by a feline thyroid cell line which is able to form follicle-like structures *in vitro*, and also in v-ras-transfected and control-transfected sublines. Transfections were performed by lipofection with pZSR (viral Harvey ras gene; neo) and pSV2-neo (control, neo only) plasmids. We have adapted a semisolid culture system composed exclusively of polymerized alginate and therefore devoid of ECM components. Feline cells embedded in alginate gels as single cells and cultured for up to 90 days formed cell clusters within 10 days. Follicle-like structures were formed in the original cell lines and also in the v-ras- and control-transfected cells. Differences in proliferation rates were observed, the v-ras-transfected cells growing up to two to three times faster than the non-transfected cells. Immunostaining was done using rabbit first antibodies directed against mouse collagen IV, human fibronectin, laminin (tumor Engelbreth–Holm–Swarm laminin), perlecan and other ECM components. For comparison, immunostaining was also performed on cryosections of nodular goiters of six hyperthyroid cats. The cell lines and their transfected clones stained strongly positive for collagen IV and fibronectin, and positively but less strongly for laminin and perlecan. The cat goiter tissue stained positively for collagen IV, laminin, perlecan, and fibronectin, and positive staining for S-laminin (containing the β2-chain) was seen in blood vessel walls in this tissue.

In conclusion, cat cell lines grow three-dimensionally in alginate beads over several weeks, they form follicle-like structures and express the same ECM components as the native cat goiter tissue. Transfection with v-ras does increase proliferation rate, but does not fundamentally alter formation of follicle-like structures and ECM expression. Alginate gel culture is a promising new tool for the study of follicular morphogenesis, polarity, the expression pattern of ECM components and of the interaction between thyrocytes and ECM. It avoids interference caused by gels composed of ECM components.

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## Introduction

As extracellular structures, basal membranes (BMs) are thought to be involved in the regulation of cellular growth and tissue differentiation, and epithelial cells usually rest upon a BM. In addition, BMs show properties of selective barriers with respect to molecule permeability, and enhance epithelial cell attachment *in vitro* (for review see Farquhar 1982 and Paulsson 1992). Interactions between follicular epithelial cells and extracellular matrix (ECM) are supposed to play an important role in development and maintenance of thyroid tissue architecture. BMs are composed of the glycoprotein laminin and its associated polypeptide entactin, the heparan sulfate proteoglycan


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perlecan, as well as collagen type IV (CoIV), the major BM component. Several variants of these BM components have been described in different tissues (e.g. blood vessels, nerves, fat cells, lymphatics etc) (Kimura *et al.* 1980, Farquhar 1982, Paulsson & Saladin 1989, Paulsson *et al.* 1991, Paulsson 1992).

In order to study *in vitro* thyroid differentiation, function and proliferation, various cell culture systems have been tested: monolayers (Ambesi-Impiombato *et al.* 1980, Ambesi-Impiombato & Perrild 1989, Derwahl *et al.* 1990, Gerber *et al.* 1991*a*,*b*, Peter *et al.* 1991*b*, Dumont *et al.* 1992), three-dimensional systems using collagen gels (Yang *et al.* 1979, Garbi & Wollman 1982, Garbi *et al.* 1984, Peter *et al.* 1987, 1991*a*–*c*, Toda & Sugihara 1990, Toda *et al.* 1993), agarose gels (Kimura *et al.* 1980, Benya & Shaffer 1982, Aydelotte *et al.* 1986*a*,*b*, Bruckner *et al.* 1989) and alginate beads (Kupchick *et al.* 1983, Guo *et al.* 1989, Häuselmann *et al.* 1994, Tognella *et al.* 1995, Glaser *et al.* 1996, Bürgi-Saville *et al.* 1997, 1998). Alginate has several advantages in comparison with the other systems. In comparison with monolayer culture it allows three-dimensional growth of thyroid cells and follicles, imitating *in vivo* conditions much more closely. Being devoid of ECM components itself (other three-dimensional culture systems have used collagen or fibronectin gels) interference with immunohistochemical analyses of ECM components is avoided. Furthermore, by removing calcium with a chelating agent the alginate beads can be turned back into a liquid cell suspension, allowing harvesting of the cells for further experiments if required. Thus the alginate bead culture system offers an interesting alternative for investigations of the ECM, permitting, for example, the analysis of ECM components by immunohistochemistry, and also the investigation of the secretion of ECM components into the extracellular space by specific immunoprecipitation methods.

