Beneficial effects of retinoic acid on extracellular matrix degradation and attachment behaviour in follicular thyroid carcinoma cell lines

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Abstract

The prognosis of patients with metastasised follicular thyroid carcinoma (FTC) is limited, necessitating the search for new treatment options. Beneficial effects of retinoids have been suggested in thyroid cancer and the present study was performed to investigate the effects of retinoic acid (RA) on important determinants of metastatic behaviour in FTC: the disengagement of tumour cells from the primary tumour and the degradation of extracellular matrix, focusing on the role of the plasmin activation system and the integrin and E-cadherin families of attachment molecules. Three FTC cell lines were studied: FTC-133, derived from the primary tumour; and FTC-236 and FTC-238, derived from metastases. FTC cell lines were cultured with 0.1, 1 and 10 μ M 13-*cis*-RA or with the solvent DMSO for 1 and 5 days. Extracellular matrix degradation by these cell lines was studied by assessing the 48-h release of radioactivity from ³⁵S-methionine labelled extracellular matrix proteins synthesised by the MC3T3 cell line coated onto plastic. The involvement of constituents of the plasmin activation system was investigated by semi-quantitative RT-PCR and zymography. Attachment to extracellular matrix was studied by determining the number of adhering FTC cells to extracellular matrix coated onto plastic, 3 h after seeding. The involvement of attachment molecules was

studied by RT-PCR with primers for integrin subclasses and E-cadherin and immunofluorescence for E-cadherin. Five days culturing with 10 μ M RA reduced the degradation of extracellular matrix significantly in all cell lines: FTC-133 by 35%, FTC-236 by 74% and FTC-238 by 31%. Zymography revealed diminished activity of urokinase type plasminogen activator (uPA) in FTC-236 and FTC-238, but not in FTC-133 cultured with RA. mRNA expression of the uPA receptor was diminished in FTC-236. In the attachment assay, 10 μ M RA for 5 days increased the number of adherent cells to extracellular matrix significantly by 91% in FTC-133, 64% in FTC-236 and 87% in FTC-238. No effects of RA on integrin or E-cadherin mRNA expression were observed. Immunofluorescence, however, revealed enhanced organisation of E-cadherin along the cell membrane by RA treatment. In conclusion, the present study demonstrates beneficial effects of RA on important determinants of metastatic behaviour in FTC cell lines, e.g. decreased degradation of extracellular matrix which may in part be explained by effects on the plasmin activation system and enhanced attachment to extracellular matrix. These findings may add to the explanations for beneficial effects of retinoids in thyroid cancer.

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Introduction

Although the overall prognosis of differentiated thyroid cancer is favourable due to the combined treatment of surgery and radioactive iodine, about 50% of patients with distant metastases of follicular thyroid carcinoma (FTC) die within 10 years after the diagnosis (Hundahl *et al.* 1998). Important events in the metastatic process are the disengagement of tumour cells from the primary tumour and the degradation of extracellular matrix. The adherence of

cells to neighbouring cells and extracellular matrix is mediated by attachment molecules (Akiyama & Yamuda 1993). E-cadherin is a transmembrane protein that is usually located at adherens junctions in epithelial cells and mediates cell–cell adhesion. Integrins are transmembrane molecules consisting of α and β subunits and mediate cellular attachment to extracellular matrix (Ruoslahti 1991, Serini *et al.* 1996). The β_1 subunit associates with a number of α subunits and constitutes the largest integrin subfamily. In many malignant tumours, loss of expression

