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Fibronectin is an Estrogen-repressed Protein in RUCA-I Rat Endometrial Adenocarcinoma Cells

Günter Vollmer,* Anne-Christine Hopert, Nicola Ellerbrake, Winfried Wünsche and Rudolf Knuppen

Institut für Biochemische Endokrinologie, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany

We recently established and characterized two rat endometrial adenocarcinoma cell lines which we called RUCA-I and RUCA-II. Despite high estrogen receptor levels neither cell line responded to estradiol in conventional cell culture conditions on plastic and in the presence of charcoal stripped fetal calf serum. We further demonstrated that culturing of these cells on a reconstituted basement membrane induced the estrogen responsiveness for both proliferation and gene expression. Particularly, the expression of components of the complement C3 system, which represent major estradiol inducible proteins in the rat uterus *in vivo*, were found to be under the control of estrogens and antiestrogens. In this paper the search for estrogen repressed proteins is reported. For this purpose secretory proteins of RUCA-I cells were metabolically labelled with ^{35}S-methionine and tested for the presence of estrogen-repressed, antiestrogen-inducible protein species. Analyzing cell culture supernatants of RUCA-I cells by polyacrylamide gel electrophoresis under reducing conditions a protein with an apparent size of approx. 250–270 kDa became conspicious. The formation and secretion of this protein was suppressed by estradiol and induced by the antiestrogen ICI 164384. Gel electrophoresis performed under non-reducing conditions and hyaluronidase digestion showed that this estrogen-repressed protein represents a dimeric glycoprotein. By immunoprecipitation this glycoprotein was identified as fibronectin. Investigations of steady state mRNA levels of fibronectin by rtPCR suggested a post-transcriptional regulation of this molecule by estradiol. This is the first report on repression of components of the extracellular matrix by estradiol and induction by the complete antiestrogen ICI 164384. The consequences of this finding in regard to growth and invasion of endometrial tumors are discussed.

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INTRODUCTION

The number of suitable cell culture models to study hormonal functions in endometrial cancer cells is rather limited, because either endometrial tumor cell lines of human and rodent endometrial adenocarcinoma do not express steroid hormone receptors or if they express e.g. the estrogen receptor (ER) *in vitro*, they respond to estrogens and antiestrogens rather marginally [1–4]. Recently we described the establishment and characterization of two ER-positive rat endometrial adenocarcinoma cell lines, which we named RUCA-I and RUCA-II [5]. If cultured under standard cell culture conditions, none of the cell lines responded to estradiol treatment and only limited to treatment with the antiestrogen tamoxifen. Using matrigel as substrate, in order to provide a more organotypical surrounding for RUCA-I cell cultures, we were able to induce functional differentiation of these cells. Both proliferation and gene expression could be modulated in response to estrogens and antiestrogens. Particularly striking was the inductory effect of estrogens and the inhibitory effect of the antiestrogen ICI 164384 on the expression of complement C3 components [6], which represent major estrogen inducible proteins in the immature rat uterus *in vivo* [7, 8].

The hormone, particularly estrogen, dependency of the expression of components of the complement C3 system in the rat uterus is well studied and documented

*Correspondence to G. Vollmer.
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[9–12]. Components of the complement C3 system have therefore to be regarded as highly valuable marker molecules to study molecular mechanisms of estrogen-dependency of gene expression in the rat uterus and endometrium. However, little is known about estrogen-repressed functions in endometrial tissue. There are some reports in the literature on the repression of gene expression by steroid hormone receptors, vitamin receptors or other members of the superfamily of ligand dependent transcription factors. The most extensively studied example is the repression of the rat TRPM-2 gene by androgens in the ventral prostate [13, 14]. Additionally, there exists a single report on estrogenic repression of gene expression. By differential hybridization analysis the MGT1 gene was shown to be estrogen repressed in T47D mammary carcinoma cells [15].

In this paper we report on the search for estrogen-repressed proteins in RUCA-I rat endometrial adenocarcinoma cells using ^{35}S-methionine labelling of cell culture supernatants prior to gel electrophoresis. We report that the formation and secretion of a dimeric glycoprotein with an apparent molecular weight of 250–270 kDa per subunit is repressed by estradiol. By immunoprecipitation we identified this protein as fibronectin.

MATERIALS AND METHODS

Cell culture

From RUCA-I cells large quantities of cells of passages 10, 30 and 50 were grown, frozen and subsequently stored in liquid nitrogen. This enabled us to use the same stocks of cells for all experiments. Prior to experimental use, RUCA-I cells were precultured for one passage in DMEM/F12 medium without phenol red containing 10% fetal calf serum and for two passages in the above medium containing charcoal stripped serum. Thereafter cells were harvested and 200,000 cells were seeded on top of a layer (300 µl) of ECM-substrate (Harbour matrix; Cell Systems, Germany) per well of a 24-well plate in the presence of 2 ml serum-free defined medium (SFDM). The SFDM was composed of DMEM/F12 and contained additionally 2 µg/ml insulin, 4 mM glutamine, 40 µg/ml transferrin, 10^{-8} M hydrocortisone, 2×10^{-8} M sodium selenite, and 1 µg/ml putrescine. The cells were cultured in a humidified 5% CO_2 atmosphere at 37°C and medium was changed twice a week except for matrigel cultures, which were fed daily.

Hormonal treatment

For hormonal treatment we used estradiol (10^{-9}-10^{-7} mol/l) as agonistic ligand, as antiestrogen we used ICI 164384 (kindly provided by Dr A. E. Wakeling, Zeneca Chemicals, Macclesfield, U.K.) in concentration from 5×10^{-9} to 5×10^{-7} mol/l. This means hormonal components were used in approximately equipotent concentrations because of the relative binding affinity of ICI 164384 to the ER which is approx. 5-fold weaker than that of estradiol [16]. Prior to hormonal treatment, cells were seeded on ECM in the above numbers and precultured in SFDM on the substrate for 24–48 h. Thereafter cells were incubated for 48 h with estradiol or ICI 164384. Control cultures received ethanol, which was used as a vehicle. Medium was changed daily and hormonal treatment was repeated. In some experiments additional controls were included by culturing RUCA-I cells in 24-well plates on plastic in the presence of DMEM/F12 medium containing 5% fetal calf serum or SFDM and the above concentrations of hormone or anti-hormone.