Thyroid follicular cell proliferation, differentiation and functions have been thoroughly investigated (for review see Ambesi-Impiombato & Perrild 1989, Studer *et al.* 1989, Ericsson & Fredriksson 1990, Dumont *et al.* 1992, Dumont & Vassart 1994, Ekholm 1994, Gentile *et al.* 1994, Studer & Gerber 1994, Peter *et al.* 1996). So far, much less but nevertheless fascinating research has been done on the structure and metabolism of ECM of thyroid tissue (Garbi & Wollman 1982, Alquier *et al.* 1983, Garbi *et al.* 1984, 1988, 1990, Wadeleux *et al.* 1985, Demeure *et al.* 1992, Prabakaran *et al.* 1993, 1996, Vitale *et al.* 1993, 1994, 1995, Andre *et al.* 1994, Lemansky *et al.* 1994, Tognella *et al.* 1995, Arvan *et al.* 1997, Bürgi-Saville *et al.* 1997, 1998). Ultrastructural studies indicate that BM formation or maintenance can be achieved in cultures of thyroid cells (Alquier *et al.* 1983, Garbi & Wollman 1982, Garbi *et al.* 1988). Cultured porcine cells have been shown to synthesize type I, II and IV collagens as well as a heparan sulfate glycosaminoglycan (Wadeleux *et al.* 1985) and also a laminin variant containing an α2-chain (merosin) (Andre *et al.* 1994). Synthesis of laminin, CoIV and fibronectin has been demonstrated in the thyroid epithelial cell line FRTL5 (Ambesi-Impiombato *et al.* 1980, Ambesi-Impiombato & Perrild 1989) and also the deposition of the extracellular material at the basolateral surface of the cells and its organization into a BM (Garbi *et al.* 1988). We have recently demonstrated the maintenance of an ECM containing fibronectin, CoIV, laminin and perlecan both in FRTL5 cells and in primary cultures of rat thyroid cells (Bürgi-Saville *et al.* 1998) and in primary cultures of normal human thyroid tissue, also in alginate beads (Bürgi-Saville *et al.* 1997).

In the present study we report the investigation of ECM synthesis by different feline thyroid cell lines (Romcat, Petcat) (Peter *et al.* 1987, 1991*a*–*c*, Gerber *et al.* 1991*a*,*b*, 1994) and v-ras-transfected sublines. For transfection, we have introduced the pZSR plasmid harboring the viral Harvey ras gene or the pSV2-neo plasmid only (as control) into the Romcat cell line (Gerber *et al.* 1991*a*,*b*, 1994). Embedded into alginate gel beads, three-dimensional growth of the cells could be observed. The cell clusters and follicle-like structures growing out from the cat cells were able to synthesize different ECM proteins such as CoIV, laminin, perlecan and fibronectin. We also investigated whether there is an evident connection between follicle-forming capacity and ECM component production. Moreover, the relationship between ECM production, cell proliferation (measured by staining of the Ki-67 cell proliferation-associated antigen) and transfection status has been examined.

## Materials and Methods

### *Thyroid tissue, cell lines and culture conditions*

Thyroid tissue of hyperthyroid cats (Peter *et al.* 1987, Gerber *et al.* 1994) was obtained from Dr M E Peterson, Cornell University, New York, NY, USA. Different cell lines have been established from clinically well-characterized toxic nodular cat goiters and characterized as described (Peter *et al.* 1987, 1991*a*–*c*, Gerber *et al.* 1991*a*,*b*, 1994) (Table 1). In this study, we mainly used the Romcat cell line and eight v-ras-transfected sublines as well as two sublines transfected only with pSV2-neo (see below) (Table 1).

Cells were cultured as described (Peter *et al.* 1987, 1991*a*–*c*, Gerber *et al.* 1991*a*,*b*) in Coon's modified Ham F-12 medium (Seromed, Biochrom KG, Berlin, Germany), supplemented initially with 5%, in later experiments with 1% newborn calf serum (Seromed) and six hormones, i.e. insulin, 10 μg/ml (Sigma, Division of Fluka Chemicals, Buchs, Switzerland), transferrin, 5 μg/ml (Sigma), somatostatin, 10 ng/ml (Sigma), glycyl-L-histidyl-L-lysine, 10 ng/ml (Sigma), hydrocortisone, 3·2 ng/ml (Sigma), thyrotropin from bovine pituitary (Sigma) at a concentration of 0·1 mU/ml. Penicillin,

**Table 1** Characteristic features of cell lines tested

| | Thyroglobulin[1] | Cytokeratin[1] | Vimentin[1] | Morphology | Follicle-forming capacity |
|---|---|---|---|---|---|
| **Cell type** | | | | | |
| Petcat 1 | + | (+) | + | Epithelial | + |
| Petcat 2 | + | + | + | Epithelial | + |
| Petcat 3 | + | − | + | Mesencymal | − |
| Petcat 4 | + | − | + | Mesenchymal | − |
| Romcat, control non-transfected | + | + | + | Epithelial | ++ |
| Romcat, pSV2-neo transfected, clone 1 | | | | Epithelial | ++ |
| Romcat, pSV2-neo transfected, clone 2 | | | | Epithelial | ++ |
| Romcat, pZSR transfected, clone 1 | | | | Epithelial | + |
| Romcat, pZSR transfected, clone 2 | | | | Epithelial | + |
| Romcat, pZSR transfected, clone 3 | | | | Epithelial | ++ |
| Romcat, pZSR transfected, clone 4 | | | | Epithelial | +++ |
| Romcat, pZSR transfected, clone 5 | | | | Epithelial | +++ |
| Romcat, pZSR transfected, clone 6 | | | | Epithelial | + |
| Romcat, pZSR transfected, clone 7 | | | | Epithelial | + |
| Romcat, pZSR transfected, clone 8 | | | | Epithelial | + |
| FRTL-5 | + | + | | Epithelial | − |

[1]Positive (+) or negative (−) immunohistochemical staining with anti-thyroglobulin, anti-cytokeratin and anti-vimentin antibodies (Gerber *et al.* 1991*b*).