of E-cadherin and altered expression of integrins have been related to metastatic potential and poor prognosis (Juliano 1993, Serini *et al.* 1996). We have recently reported on integrin expression and attachment behaviour in FTC cell lines (Smit *et al.* 1998). In malignant tumours, an important role in the degradation of extracellular matrix is assigned to the plasmin activation system (Andreasen *et al.* 1997). Plasmin is a serine protease which degrades extracellular matrix proteins such as fibronectin, laminin and proteoglycans (Vassalli *et al.* 1991). Plasminogen can be converted to plasmin by the urokinase-type plasminogen activator (uPA) or the tissue-type plasminogen activator (tPA) system (Vassalli *et al.* 1991, Andreasen *et al.* 1997). uPA exerts its effects after binding to the uPA receptor (uPAR) which is present on the cell membrane (Mondino *et al.* 1999). The presence of uPAR has been demonstrated in many malignant tumours and uPA-mediated plasminogen activation appears to be an important pathway in tumour invasion (Gaffney *et al.* 1994, Costantini *et al.* 1996, Xing & Rabbani 1996). uPA can be inactivated by plasminogen activator inhibitors (PAI-1 and PAI-2) (Schneiderman & Loskutoff 1991, Vassalli *et al.* 1991). Matrix metalloproteinases (MMPs), like MMP-2 and MMP-9, are a class of enzymes that are involved in matrix degradation by malignant tumours as well and can be activated by plasmin or independently of the plasmin activation system. MMP activity can be inhibited by tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) (Parsons *et al.* 1997, Pijuan-Thompson *et al.* 1999). The expression of components of the plasmin activation system in thyroid cancer tissue samples has been demonstrated (Ito *et al.* 1996). We have demonstrated the involvement of the plasmin activation system in matrix degradation by FTC cell lines (Smit *et al.* 1999).

Although radioiodine is the mainstay of therapy for metastasised thyroid carcinoma (Schlumberger *et al.* 1996), alternative treatment modalities are being investigated (Smit *et al.* 2000). Retinoids are derivatives of vitamin A (retinol). Beneficial effects of retinoids have been reported in patients with promyelocytic leukaemia, melanoma and several types of carcinoma (Castaigne *et al.* 1990, Lotan 1991, McBurney *et al.* 1993). *In vitro* data suggest that retinoids inhibit tumour invasion. This has been observed in prostate and other carcinomas (Webber & Waghray 1995, Vo *et al.* 1998, Benbow *et al.* 1999), meningioma (Pereda *et al.* 1999) and melanoma (Hendrix *et al.* 1990). One of the explanations may be decreased activity of uPA or tPA or enhanced expression of TIMPs (Hendrix *et al.* 1990, Waghray & Webber 1995, Webber & Waghray 1995). Furthermore, some recent studies report enhanced attachment of tumour cells to extracellular matrix by retinoids (Matarrese *et al.* 1998, Nakagawa *et al.* 1998, Pereda *et al.* 1999, Touhami *et al.* 1999). Alterations in the expression of adhesion molecules have been suggested as an explanation (Matarrese *et al.* 1998, Nakagawa *et al.* 1998, Touhami *et al.* 1999). In thyroid carcinoma cell lines

and tissue samples, expression of retinoid receptors has been investigated, suggesting decreased expression of RXR β in anaplastic thyroid carcinoma as compared with differentiated thyroid carcinoma cell lines and decreased RXR β expression in thyroid carcinoma tissues as compared with normal thyroid tissue (Schmutzler *et al.* 1998). Intervention with retinoids have been reported to ameliorate *in vitro* iodide uptake and 5' deiodinase activity (Van Herle *et al.* 1990, Schreck *et al.* 1994). In an *in vivo* study, beneficial effects on iodine uptake behaviour were suggested (Schmutzler *et al.* 1998). In the present study, we investigated the effects of 13-*cis*-retinoic acid (RA) on the degradation of extracellular matrix and the role of the plasmin activation system in FTC cell lines, and the effects of RA on the attachment of these cell lines to extracellular matrix.