Metabolic labelling of secretory proteins and immunoprecipitation

After 48 h of hormonal stimulation, secretory proteins were labelled metabolically with ^{35}S-methionine. During labelling cells were cultured for another 16 h in the above media and under the hormonal conditions described above, including the following modifications: the methionine content of the medium was reduced by 90%, instead the medium was substituted with 200 µCi/ml ^{35}S-methionine. The volume of the cell culture medium per well was reduced to 150 µl. At the end of the incubation period cell culture supernatants containing the *de novo* synthesized metabolically-labelled secretory proteins were aliquoted in a 15 µl aliquot and an aliquot containing the remainder. From the 15 µl aliquot the incorporation rate of the radioactive amino acid was determined, the remainder was used for SDS–PAGE electrophoresis. Total synthesis of secretory proteins was determined in triplicate by precipitating proteins out of 3 µl cell culture supernatant with TCA.

In order to determine whether or not investigated proteins represent glycoproteins, cell culture supernatants were digested at room termperature for 12 h with 10 mg/ml, 1 mg/ml or 0.1 mg/ml of hyaluronidase (Boehringer Mannheim, Germany) prior to electrophoretic separation.

Prior to immunoprecipitation cell culture supernatants were preabsorbed for 1 h with Sepharose 4B (Pharmacia, Freiburg, Germany). In order to immunoprecipitate fibronectin the following reagents were subsequently added to labelled cell culture supernatants: a polyclonal anti-rat fibronectin antiserum (GIBCO, Eggenstein, Germany) giving a final dilution of 1:400 or 1:2000 overnight at 4°C, 5 µl of a goat anti-rabbit IgG antiserum (Dianova, Hamburg, Germany) for 1 h at 4°C, and finally 100 µl protein-A-Sepharose overnight at 4°C (Pharmacia, Freiburg, Germany). To controls we added 125 µg of unlabelled rat fibronectin (Sigma, Deissenhofen, Germany) to prelabelled cell supernatants and prior to the immunoprecipitation procedure.

Electrophoresis

Electrophoresis was performed according to standard protocols using a discontinuous system [17], reducing and non-reducing conditions and either 5 or 7.5% polyacrylamide gels. Loading of the gel was performed after the amount of acid precipitable protein had been determined. For each experimental condition the same number of counts, meaning the same amount of protein bound ^{35}S-methionine was loaded on the gel. After electrophoresis the gels were fixed, incubated in En^{3}Hance (DuPont, Germany), dried and the gel bands were visualized by fluorography.

rt PCR of fibronectin mRNA

The expression of fibronectin and of cyclophilin, which was used as a constitutively expressed control gene, was assessed on the mRNA level with a rtPCR method. Reversed transcription was performed according to the manufacturers instruction using a standard kit (Gibco, Eggenstein, Germany). The primer sequences used for amplification of fibronectin ssDNA were forward 5′GTGTTATGACGACGGGAAGACCT; reverse 5′CTGTAGGGGTGGGCTTTGAGGTC giving rise to a 466 bp fragment. The sequences used for amplification of cyclophilin were forward 5′GGATTCATGTGCCAGGGTGG; reverse 5′CACATGCTTGCCATCCAGCC (provided by Dr R. Knauthe, Schering AG, Berlin), giving rise to a 213 bp fragment. Single stranded cDNA obtained after reverse transcription was amplified for 35 cycles [1 min 92°C (strand separation), 1 min 55 C (annealing), 1 min 72°C (extension)] using the primer pairs described above. To enhance sensitivity ssDNA was amplified in the presence of Digoxigenin-dUTP. 5 μl PCR product of each reaction was combined, separated in an 1% agarose gel, blotted and developed using anti-Digoxigenin antibodies (Boehringer Mannheim) and luminescent substrates according to manufacturers instructions.

RESULTS

Morphology of RUCA-I cells

If RUCA-I cells were plated on a thin layer of reconstituted basement membrane, within 6 h they organized themselves into web-like structures [Fig. 1(A)]. The formation of these structures was completed after 24 h [Fig. 1(B)], thereafter only thickening of the web-like structures was observable. There were no morphological differences whether or not RUCA-I cells were cultured with or without estradiol or the antiestrogen ICI 164384. However, in contrast to RUCA-I cells cultured on plastic, RUCA-I cells cultured on extracellular matrix responded to treatment with estrogens or antiestrogens [6].

Detection of an estrogen-repressed secretory protein

Metabolic labelling of cells with ^{35}S-methionine followed by polyacrylamide gel electrophoresis is a potent tool to screen for hormonally regulated secretory proteins, which are secreted into cell culture supernatants. With this method, using a 7.5% gel and reducing conditions we identified a protein to be repressed by estradiol treatment (Fig. 2, left panel, E_2-repr.). This protein was larger than the 200 kDa standard protein,