100 IU/ml and streptomycin, 100 μg/ml were added as antibiotic agents. The culture medium was changed three times a week and cultured cells maintained at a temperature of 37°C in a humidified atmosphere with 5% carbon dioxide.

*Transfection procedure*

Transfection of Romcat cells either with the plasmid construct pZSR (kindly provided by Prof. R R Friis, University of Berne) harboring the viral Harvey ras gene (Remond *et al.* 1988) or the pSV2-neo plasmid (for transfection control purpose) was performed as described (Wagner *et al.* 1990*a*,*b*) with DOTAP (N-(1-(2,3-dioleoyl-oxy)-propyl)-N,N,N-trimethyl-ammonium-methyl-sulfate) transfection reagent (Boehringer, Mannheim, Germany). In brief, Romcat cells were plated in 60 mm culture dishes and grown to subconfluency. The transfection reagent was prepared by dilution of 25 μl DOTAP suspension with 75 μl PBS. Five micrograms of pZSR plasmid or pSV2-neo plasmid suspended in 95 μl PBS were added to this solution. The preparations were carefully mixed by gentle pipetting, and incubated for 10 min at room temperature. Finally, the mixtures were completed by adding 5 ml culture medium. The thyroid cells were then exposed to the plasmid solution. After incubation for 4 h, the transfection medium was replaced by fresh culture medium (Coon's modified F12 medium, supplemented with 5% fetal calf serum and the six hormones). The next day, the medium was changed again. Selection of successfully transfected cells (with acquired neomycin resistance) was performed by adding a medium containing Geneticin (400 μg/ml, Boehringer) (Wagner *et al.* 1990*a*,*b*). Test experiments, not presented here in detail, have shown that non-transfected feline cells die in medium containing Geneticin at a concentration of 400 μg/ml. Eight different clones of the pZSR-transfected Romcat cell line and five different clones of the pSV2-neo-transfected Romcat cell line were isolated, expanded and frozen for further investigations.

*Alginate culture technique*

Feline cells cultured as monolayers were, after trypsinization, prepared as a single cell suspension. Alginate beads were prepared as described (Häuselmann *et al.* 1994, Bürgi-Saville *et al.* 1997). In brief, $6 \times 10^6$ cells were suspended in 6 ml of a solution of 1·2% alginate (sodium salt, Sigma) in 0·9% NaCl (final concentration $10^6$ cells/ml alginate). The cell suspension was dripped from a plastic syringe into 40 ml of a slowly stirred sterile solution of 102 mM $CaCl_2$ (Merck, Dietikon, Switzerland). Upon contact with the calcium solution, the alginate formed beads of approximately 2 mm in diameter which contained cat thyroid cells. The bead-forming process, i.e. the gelation of alginate, takes about 2 min. Beads were allowed to polymerize completely in the $CaCl_2$ solution for another 8 min. Immediately after careful removal of the $CaCl_2$ solution, beads were washed in 30 ml 0·9% NaCl or PBS solution three times for 5 min, followed by a similar rinsing process in culture medium.

*Paraffin embedding process of alginate beads*

Alginate beads were washed in 0·05 M Tris buffer, pH 7·6, supplemented with 7 mM $CaCl_2$, and then fixed in buffered 4% formalin, pH 7·4, for 30 min. Before embedding in paraffin, cells were washed in Tris buffer again. Overnight, beads were dehydrated in an automatic tissue processor (Histokinette Shandon Citadel 1000). In