Materials and Methods

Cell lines and culturing conditions

Three FTC cell lines FTC-133, FTC-236 and FTC-238 were kindly donated by Dr Goretzki and Dr Simon, University of Düsseldorf, Germany. The cell lines were derived from a 42-year-old male patient with metastatic FTC. The FTC-133 cell line was derived from the primary tumour whereas the FTC-236 and FTC-238 cell lines were derived from distant metastases. The cell lines produce thyroglobulin and express thyroid peroxidase. Receptors for thyrotrophin and the retinoid receptors RXR and RAR have been demonstrated in these cell lines (Goretzki *et al.* 1990, Schmutzler *et al.* 1998). The cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) and modified HAM-F12 medium 1:1 supplemented with 10% fetal bovine serum and penicillin/streptomycin in a humidified incubator at 37 °C and 5% CO₂. During experiments, the cell lines were cultured in serum-free DMEM/HAM-F12 medium. Cells were cultured for different time periods as indicated in the experiments in medium containing 0.1, 1 or 10 μ M 13-*cis*-RA (Life Technologies-Gibco BRL, Gaithersburg, MD, USA) or DMSO as solvent of RA (control). Cells were shielded from light by aluminium foil. To prevent possible interaction with the attachment of the cells to the culture dishes, RA and DMSO were added 1 day after seeding the cells. For all assays, tumour cells were cultured until approximately 90% confluency and dissociated into single-cell suspensions from the tissue culture flasks using 0.05% EDTA in PBS for 3 min.

Degradation of extracellular matrix

Matrix degradation by the FTC cell lines was studied using an *in vitro* model of ³⁵S-methionine labelled extracellular matrix molecules (Ronday *et al.* 1997, Smit *et al.*

1999). Extracellular matrix synthesised by MC3T3 includes collagen type I and IV, fibronectin, vitronectin laminin and glycosylated molecules (Sudo *et al.* 1983, Takeuchi *et al.* 1990). In short, ^{35}S -methionine labelled extracellular matrix proteins, obtained by culturing the mesenchymal cell line MC3T3 with ^{35}S -labelled methionine, were coated overnight onto plastic 24-well plates at 4 °C and fixed with methanol 60%. The FTC cell lines were cultured for 1 or 5 days in DMSO alone or with 0.1, 1 or 10 μM RA and seeded at a density of 100 000 per well. Attachment of the cell lines to non-radioactive extracellular matrix coated onto plastic was verified by counting adhering cells in two non-overlapping microscopic fields after staining with Amido black, magnification 400 $\times$, as previously described (Van der Pluijm *et al.* 1996). After 48 h at 37 °C, supernatant and coating (cells and extracellular matrix) from each well were collected into tubes and counted in a scintillation counter. Supernatant with DMSO from wells without FTC cells was collected to assess the spontaneous release of radioactivity. To rule out cell-independent effects of RA on degradation of extracellular matrix, RA was added to ^{35}S -methionine labelled matrix, without cells. To study the involvement of the plasmin activation system in matrix degradation, FTC cells, cultured without RA, were seeded in the presence of 200 U/ml aprotinin (Trasylol), a potent inhibitor of plasmin activation. The release of radioactivity for each well is expressed as a percentage of the total radioactivity per well according to the formula:

$$\frac{A_{\text{sn}}}{A_{\text{sn}} + A_{\text{coating}}} \times 100\%$$

where A_{sn} = radioactivity (c.p.m.) of supernatants and A_{coating} = activity of extracellular matrix coating.

Matrix degradation by the FTC cell lines is reflected by the release of radioactivity in the wells in which FTC cell lines were seeded minus the release of radioactivity in the wells without cells (spontaneous release). Each FTC cell line was studied in hexaplicate.

Zymography

Fibrinolytic enzyme activity was studied by zymography as described previously (Smit *et al.* 1999). In short, cell lines were cultured in serum-free medium with DMSO and those concentrations of RA that revealed significant reduction of matrix degradation. Protein concentrations of the supernatants were determined by the method of Lowry *et al.* (1951). Supernatants were electrophoresed on 10% polyacrylamide gels with sodium dodecylsulphate (SDS-PAGE), and plasminogen activator activities were visualised on fibrin/plasminogen containing agarose underlay gels according to the Granelli-Piperno and Reich method (Granelli-Piperno & Reich 1978). Before electrophoresis, samples were incubated for 1 h at 37 °C in

2% SDS to induce activator activity in the plasminogen activator-PAI complexes. Homogenates of the breast carcinoma cell line ZR75-1 (American Type Culture Collection, Rockville, MD, USA) were used as standards. In one experiment RA was added to the supernatant of FTC cells cultured without RA, to assess whether RA affects activity of fibrinolytic enzymes directly.