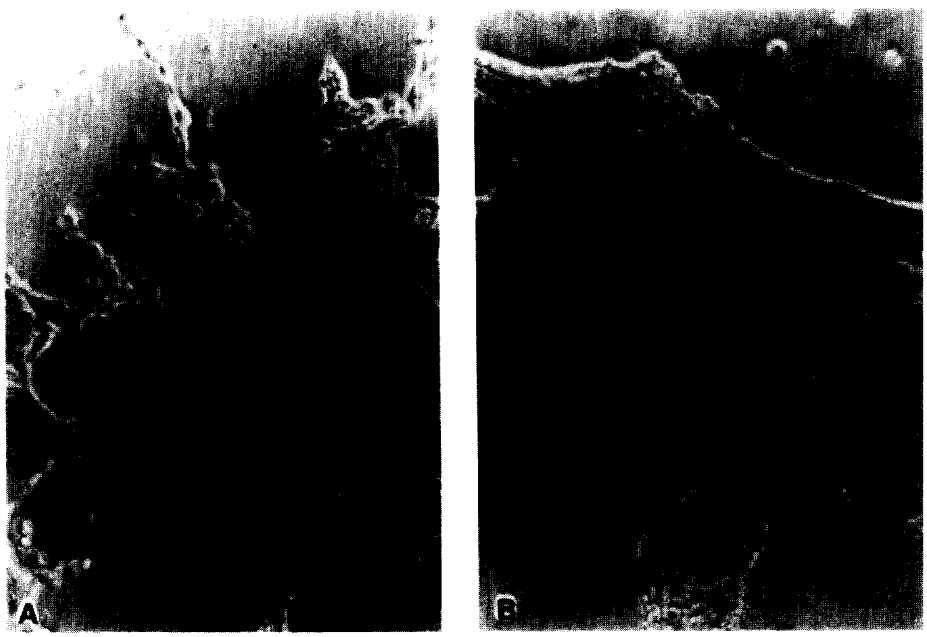

Fig. 1. Morphology of RUCA-I cells on a reconstituted basement membrane. RUCA-I cells were randomly seeded on a thin layer of a reconstituted basement membrane. Overnight they organized themselves into web-like structures (A), with single webs increasing with longer culture periods (B).

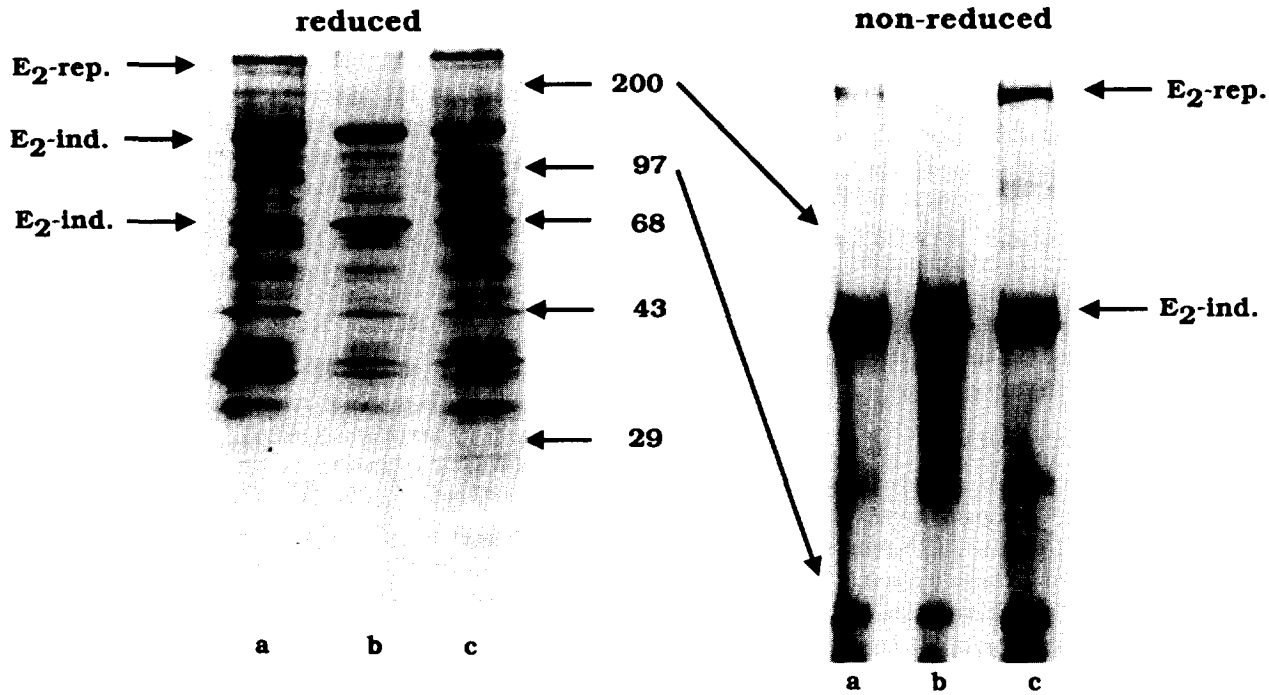

Fig. 2. SDS–gel electrophoresis of secreted proteins. Cell culture supernatants were analyzed by SDS–PAGE using 7.5% gels for reducing (left panel) and 5% gels for non-reducing (right panel) conditions. Prior to electrophoresis RUCA-I cells were cultured on a reconstituted basement membrane without (a) hormonal treatment or a treatment with estradiol (b) and the antiestrogen ICI 164384 (c).

which served as the largest molecular weight standard that was available to us. In contrast, treatment of RUCA-I cells with the antiestrogen ICI 164384 induced an increased production of this protein above untreated control values. This qualitative impression that was suggested by the fluorographs could be evidenced by semiquantitative densitometry [Fig. 3(a)]. Estradiol reduced the expression of this protein by approx. 40% whereas antiestrogen treatment lead to a 50% induction of this protein ($n = 15$).

Analysis of cell culture supernatants by gel electrophoresis using non-reducing conditions (Fig. 2, right panel) suggested a dimeric structure of the estradiol-repressed protein rather than a trimeric or a multimeric structure. Densitometric analysis of the non-reduced protein visualized the estrogenic regulation of this