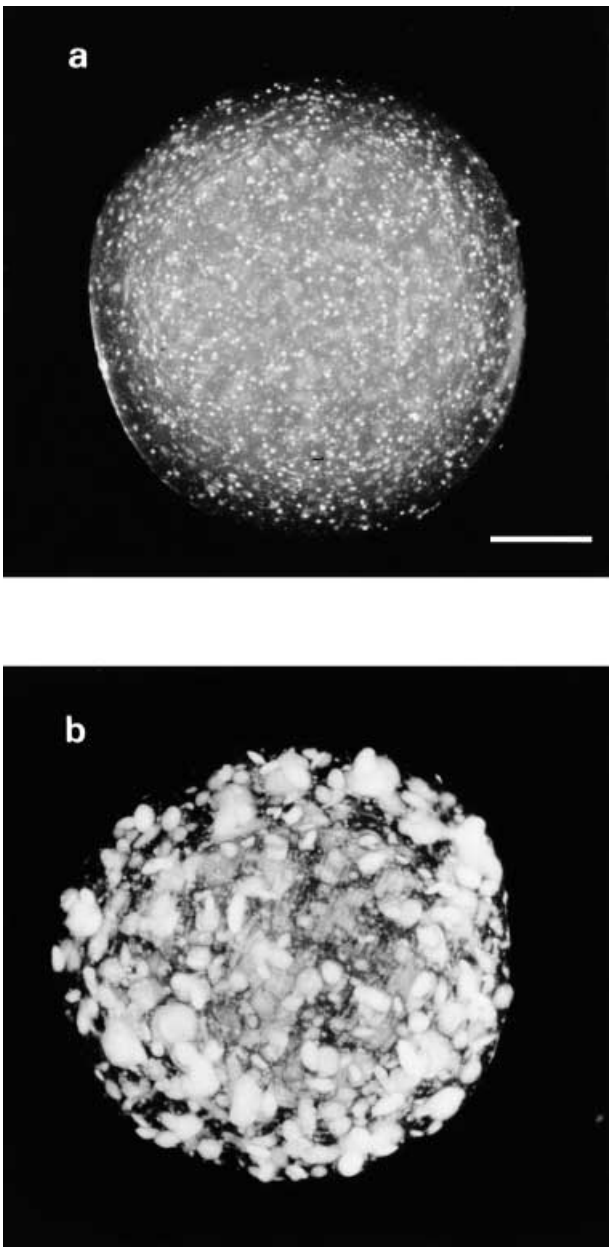


**Figure 1** Proliferation patterns of two cat thyroid cell lines growing in alginate beads: (a) Petcat 4, non-transfected, 74 days of culture, (b) Romcat, v-ras-transfected, clone 2, 67 days of culture. Bar 500 μm.

specially adapted time cycles, samples passed through a series of ethanol solutions with ascending concentrations (50–95%), followed by treatment with 2-propanol, xylol and paraffin, the whole preparation lasting 19 h. After this pretreatment, the beads were embedded in paraffin (Histo-Comp, Vogel, Giessen, Germany). Sections of 2 μm were prepared for immunohistochemical investigation.

*Immunohistochemical methods*

The sections were dewaxed with xylene and rehydrated through decreasing concentrations of ethanol. The sections were incubated overnight at room temperature with specific rabbit polyclonal primary antibodies to mouse laminin (Sigma, dilution 1:50), human fibronectin (Dako, Zug, Switzerland, dilution 1:250), mouse perlecan (kindly provided by Prof. J Hassell, University of Pittsburgh, PA, USA, dilution 1:100) and mouse CoIV (Becton Dickinson, Basel, Switzerland, dilution 1:250) (Studer *et al.* 1992, Bürgi-Saville *et al.* 1997). MIB-1, a monoclonal mouse antibody to the Ki-67 proliferation-associated antigen (Dianova, Hamburg, Germany, dilution 1:50), was used as a cell proliferation marker. For this antigen, the slides were microwave pretreated in citric acid (10 mM, pH 6) for 5 min at 750 W. After 10 min cooling, this process was repeated twice followed by rinsing in water and PBS. After washing the slides with PBS for 15 min, an alkaline phosphatase-coupled swine anti-rabbit immunoglobulin (Dako, dilution 1:50) was applied for 7 h. For Ki-67 antigen detection, the monoclonal mouse antibody was followed by a biotinylated goat anti-mouse immunoglobulin (Dako, dilution 1:250). This complex was detected by an alkaline phosphatase-conjugated ExtrAvidin (Sigma, dilution 1:250). Thereafter, slides were rinsed with PBS for 15 min and exposed to the substrate solution (3 mg fast red TR salt (Sigma), 4 mg naphthol AS-MX phosphate sodium salt (Sigma) and 1·5 mg levamisole (Sigma), dissolved in 5 ml 0·1 M veronal acetate buffer, pH 8·3) for 20–30 min. The sections were rinsed in running tap water overnight and were counterstained with aqueous Ehrlich's hematoxylin. Finally, slides were sealed with Kaiser's glycerin gelatin (Merck). In controls for non-specific binding, the specific primary antibody was omitted in the first incubation step.

The expression of the v-ras gene product in the v-ras-transfected cell was detected using a monoclonal antibody against ras protein (Oncogene Research Products, Cambridge, MA, USA). Monolayer cultures were fixed with methanol/acetic acid and incubated overnight with the primary antibody followed by a biotinylated goat anti-mouse immunoglobulin and ExtrAvidin as described above.