Semi-quantitative PCR and immunofluorescence for E-cadherin

Total RNA from the three cell lines, cultured during 3 h, 6 h, 24 h, 48 h and 5 days with the above-mentioned DMSO and RA concentrations was isolated according to Chomczynski (RNA-zol method) (Chomczynski & Sacchi 1987). The RNA was reverse-transcribed and used as a template for PCR amplification. The internal standard pQA1 (Bouaboula *et al.* 1992, Van Bezooijen *et al.* 1998) was used to standardise cDNA for β_2 microglobulin expression by competitive PCR. PCR was performed using primers for the following integrins (extracellular matrix protein ligand are in parentheses): β_1 (fibronectin, vitronectin, collagen), β_3 (vitronectin), β_4 (laminin), β_5 (vitronectin), β_6 (fibronectin), α_1 and α_2 (collagen), α_3 (laminin), α_4 and α_5 (fibronectin), α_6 (laminin), α_8 (fibronectin, vitronectin, tenascin), α_9 (tenascin) and α_v (vitronectin); E-cadherin; uPA, uPAR, tPA, PAI-1, PAI-2; MMP-2 and MMP-9; TIMP-1 and TIMP-2. Primer sequences are available from the authors upon request. PCR conditions were 95 °C for 5 min, 29–38 cycles of 95 °C for 20 s, 56 °C for 60 s and 72 °C for 30 s, followed by extension at 72 °C for 3 min. The PCR products were resolved on 1.5% agarose gels and visualised by ethidium bromide staining. Oligonucleotides for PCR amplification were synthesised by Isogen (Maarssen, The Netherlands) or Eurogentec (Seraing, Belgium). In addition, E-cadherin protein expression was investigated by immunofluorescence using a mouse anti-human monoclonal antibody against E-cadherin (Transduction Laboratories, Toronto, Canada). The canine kidney cell line MDCK was used as a positive control.

Cell attachment assay

The attachment of FTC cells to extracellular matrix was performed as described previously (Van der Pluijm *et al.* 1996, Smit *et al.* 1998, 1999). In short, extracellular matrix derived from the MC3T3 cell line as described in the 'Degradation of extracellular matrix' section was coated onto 96-well plates overnight at 4 °C. Methanol (100 μl 60%) was added to each well for 2 h at 4 °C and the wells were washed for 30 min at 4 °C with washing buffer (50 mM Tris-HCl (pH 7.8), 110 mM NaCl, 5 mM CaCl_2 , 0.1 mM phenylmethylsulphonyl fluoride, 1%

Figure 1 Effects of 13-*cis*-RA and aprotinin (Trasylol; 200 U/ml) on degradation of ^{35}S -methionine labelled extracellular matrix synthesised by the MC3T3 cell line coated onto plastic. The three FTC cell lines were cultured for 5 days with RA 0.1, 1 and 10 μM or DMSO and seeded at a density of 100 000 per well. After 48 h, radioactivity in the supernatant and coating were measured. Release of radioactivity is expressed as the radioactivity of the supernatant as a percentage of total radioactivity per well. Matrix degradation is reflected by the release of radioactivity from wells containing FTC cells minus the release of radioactivity from wells without cells. For formula, see text. Results are expressed as means $\pm$ S.D. * $P < 0.05$; # $P < 0.01$.

BSA, and 0.1 μM sodium azide) to block unbound sites on the plastic. The FTC cell lines were cultured for 1 and 5 days with RA and DMSO concentrations as mentioned. The FTC cells were dissociated with 0.05% EDTA. Cells were seeded in serum-free medium (DMEM/HAM-F12) supplemented with glutamine and 0.5% insulin, transferrin and selenium solution (ITS, Life Technologies-Gibco BRL) at a density of approximately 8000 per well. The 96-well plates were incubated at 37 °C for 3 h. After washing, the adhering cells were fixed with methanol and stained with Amido Black. Two non-overlapping microscopic fields within each well were counted (magnification $300\times$). To study whether RA or DMSO affect attachment directly, in a separate experiment, RA and DMSO were added *after* seeding FTC cells onto the 96-well plates that were pre-cultured without RA.