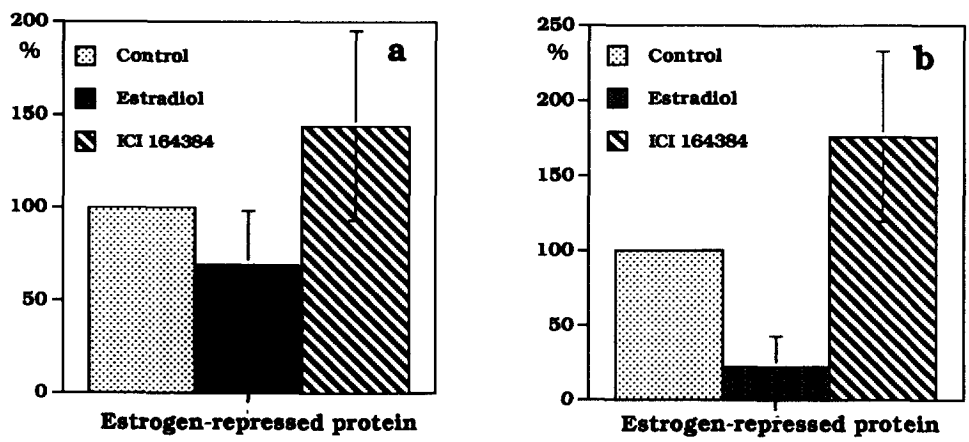

Fig. 3. Densitometric analysis of the estrogen repressed protein. For semiquantitative evaluation the estrogen-repressed protein was analyzed by densitometry. 15 gels run under reducing (a) and 5 gels run under nonreducing conditions (b) were subjected to densitometry. For comparison, values obtained for the untreated control were set to 100% and values for experimental conditions were calculated accordingly. Densitimetric results were subjected to statistical analysis. Using a Students t-test analysis experimental conditions were compared to untreated controls. Effects of estrogen treatment ($P = 0.009$ (reducing conditions) and $P = 0.0001$ (non-reducing conditions)) showed a higher significance than effects resolvable following treatment with ICI 164384 ($P = 0.048$ (reducing conditions) and $P = 0.0037$ (non-reducing conditions)).

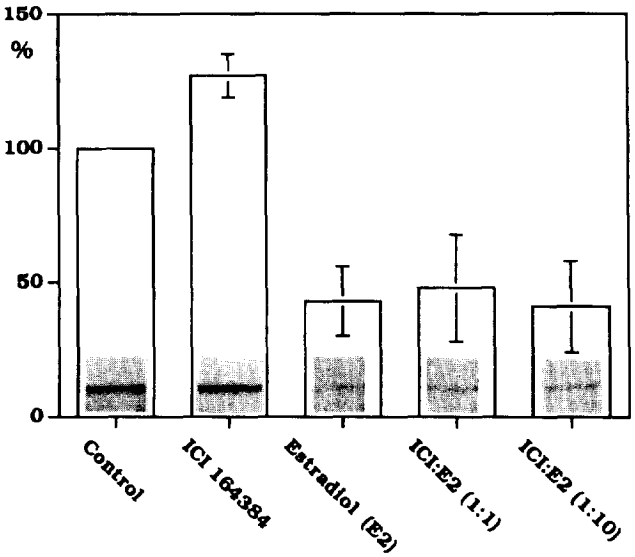

Fig. 4. Competition experiments. The induction of the estrogen repressed protein by the antiestrogen ICI 164384 was used for the assessment of the competing activity of estradiol. Shown are a untreated control, the effects of the antiestrogen ICI 164384 and of estradiol if given alone, or the effects of combinations of antihormones and hormones at equipotent (1:1) concentrations and a 10-fold excess of estradiol (1:10). Columns represent mean values and standard deviations of four independent experiments.

protein by far more clearly than did densitometric analysis of the protein gel that was run under reducing conditions [Fig. 3(b)]. Treatment with estradiol reduced the expression of this protein to 25% of control values, whereas treatment with the antiestrogen ICI 164384 exhibited an approx. 60% induction of the formation and secretion of this protein ($n = 5$).

Competition experiments revealed the estradiol dependency of the repression of synthesis of this protein in RUCA-I cells. ICI 164384 at a concentration of 5×10^{-7} mol/l stimulated an increased production of this glycoprotein by RUCA-I cells, whereas estradiol not only counteracted this stimulation but also reduced basic, unstimulated levels of expression (Fig. 4). The stimulation by ICI 164384 was found to be significantly inhibited by equipotent concentrations [16] of 10^{-7} mol/l of estradiol (Fig. 4) as well as by a 10-fold excess of estradiol (Fig. 4).

Hyaluronidase digestion of cell culture supernatants suggested that the estrogen-repressed, ICI 164384-induced protein of RUCA-I cells is a glycoprotein with an estimated glycomoiety of approx. 20–30 kDa, and a protein moiety (Fig. 5) larger than the largest marker protein, which was 200 kDa in size.

Identification of the estradiol repressed protein

From the potential size of the estradiol repressed protein and its presumptive dimeric glycoprotein nature we hypothesized that the estradiol-repressed protein may be identical to fibronectin. We tested this hypothesis by an immunoprecipitation experiment. With a rabbit anti-rat fibronectin antiserum (Gibco Eggenstein, Germany) we were able to immunoprecipitate the estrogen-repressed protein (Fig. 6). The specificity of the reaction was checked by a competition experiment with purified, unlabelled rat fibronectin added to cell culture supernatants prior to immunoprecipitation. The unlabelled fibronectin competed the metabolically labelled fibronectin out of the antibody binding (Fig. 6).

Analyis of fibronectin mRNA expression

Fibronectin mRNA levels from cultured RUCA-I cells were analyzed by rtPCR. Interestingly, in either experimental condition involving matrigel cultures and independent of a hormonal treatment we found fibronectin mRNA expression (Fig. 7). On plastic in the presence of charcoal stripped serum, expression of fibronectin mRNA appeared to be completely down regulated [Fig. 7(f)].

DISCUSSION

Unopposed action of estrogens on endometrial cells has to be regarded as a major risk factor for endometrial carcinogenesis of humans and rodents [18–20]. It is therefore of importance to know precisely the molecular and cellular mechanism of estrogen action in normal and malignant endometrial cells. We are interested in the estrogen dependency of gene expression in endometrial tumor cells. However, the number of hormone receptor positive, hormone responsive endometrial