Immunofluorescent staining was performed in unfixed 6 μm cryosections of nodular goiters of six hyperthyroid cats and in samples of cultured cells released from the alginate beads as previously described (Bürgi-Saville *et al.* 1997) and applied to eight-well microscope slides (Veenstra & Dowale 1992). The cell samples were fixed for 10 min in acetone at room temperature. Following washing for 30 min in 50 mM Tris, 150 mM NaCl, pH 7·4 containing 1% BSA to block non-specific binding sites, sections were incubated for 1 h with monoclonal primary antibodies as follows: human CoIV (diluted 1:250), and bovine laminin β2-chain

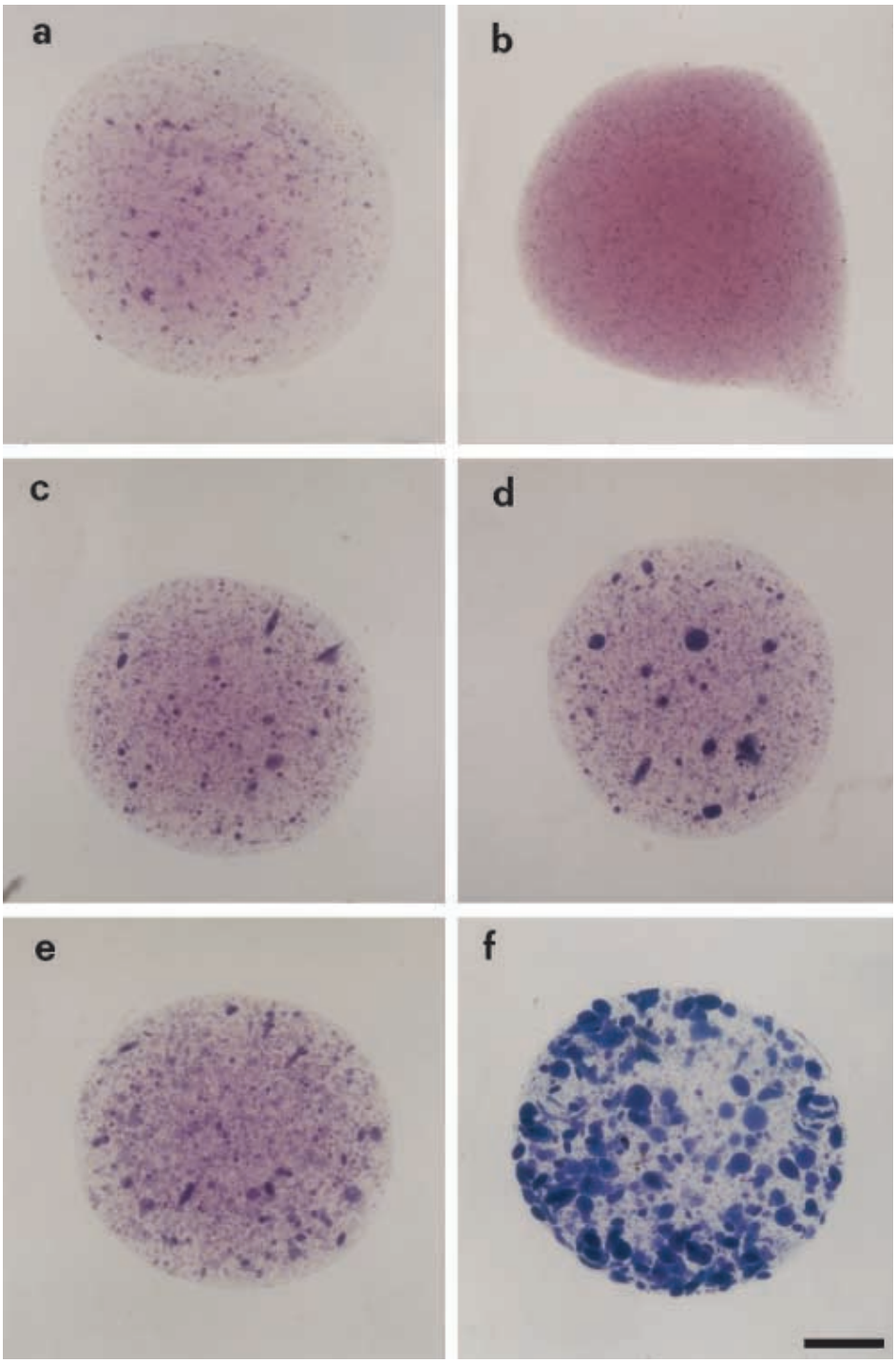


**Figure 2** Cat thyroid cell lines growing in alginate beads, cultured with six-hormone medium, 5% calf serum: (a) Romcat, non-transfected, 29 days of culture, (b) Petcat, clone 2, 41 days of culture, (c) Romcat, v-ras-transfected, clone 7, 35 days of culture, (d) Romcat, v-ras-transfected, clone 7, 50 days of culture, (e) Romcat, v-ras-transfected, clone 2, 35 days of culture, (f) Romcat, v-ras-transfected, clone 2, 50 days of culture. Staining with crystal violet 0·1%. Bar 500 µm.