Statistics

All results are presented as means $\pm$ S.D. Comparison with controls was performed using a two-tailed unpaired Student's *t*-test or analysis of variance. A *P* value < 0.05 was considered significant.

Results

Effects of RA on degradation of extracellular matrix

All cell lines attached rapidly (< 1 h) to non-radioactive serum free conditioned medium coated onto plastic. No differences in attachment were observed between the three cell lines. No proliferation of the cell lines was observed in the 48 h after seeding (data not shown). Data on matrix degradation are given in Fig. 1. Release of radioactivity during 48 h in wells with DMSO alone, without cells, was $14 \pm 4\%$ of total radioactivity. This did not differ from wells with 10 μM RA without cells ($15 \pm 2\%$). Differences between the three cell lines in matrix degradation were observed: matrix degradation (release of radioactivity observed in wells containing FTC cells minus spontaneous release (14%)) in FTC-133 with DMSO alone was more prominent than in the other cell lines cultured with DMSO alone – FTC-133: $16 \pm 2\%$ (calculated from $30 \pm 2\%$ release of radioactivity in FTC-133 containing wells minus 14% spontaneous release); FTC-236: $13 \pm 1\%$ ($27 \pm 1\% - 14\%$); and FTC-238: $13 \pm 1\%$ ($27 \pm 1\% - 14\%$), $P < 0.05$ vs FTC-133. Addition of aprotinin inhibited matrix degradation to a considerable

Figure 2 Zymographic analysis of plasminogen activator activity in FTC cell lines cultured for 5 days with 10 μ M 13-*cis*-RA or DMSO. In FTC-133, no expression of uPA-PAI complexes (110 kDa) is observed in contrast with FTC-236 and FTC-238. The uPA observed at zymography is the 54 kDa variant. RA treatment results in decreased uPA and uPA-PAI activity in FTC-236 and FTC-238, whereas in FTC-133 uPA activity is similar. Control is the ZR75-1 cell line.

extent in all cell lines. In the FTC cell lines cultured for 1 day with RA, no effects on matrix degradation were observed. In the FTC cell lines cultured for 5 days with RA, significant reductions in matrix degradation were observed with 10 μ M RA: in FTC-133 extracellular matrix degradation was reduced to $10 \pm 1\%$ (24%–14%) and in FTC-238 to $8 \pm 1\%$ (22%–14%) ($P < 0.05$ vs FTC cells cultured with DMSO only). In FTC-236, matrix degradation by cells cultured for 5 days with 10 μ M RA was $3 \pm 2\%$ (17%–14%, $P < 0.01$ vs FTC-236 cultured with DMSO only).

Zymography

Zymography was performed with cells cultured for 5 days with 10 μ M RA as this condition revealed significant reductions in extracellular matrix degradation. The results of zymography are given in Fig. 2. All three cell lines exhibited uPA activity (54 kDa), whereas no tPA activity could be found. Clear uPA-PAI complexes (110 kDa) were observed in FTC-236 and FTC-238 in contrast with FTC-133, as observed before (Smit *et al.* 1999). Supernatants of cells cultured for 5 days together with 10 μ M 13-*cis*-RA revealed a clear decrease of uPA activity in FTC-236 and FTC-238, with the most prominent reduction in FTC-236. In the FTC-133 cell line, uPA activity appeared not to be influenced by RA. Expression of

uPA-PAI complexes was decreased in FTC-236 and FTC-238.