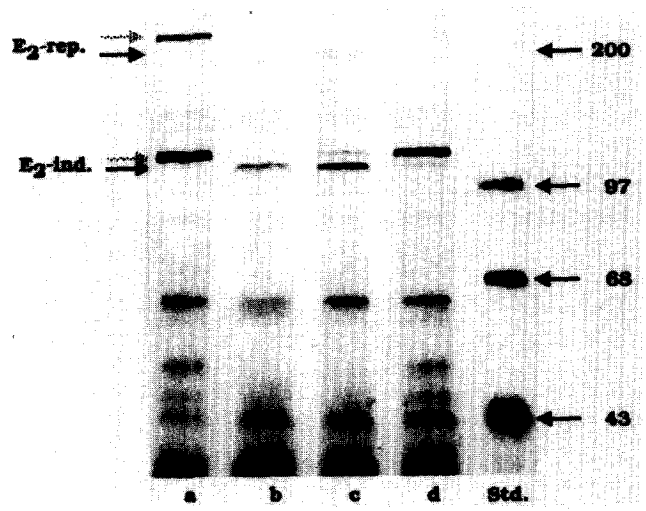

Fig. 5. Hyaluronidase treatment. The molecular nature of the estrogen repressed protein was further analyzed by treatment of cell culture supernatants from ICI 164384 stimulated cells [shown in (a)] or after treatment of these supernatants with hyaluronidase at concentrations of 10 mg/ml (b), 1 mg/ml (c), or 0.1 mg/ml (d). Dashed arrows represent the undigested molecule, solid arrows indicate electrophoretic mobility after treatment of cell culture supernatants with hyaluronidase.

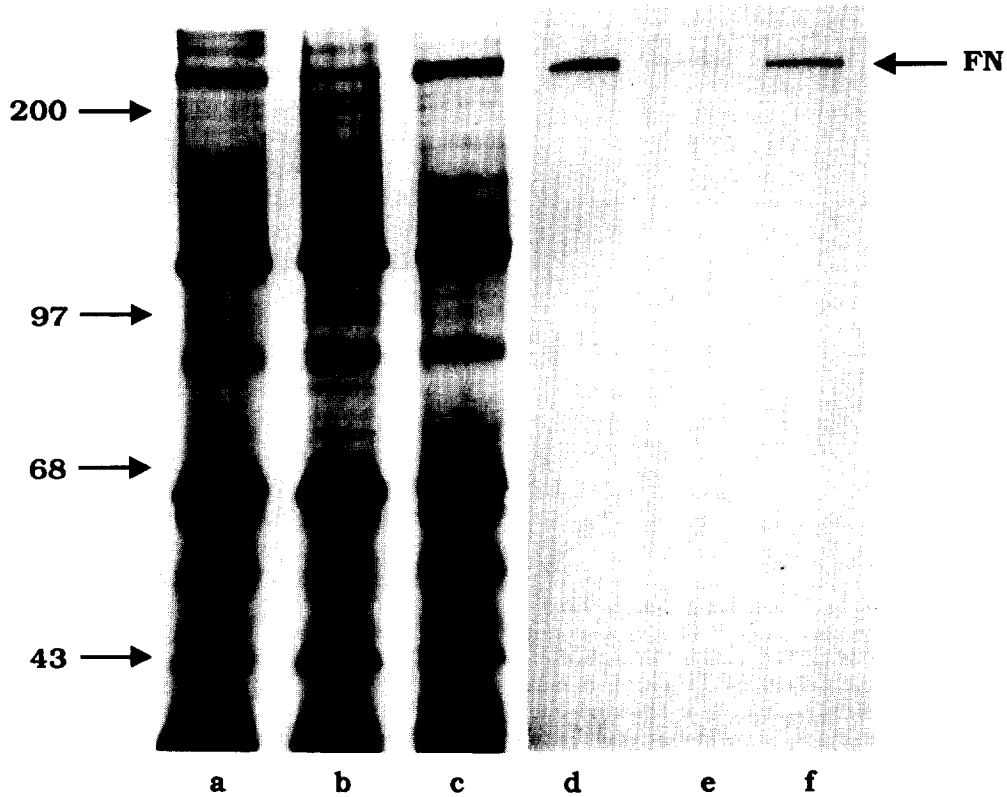

Fig. 6. Immunoprecipitation of the estrogen-repressed protein with anti-fibronectin antibodies. To identify the estrogen repressed protein cell culture supernatants were immunoprecipitated with a polyclonal anti-rat-fibronectin antiserum. Shown is a SDS–PAGE using reducing conditions of unprecipitated cell culture supernatants from untreated controls (a), from estradiol treated cultures of RUCA-I cells (b) and from cells treated with ICI 164384 (c). Immunoprecipitation was carried out from supernatants of cells treated with ICI 164384 with 1:400 (d) and 1:2000 (f) dilutions of the antibody. To test specificity of the precipitation reaction some cell supernatants received 125 μg of unlabelled fibronectin prior to immunoprecipitation (e).

derived *in vitro* cell culture models is relatively small. We succeeded in the establishment of the ER-positive cell line RUCA-I [5] which responds to both estrogens and antiestrogens if cultured on top of a reconstituted basement membrane as cell culture substrate [6]. In the paper presented here we were able to demonstrate that the expression of the extracellular matrix glycoprotein fibronectin by RUCA-I cells is repressed by estradiol and highly induced by the pure antiestrogen ICI 164384. Further, mechanistic studies provided a first clue that estrogenic regulation of fibronectin expression does not occur on a transcriptional level and presumably occurs posttranscriptionally.

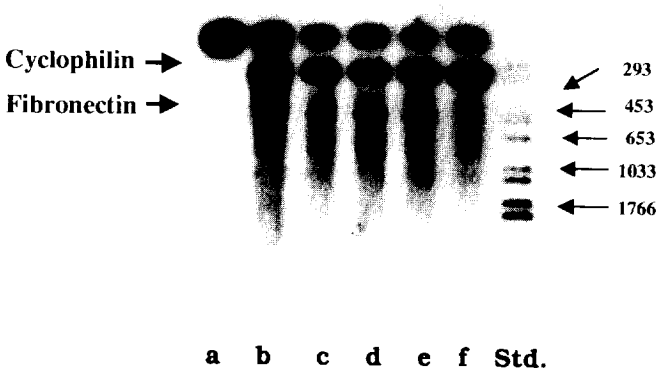

Fig. 7. Detection of fibronectin mRNA expression by rtPCR. To obtain a first molecular clue for the regulation of fibronectin, mRNA expression was analyzed by rtPCR. A 466 bp fragment representing fibronectin was detected in all cultures on reconstituted basement mebrane (b–e) without a hormonal treatment (e) or after treatment with estradiol (d) or with ICI 164384 (b,c). This fragment could not be detected in the negative control (a) and in RUCA-I cells which were cultured on plastic in the presence of serum (f). Cyclophilin was used as an internal control.