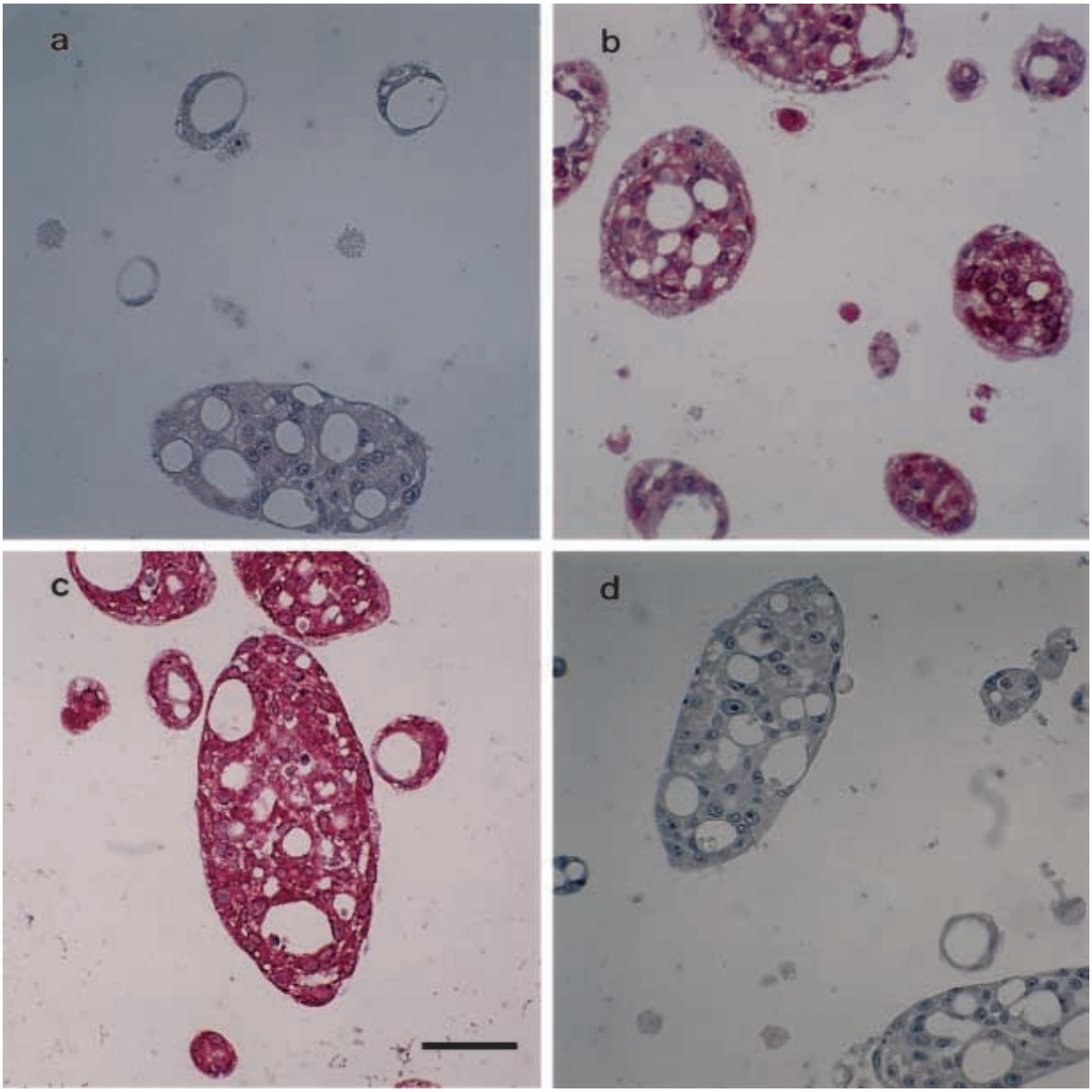


**Figure 3** Immunohistochemical staining for ECM components in Romcat cell line, v-ras-transfected, clone 4, cultured with six-hormone medium, 5% calf serum. (a) Laminin antibody (L9393), 11 days of culture, (b) fibronectin antibody, 18 days of culture, (c) CoIV antibody, 18 days of culture, (d) negative control. Bar 50 µm.

(S-laminin, 1:250) (developed by Dr Heinz Furthmayr and Dr Joshua Saves respectively), which were obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, USA and the Department of Biological Sciences, University of Iowa, IA, USA. Rabbit polyclonal antibodies to human fibronectin (Dako 1:250), mouse perlecan (as described above; 1:400) and mouse Engelbreth–Holm–Swarm (EHS) laminin (1:50) were also tested. The EHS laminin antibody was kindly provided by Dr A Lindblom, University of Berne. The sections were then incubated for 45 min with anti-mouse or anti-rabbit rhodamine-conjugated secondary antibodies (Dako, 1:25), washed and sealed using poly-DABCO.