Semi-quantitative RT-PCR and immunofluorescence for E-cadherin

RT-PCR revealed no mRNA expression at any point of time of tPA, PAI-2, integrin subunits β_4 , β_6 and α_3 , α_6 , α_7 and α_8 ; MMP-9 and TIMP-1. mRNAs of uPA (Fig. 3a), MMP-2, TIMP-2 and integrin subunits β_1 , β_3 , β_5 and α_1 , α_2 , α_5 and α_v were expressed in all three cell lines to the same extent at all points of time and no influence of RA was observed. As observed previously (Smit *et al.* 1999) PAI-1 mRNA expression was lower in FTC-133 than in FTC-236 and FTC-238 without influence of RA (Fig. 3a). Integrin α_9 expression was lower in FTC-236 than in FTC-133 and FTC-238, without effect of RA, whereas α_4 mRNA was more prominent in FTC-236 (Fig. 3b). E-cadherin and uPAR mRNA expression were only detectable after 48 h culture (Fig. 3c). E-cadherin mRNA expression was not influenced by RA. The only effect of RA was observed on uPAR mRNA expression: uPAR mRNA expression was clearly diminished in FTC-236 cultured with RA, whereas it was unaffected in FTC-133 and FTC-238 (Fig. 3c).

Immunofluorescence of E-cadherin did not reveal increased expression of E-cadherin. However, a tendency

Figure 3 RT-PCR of integrin subunits, constituents of the plasmin activation system and E-cadherin. (a) uPA and PAI-1 mRNA expression of FTC cells cultured for 6 h in 10 μ M 13-*cis*-RA or DMSO, 29 cycles, 56 °C. (b) Integrin α_4 and α_9 mRNA expression of FTC cells cultured for 3 and 6 h in 10 μ M RA, 33 cycles. (c) uPAR and E-cadherin mRNA expression of FTC cells cultured for 48 h in 10 μ M RA, 38 cycles C, DMSO; R, RA.

of E-cadherin to organise along the cell membrane could be observed in 23% of the cells cultured with 10 mM RA, assessed by counting three fields of vision for the FTC cell lines (magnification 400 $\times$) (Fig. 4).

Cell attachment assay

The effects of RA on attachment of the FTC cell lines to extracellular matrix are given in Fig. 5. RA added to the FTC cells after seeding did not influence the attachment. After 1 day, only FTC-133 cultured with 10 μ M RA showed increased attachment (+89%, $P<0.05$). After 5 days, attachment in all three cell lines cultured with 10 μ M RA was enhanced strongly, as compared with

DMSO controls – FTC-133: +91% ($P<0.01$); FTC-236: +64% ($P<0.01$); and FTC-238: +87% ($P<0.05$).

Discussion

The present study was performed to assess the putative beneficial effects of 13-*cis*-RA on degradation of extracellular matrix and the attachment to extracellular matrix in FTC cell lines. A clear reduction in extracellular matrix degradation by RA was observed in all three cell lines. The most prominent effect was observed in the FTC-236 cell line. Zymography revealed clear reductions in uPA activity in FTC-236 and FTC-238 cultured for 5 days

Figure 4 Immunofluorescence of FTC cell lines cultured with 10 μ M 13-*cis*-RA or DMSO for 5 days. Immunofluorescence was performed with a mouse anti-human monoclonal antibody and a goat anti-human fluorescence labelled second antibody. The MDCK cell lines was used as a positive control. Magnification is 300 $\times$. (a) FTC-133 cultured with DMSO. (b) FTC-133 cultured with RA: cell-cell contact is more prominent. The arrow indicates organisation of E-cadherin along the cell membrane as observed in 23% of cells.

with 10 μ M RA, with the most impressive reduction in FTC-236, whereas no effects were observed in FTC-133. Decreased activity of uPA-PAI complexes in FTC-236

and FTC-238 were observed as well, so that the decreased uPA activity cannot be explained by increased binding to PAI. No effects of RA on uPA mRNA were observed.

Figure 5 Attachment of FTC cell lines, cultured for 1 and 5 days in 0.1, 1 and 10 μ M 13-*cis*-RA or DMSO (control) to extracellular matrix coated onto plastic. Cells were counted 3 h after seeding. Attachment is reflected as the number of cells/area at a magnification of 400 $\times$. Results are expressed as means $\pm$ s.d. * P <0.05; # P <0.01.

However, uPAR mRNA expression in FTC-236 appeared to be decreased by RA. Thus, decreased matrix degradation by RA in FTC-236 and FTC-238 may be explained by decreased uPA activity, which appears to be post-translational. In FTC-236, the decreased uPAR mRNA expression may contribute to the effect, as uPAR is a prerequisite for uPA activity (Mondino *et al.* 1999). The finding of decreased uPAR mRNA expression by RA has not been reported before.