Various findings of our investigations have to be discussed, in particular the observation of hormonal repression of fibronectin biosynthesis, the potential molecular mechanism of regulation of fibronectin expression as well as the potential oncologic impact of the findings described above.

Whereas numerous hormone induced genes have already been identified in many experimental systems, the number of repressed genes and proteins by steroid hormones and vitamins acting through nuclear transcription factors is still comparatively small [1, 21, 22]. This is surprising since down regulation of gene expression potentially has the same impact on regulation of tissue function as induction of gene expression. For estrogens only a single report exists of estradiol being capable to repress the expression of the MGT1 gene in

T47D mammary carcinoma cells [15]. Fibronectin glycoprotein now has to be regarded as the second protein identified to be negatively regulated by estradiol.

We also briefly addressed the question of regulation of fibronectin expression. Our results suggest that it is not transcriptionally regulated, however, final clarification of this question is the subject of future experiments using Northern blot and RNase protection assays. Although estrogenic control of fibronectin glycoprotein neither in the endometrium nor in other tissues has been described so far, other steroid hormones and vitamins have been implicated to be important in the regulation of fibronectin expression. Glucocorticoids have been demonstrated to down regulate fibronectin expression in placenta [23, 24] and in parietal bones [25], but, on the other hand, have been shown to be stimulatory in HT 1080 human fibrosarcoma cells [26]. Progestins have also been discussed as being involved in regulation of fibronectin production. They were stimulatory for human endometrial stromal cells [27], for apical secretion in guinea pig endometrial epithelial cells [28], and chicken granulosa cells [29]. In contrast, progestins were described to down-regulate oncofetal fibronectin in placental cytotrophoblast cells, however, in this paper the authors speculated that medroxyprogesterone acetate acted in a glucocorticoid-like fashion [30]. Vitamins, particularly retinoic acid derivatives, and phorbolesters have also been demonstrated to regulate fibronectin expression, thereby exerting antagonistic effects. This is particularly interesting since both classes of substances can potentially act through specific responsive elements [31–33].

To get a closer molecular view of the potential mechanism of the regulation of fibronectin expression, regulatory elements identified within the fibronectin promotor have to be reviewed. Within a 3.7 kb EcoR1 fragment containing a 2.7 kb of flanking sequence, a GC-rich region, an AT-rich region, an ATATAA sequence, a sequence homologous to a binding site for the SP1 transcription factor, and initially one [34], later on two additional cAMP responsive elements have been identified [35]. These cAMP responsive elements may be involved in estrogenic regulation of fibronectin. At least for up-regulation of the progesterone receptor in cell cultures of immature rat endometrial cells, treatment with 8-bromo-cAMP or treatment procedures that give rise to intracellular cAMP-levels could substitute for estradiol [36]. Additionally, in another study the same authors have shown, that those agents that influence cyclic AMP-levels also stimulate the ER mediated transcription from a tandem estrogen response element linked to a CAT-reporter gene [37]. However, the same study revealed that the phosphorylation status of the ER is not intimately involved in transcriptional activation, since the degree of phosphorylation was indistinguishable in estradiol treated and ICI 164384 treated cells. In addition to the cAMP-responsive elements, so far no responsive elements for estrogens, glucocorticoids, progestins or retinoic acid derivatives have been identified. This implicates that down-regulation of fibronectin expression by estradiol in our system, or glucocorticoids and retinoic acid in other experimental systems, is possibly due to mechanisms other than a responsive element. In the case of dexamethasone it had been shown that regulation of fibronectin occurs posttranscriptionally by stabilization of fibronectin mRNA, whereas forskolin (an activator of the adenylate cyclase) and TGF-β directly regulate fibronectin expression on a transcriptional level [26, 38].

The latter finding implicates that indirect, growth factor and/or cAMP mediated regulation of fibronectin biosynthesis by estrogens is an attractive hypothesis to explain down-regulation of fibronectin expression by estrogens for several reasons. In MCF-7 breast cancer cells production of TGF-β is under the control of estrogens, an increased production is stimulated by antiestrogens [39]. Further, TGF-β is a potent stimulator of fibronectin expression and its incorporation into the extracellular matrix [40, 41]. Our current working hypothesis is that estrogen treatment modulates TGF-β levels which in turn regulate fibronectin biosynthesis and secretion. This hypothesis could further explain comparatively high levels of fibronectin production in untreated control cultures, since reconstituted basement membrane preparations are known to contain significant amounts of TGF-β bound to the matrix preparation [42]. Another family of growth factors and growth factor binding proteins that is important for uterine function and that had additionally been implicated with regulation of fibronectin expression, at least in thoracic aortic smooth muscle cells and glomerular mesangial cells of the rat [43], is the family of IGF's and IGF-binding proteins. The growth factor IGF-I, which is down-regulated by the pure antiestrogen ICI 182780 [44], has been demonstrated to mimick estrogenic regulation of progesterone receptor expression in the rat uterus [36] whereas uterotropic action of estradiol in rat is asssociated with inhibiton of uterine insulin-like growth factor binding protein 3 [45].

Another mechanism for down regulation of fibronectin has been described. Transfection of resting rat 3Y1 cells with the Ela adenovirus resulted in a dramatic down-regulation of fibronectin expression. Down-regulation thereby occurred on the transcriptional level by recruitment of various Ela-responsive negative factors, that bind to the promoter region of fibronectin [46]. Further, it has been reported that Ela represses AP-1, a promoter element recognized by the family of fos and jun transcription factors [47]. In the rat uterus these early, immediate response genes are regulated by estradiol [48, 49]. Provided estrogen treatment activates the same or a similar subset of factors as

described for Ela, this represents another potential mechanism of negative regulation of fibronectin expression by estradiol.