**Table 2** Immunohistochemical staining

| | Culture time (days) | Antibody[1] | Staining intensity | Follicle forming capacity |
|---|---|---|---|---|
| **Romcat non-transfected** | 35 | L | ++ | ++ |
| | | F | + | |
| | | C | + | |
| **Romcat transfected** | | | | |
| Clone 1 | 35 | L | +++ | + |
| | | F | +++ | |
| | | C | +++ | |
| Clone 2 | 33 | L | + | + |
| | | F | ++ | |
| | | C | ++ | |
| Clone 3 | 35 | L | ++ | ++ |
| | | F | ++ | |
| | | C | +++ | |
| Clone 4 | 33 | L | ++ | +++ |
| | | F | +++ | |
| | | C | +++ | |
| Clone 5 | 35 | L | + | +++ |
| | | F | ++ | |
| | | C | +++ | |
| Clone 6 | 35 | L | ++ | + |
| | | F | +++ | |
| | | C | ++/+++ | |
| Clone 7 | 35 | L | +++ | + |
| | | F | ++/+++ | |
| | | C | ++++ | |
| Clone 8 | 33 | L | +/++ | + |
| | | F | ++ | |
| | | C | +++ | |

[1]L=anti-laminin antibody, F=anti-fibronectin antibody, C=anti-CoIV antibody.

## Results and Discussion

### *Alginate culture system*

Feline cell lines embedded in alginate gel matrix as single cells were cultured for up to 90 days. They formed cell clusters within 10 days. Proliferation rates differed considerably between cell lines and their sublines. Romcat cells grew faster than Petcat cells and the v-ras-transfected Romcat sublines grew two to three times faster than the non-transfected Romcat cell line (Figs 1 and 2), as estimated from the labeling index for the proliferation-associated Ki-67 antigen.

Petcat cells, which grow well in collagen gel (Peter *et al.* 1987, 1991*a*–*c*, Gerber *et al.* 1991*a*,*b*, 1994) and about as fast as Romcat cells, generally showed very low growth rates in alginate. Obviously Petcat cells depend – more than Romcat cells – on ECM components in the culture system for optimal three-dimensional growth *in vitro*.

### *Expression patterns of ECM components*

Immunohistochemical studies showed that cat goiter tissue stained positively for CoIV, laminin (tumor EHS laminin), perlecan and fibronectin. S-laminin (αβ-chain variant), selectively found in blood vessel walls in human tissue, was also found in vessel walls of cat thyroids.

In the studies of the cell lines, the following ECM components were investigated immunohistochemically: laminin (tumor EHS laminin), perlecan, CoIV and fibronectin. Results of immunohistochemical staining are shown in Fig. 3 and summarized in Table 2. Positive staining of variable intensity was seen for all four ECM components in the Romcat cell line and their v-ras-transfected subclones. The Romcat cat cell lines express therefore the same ECM components as the native cat goiter tissue. Negative controls were completely unstained. For laminin, subclones 1 and 7 (with remarkable low follicle-forming capacity) showed a relatively high staining intensity. Medium staining intensity was found in non-transfected Romcat cells as well as in the transfected clones 3, 4, 6 and 8, whereas clone 2 and 5 showed low staining reaction. For fibronectin, non-transfected Romcat cells were the only cell type to stain only weakly positive, contrary to all transfected clones with medium (clones 2, 3, 5 and 8) up to intensive staining properties. CoIV expression was found to a rather high degree in almost all

transfected clones, i.e. clones 1 and 3–8. Clone 2 and non-transfected Romcat cells were the only ones with less intense staining. Perlecan expression was found in all clones examined (data not shown).

Immunohistochemical staining for ras in clone 5, with a high follicle-forming capacity and clones 6 and 7, with a low follicle-forming capacity, was found to be comparable. This suggests no direct correlation between the expression of v-ras and the follicle-forming capacity.

### *Follicle formation*

The formation of follicle-like structures is illustrated in Figs 3 and 4 and summarized in Table 1. Immunohistochemical staining intensity of the ECM components was in general independent of the variable follicle-forming capacity in the Romcat cell line and their subclones, although subclones 1 and 7, being the two transfected subclones with remarkable low follicle-forming capacity, showed a comparable high staining intensity with respect to EHS laminin. The two pSV2-neo-transfected cell lines studied showed comparable follicle-forming capacity.

### *Transfection*

In the wider context of the question of ECM expression in the course of the neoplastic transformation, it was the aim of this study to find out whether ECM production and follicle formation by feline thyrocytes are altered with v-ras-gene expression. Transfection with v-ras did indeed increase the proliferation rate of the cell lines, but it did not fundamentally alter the expression pattern of the ECM components – as determined by immunohistochemistry – and the formation of follicle-like structures (Tables 1 and 2). Whether the increased follicle-forming capacity found in clones 4 and 5 is due to the v-ras-transfection is not clear. The lack of a correlation between ras protein expression and the follicle-forming capacity suggests no direct effect. But the site of incorporation of the v-ras gene into the host genome may affect the follicle-forming capacity. However, this question can only be solved with an inducible v-ras expression system.