Most studies performed so far investigated invasion of tumour cells through artificial membranes and not degradation of extracellular matrix *per se*. However, the decreased activity of plasmin activators is in line with studies on invasive behaviour in prostate carcinoma cell lines and melanoma (Hendrix *et al.* 1990, Waghray & Webber 1995, Webber & Waghray 1995). Although RA reduced extracellular matrix degradation by FTC-133, no clear effects of RA on uPA activity at zymography was observed. Apparently, other enzymes involved in degradation of extracellular matrix may play a role. RA has been reported to decrease mRNA expression of MMPs (Hendrix *et al.* 1990, Schoenemark *et al.* 1999). However, we did not find effects of RA on MMP mRNA expression. No effects of RA on expression of TIMPs were observed either. The more prominent matrix degradation in FTC-133 as compared with the other cell lines is in line with earlier experiments and could be correlated to diminished PAI mRNA expression in FTC-133.

Attachment of the cell lines to extracellular matrix was enhanced considerably by RA. In recent reports, similar effects of RA on the attachment of other tumour cell lines to extracellular matrix have been observed (Matarrese *et al.* 1998, Nakagawa *et al.* 1998, Pereda *et al.* 1999, Pijuan-Thompson *et al.* 1999). Several explanations can be proposed. First, RA treatment may affect the expression of attachment molecules, such as integrins or E-cadherin. In the literature no consistent effects of RA on integrins are described, some studies reporting increased, unaltered or decreased expression of integrin subclasses α_v , α_3 , α_4 , α_5 , β_1 , β_3 , β_4 and β_5 (Dedhar *et al.* 1991, Morini *et al.* 1999, Pijuan-Thompson *et al.* 1999, Touhami *et al.* 1999). We found no effects of RA on integrin mRNA expression. The differences in α_4 and α_9 integrin mRNA expression as observed between the cell lines were not influenced by RA. In two studies in human malignant tumours, RA was reported to increase E-cadherin mRNA and protein expression (Hoang-Vu *et al.* 1998, Matarrese *et al.* 1998). In the present study, we found an indication that RA treatment had an effect on the organisation of E-cadherin. Interestingly, this was also observed in a study of RA in colon carcinoma (Nakagawa *et al.* 1998). An alternative explanation for the enhanced attachment of the thyroid carcinoma cell lines to extracellular matrix by RA treatment in this study may be the decreased proteolytic activity of the cell lines. It was found previously that the expression of RAR α , β and γ and RXR α and β were

expressed by all FTC cell lines but that RAR α and RAR β expression were decreased in FTC-238 as compared with the other FTC cell lines (Schmutzler *et al.* 1998). The differences in RA response by the three cell lines can therefore not be related to differences in retinoid receptor expression.

As the three cell lines are derived from the same patient, the differences between the cell lines are remarkable. Clonal selection within the primary tumour resulting in subclones with different characteristics with respect to the plasmin activation system may be responsible for these differences. Another possibility is that the microenvironment from which the metastatic cells were derived has influenced their phenotype.

In conclusion, the present study demonstrates the beneficial effects of RA on important characteristics of metastatic behaviour in three related FTC cell lines. The degradation of extracellular matrix was decreased whereas the attachment to extracellular matrix was enhanced. Evidence is found for effects of RA on the plasmin activation system, e.g. diminished uPA activity and uPAR mRNA expression. However, other mechanisms, independent of the plasmin activation system, may be involved as well. The beneficial effects on attachment to extracellular matrix may be explained by decreased proteolytic activity or enhanced functional E-cadherin expression. As these studies were performed in cell lines only, the implications of our findings for clinical practice are not clear and may not be translated unconditionally to the clinic. It would be worthwhile to investigate the relation between the expression of retinoid receptor subclasses and clinical behaviour in tissue samples derived from thyroid carcinoma. However, the results of the present study may add to the understanding of the mechanisms of the beneficial effects of retinoids as observed in thyroid carcinoma and offer perspectives for further investigations into the application of retinoids in thyroid carcinoma.

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