Finally the potential role of fibronectin in endometrial adenocarcinoma cells has to be discussed. Delineated from previously published findings fibronectin could participate in two processes that give tumor cells a growth advantage over other cells or increase their motility. Fibronectin is a very potent adhesion factor usually promoting tight adherance of cells including tumor cells [50]. The decreased production of this adhesion factor in the presence of estradiol, could possibly contribute to the more aggressive growth of the tumor in the presence of hormone, as has recently been suggested for retinoic acid [33]. In this way down regulation of fibronectin could support invasive and metastatic growth of tumor cells. This may increase both their motility and their capability to survive once they have left the primary tumor.

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REFERENCES

1. Holinka C. F., Hata H., Kuramoto H. and Gurpide E.: Effects of steroid hormones and antisteroids on alkaline phosphatase activity in human endometrial cancer cells (Ishikawa Line). *Cancer Res.* **46 (1986)** 2771–2774.
2. Tabibzadeh S. S., Kaffka K. L., Kilian and P. L. and Satyaswaroop P. G.: Human endometrial cell lines for studying steroid and cytokine action. *In Vitro Cell Dev. Biol.* **26** (1990) 1173–1179.
3. Wiehle R. D., Helftenbein H. G., Land H., Neumann K. and Beato M.: Establishment of rat endometrial cell lines by retroviral mediated transfer of immortalizing and transforming oncogenes. *Oncogene* 5(1980) 787–794.
4. Helftenbein G., Wiehle R. D. and Beato M.: Establishment of temperature-dependent cell line from rat endometrium by retroviral infection. *Eur. J. Cell Biol.* **56** (1991) 49–57.
5. Schütze N., Kraft V., Deerberg F., Winking H., Meitinger D., Ebert K., Knuppen R. and Vollmer G.: Functions of estrogens and anti-estrogens in the rat endometrial adenocarcinoma cell lines RUCA-I and. *Int. J. Cancer* 52 (1992) 942–949.
6. Vollmer G., Ellerbrake N., Hopert A-C., Knauthe R., Wünsche W. and Knuppen R.: Extracellular matrix induces hormone responsiveness and differentiation in RUCA-I rat endometrial adenocarcinoma cells. *J. Steroid Biochem. Molec. Biol.* 52 (1995) 259–269.
7. Sundstrom S. A., Komm B. S., Leon H. P-d., Yi Z., Teuscher C. and Lyttle C. R.: Estrogen regulation of tissue-specific expression of complement C3. *J. Biol. Chem.* **264** (1989) 16,941–16,947.
8. Kuivanen P. C., Capulong R. B., Harkins R. N. and DeSombre E. R.: The estrogen-responsive 110 K and 74 K uterine secretory proteins are structurally related to complement C3. *BBRC* 158 (1989) 898–905.
9. Galman M. S., Sundstrom S. A. and Lyttle C. R.: Antagonism of estrogen- and antiestrogen-induced uterine complement component C3 expression by ICI 164384. *J. Steroid Biochem.* **36** (1990) 281–286.
10. Sundstrom S. A., Komm B. S., Xu Q., Boundy V. and Lyttle C. R.: The stimulation of uterine complement component C3 gene expression by antiestrogens. *Endocrinology* **126** (1990) 1449–1456.
11. Howe R. S., Lee Y. H., Fischkoff S. A., Teuscher C. and Lyttle C. R.: Glucocorticoid and progestin regulation of eosinophil chemotactic factor and complement C3 in the estrogen treated rat. *Endocrinology* **126** (1990) 3193–3199.
12. Brown E. O., Sundstrom S. A., Komm B. S., Yi Z., Teuscher C. and Lyttle C. R.: Progesterone regulation of estradiol-induced uterine secretory protein, complement C3. *Biol. Reprod.* 42 (1990) 713–719.
13. Tenniswood M. P., Guenette R. S., Lakins J., Mooibroek M., Wong P. and Welsh J.: Active cell death in hormone dependent tissues. *Cancer Metast. Rev.* 11 (1992) 197–220.
14. Wong P., Pinault J., Lakins J., Taillefer D., Leger J., Wang C. and Tenniswood M. P.: Genomic organization and expression of the rat TRPM-2 (clusterin) gene, a gene implicated in apoptosis. *J. Biol. Chem.* **268** (1993) 5021–5031.
15. Manning D. L. and Nicholson R. I.: Isolation of pMGT1: a gene that is repressed by oestrogen and increased by antiestrogens and antiprogestins. *Eur. J. Cancer* **29A** (1993) 759–762.
16. Wakeling A. E.: Comparative studies of the effects of steroidal and nonsteroidal oestrogen antagonists on the proliferation of human breast cancer cells. *J. Steroid Biochem.* **34** (1989) 183–188.
17. Laemmli U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680–685.
18. Silverberg S. G.: New aspects of endometrial carcinoma. *Clin. Obstet. Gynecol.* **11** (1970) 189–208.
19. Deerberg F., Rehm S. and Rapp K. G.: *Survival Data and Age-associated Spontaneous Diseases of Two Strains of Rats.* Fischer, Stuttgart.
20. Newbold R. R., Bullock B. C. and McLachlan J. A.: Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res.* **50** (1990) 7677–7681.
21. Nicholson R. C., Mader S., Nagpal S., Leid M., Rochette-Egly C. and Chambon P.: Negative regulation of the rat stromelysin gene promotor by retinoic acid is mediated by an AP 1 binding site. *EMBO J.* **9** (1990) 4443–4454.
22. Leger J. G., Montpetit M. L. and Tenniswood M. P.: Characterization and cloning of androgen repressed mRNAs from rat ventral prostate. *BBRC* **147** (1987) 196–203.
23. Guller S., Wozniak R., Krikun G., Burnham J. M., Kaplan P. and Lockwood C. J.: Glucocorticoid suppression of human placental fibronectin expression: implications in the uterine-placental adherence. *Endocrinology* **133** (1993) 1139–1146.
24. Guller S., Markiewicz L., Wozniak R., Burnham J. M., Wang E. Y., Kaplan P. and Lockwood C. J.: Developmental regulation of glucocorticoid-mediated effects on extracellular matrix protein expression in the human placenta. *Endocrinology* **134** (1994) 2064–2071.
25. Gronowicz, G. A., DeRome M. E. and McCarthy M. B.: Glucocorticoids inhibit fibronectin synthesis and messenger ribonucleic acid levels in cultured fetal rat parietal bone. *Endocrinology* **128** (1991) 1107–1114.
26. Dean D. C., Newby R. F. and Burgeois S.: Regulation of fibronectin biosynthesis by dexamethasone, transforming growth factor beta and cAMP in human cell lines. *J. Cell Biol.* **106** (1988) 2159–2170.
27. Zhu H. H., Huang J. R., Mazela J., Elias J. and Tseng L.: Progestin stimulates the biosynthesis of fibronectin and accumulation of fibronectin mRNA in endometrial stromal cells. *Hum. Reprod.* 7 (1992) 141–146.
28. Mularoni A., Nahfoudi A., Beck L., Coosemans V., Bride J., Nicollier M. and Adessi G. L.: Progesterone control of fibronectin in guinea pig endometrium. *Endocrinology* **131** (1992) 2127–2132.
29. Asem E. K., Conkright M. D. and Novero R. P.: Progesterone stimulates fibronectin production by chicken granulosa cells *in vitro*. *Eur. J. Endocr.* **130** (1994) 159–165.
30. Guller S., LaCroix N. C., Kirkun G., Wozniak R., Markiewicz L., Wang E. Y., Kaplan P. and Lockwood C. J.: Steroid regulation of oncofetal fibronectin expression in human cytotrophoblasts. *J. Steroid Biochem. Molec. Biol.* **46** (1993) 1–10.
31. Scita G. and Wolf G.: Retinoic acid and beta-carotene inhibit fibronectin synthesis and release by fibroblasts: antagonism to phorbol ester. *Carcinogenesis* **15** (1994) 1043–1048.
32. Scheidl H., Chandra T., Gmeiner B., Zerlauth G., Scita G. and Wolf G.: The effect of diacylglycerols on fibronectin release and its reversal by retinoic acid in cell culture. *Carcinogenesis* **12** (1991) 1791–1794.
33. Varani J., Gibbs D. F., Inman D. R., Shah B., Fligiel S. E. and Voorhees J. J.: Inhibition of epithelial cell adhesion by retinoic