### *Conclusions*

Cat thyroid cell lines – as isolated human normal and goiter cells (Bürgi-Saville *et al.* 1997), rat thyroid follicles (Glaser *et al.* 1996, Bürgi-Saville *et al.* 1997*a*), and some non-thyroidal cells (Guo *et al.* 1989, Häuselmann *et al.* 1994) – grow three-dimensionally in a calcium alginate gel matrix over several weeks. They form follicle-like structures and express the same ECM components as the native cat goiter tissue. However, cell lines which grow at similar rates in collagen gel may differ considerably in growth rates when cultured in alginate, possibly because some depend more than others on ECM components in the culture system for optimal three-dimensional growth *in vitro*.

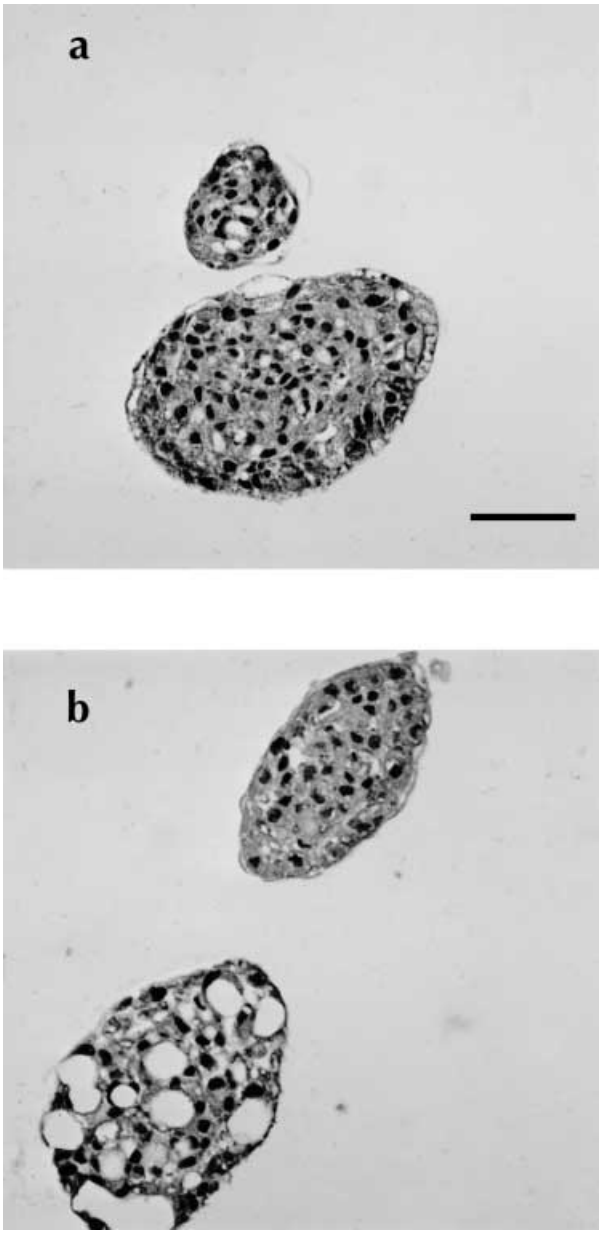


**Figure 4** Follicle formation capacity. (a) Low capacity: Romcat, v-ras-transfected, clone 7, 33 days of culture, (b) high capacity: Romcat, v-ras -transfected, clone 4, 33 days of culture; (see also Table 2). Bar 50 μm.

The expression pattern of ECM components, as determined by immunohistochemistry, was not correlated with the variable follicle-forming capacity of the cell lines, i.e. cell lines with a greater propensity to form follicles express

the ECM components investigated to the same degree as cell lines with a lesser propensity to form follicles.

However, transfection with v-ras did indeed increase the proliferation rate of the cell lines, but it did not fundamentally alter the expression pattern of the ECM components and the formation of follicle-like structures. The preservation of the functional differentiation is in line with reports by others on the effect of viral Harvey ras and mutated human Harvey ras transfections (Nakagawa *et al.* 1987, Wynford-Thomas *et al.* 1990). However, both studies reported a decrease of cell proliferation, where we found in contrast an increase. This difference may be due to the cell lines used or the cell culture system used. The latter explanation would be also supported by the differences found in the growth behavior of the Petcat and Romcat cell lines in collagen gel (Peter *et al.* 1987, 1991*a*–*c*, Gerber *et al.* 1991*a*,*b*, 1994) and alginate beads.

As described before for non-thyroidal cells (Guo *et al.* 1989, Häuselmann *et al.* 1994), alginate bead gel culture is a promising new tool for the study of follicular morphogenesis, cell polarity, the expression pattern of ECM components and of the interaction between thyrocytes and ECM. Being free of ECM components, it avoids interference caused by gels composed of ECM components.

## Acknowledgements

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