acid. Relationship to reduced extracellular matrix production and alteration in Ca^{2+} levels. *Am. J. Path.* **138** (1991) 887–895.
34. Dean D. C., Bowlus C. L. and Burgeois S.: Cloning and analysis of the promoter region of the human fibronectin gene. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 1876–1880.
35. Bowlus C. L., McQuillan J. J. and Dean D. C.: Characterization of three different elements in the 5′-flanking region of the fibronectin gene which mediate a transcriptional response to cAMP. *J. Biol. Chem.* **266** (1991) 1122–1127.
36. Aronica S. M. and Katzenellenbogen B. S.: Progesterone receptor regulation in uterine cells: Stimulation by estrogen, cyclic adenosine 3′,5′-monophosphate, and insulin-like growth factor I and suppression by antiestrogens and protein kinase inhibitors. *Endocrinology* **128** (1991) 2045–2052.
37. Aronica S. M. and Katzenellenbogen B. S.: Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. *Molec. Endocr.* 7 (1993) 743–753.
38. Chandler L. A. and Bourgeois S.: Posttranscriptional down-regulation of fibronectin in N-ras-transformed cells. *Cell Growth Diff.* **2** (1991) 379–384.
39. Knabbe C., Lippman M. E., Wakefield L. M., Flanders K. C., Kassid A., Derynck R. and Dickson R. B.: Evidence that transforming growth factor-beta is a hormonally regulated negative growth factor in human breast cancer cells. *Cell* **48** (1987) 417–428.
40. Ignotz R. A. and Massague J.: Transforming growth factor-β stimulates expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem.* **261** (1986) 4337–4345.
41. Penttinen R. P., Kobayashi S. and Bornstein P.: Transforming growth factor β increases mRNA for matrix proteins both in the presence and in the absence of changes in mRNA stability. *Proc. Natn. Acad. Sci. (USA)* **85** (1988) 1105–1108.
42. Vukicevic S., Kleinman H. K., Lyten F. P., Roberts A. B, Roche N. S. and Reddi A. H.: Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. *Exp. Cell Res.* **202** (1992) 1–8.
43. Tamaroglio T. A. and Lo C. S.: Regulation of fibronectin by insulin-like growth factor-I in cultured rat thoracic aortic smooth muscle cells and glomerular mesangial cells. *Exp. Cell Res.* **215** (1994) 338–346.
44. Huynh H. and Pollak M.: Insulin-like growth factor I gene expression in the uterus is stimulated by tamoxifen and inhibited by the pure antiestrogen ICI 182780. *Cancer Res.* **53** (1993) 5585–5588.
45. Huynh H. and Pollak M.: Uterotrophic action of estradiol and tamoxifen are associated with inhibition of uterine insulin-like growth factor binding protein 3 gene expression. *Cancer Res.* **54** (1994) 3115–3119.
46. Nakamura T., Nakajima T., Tsunoda S., Nakada S., Oda K., Tsurui H. and Wada A.: Induction of E1A-responsive negative factors for transcription of the fibronectin gene in adenovirus E1-transformed rat cells. *J. Virol.* **66** (1990) 6436–6450.
47. Offringa R., Gabel S., Dam H. V., Timmers M., Smitzs A., Zwart R., Stein B., Bos J. L., Eb A. V. D. and Herrlich P.: A novel function of the transforming domain of Ela: repression of AP-1 activity. *Cell* **62** (1991) 527–538 .
48. Weisz, A. and Bresciani F.: Estrogen induces expression of c-fos and e-mye protooneogenes in the rat uterus. *Molec. Endocr.* **2** (1988) 816–824.
49. Chiapetta C., Kirkland J. L., Loose-Mitchell D. S., Murthy L. and Stancel G. M.: Estrogen regulates expression of the jun family of protooncogenes in the uterus. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 113–123.
50. Humphries M. J., Obara M., Olden K. and Yamada K. M.: Role of fibronectin in adhesion, migration, and metastasis. *Cancer Invest.* 7 (1989) 373–393.