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Stromal Expression of Tenascin is Inversely Correlated to Epithelial Differentiation of Hormone Dependent Tissues

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We were previously investigating the expression of the extracellular matrix glycoprotein tenascin in normal and malignant endometrial tissues of humans and rodents. These studies suggested that the expression of tenascin was induced by proliferating epithelia (normal and particularly malignant) and was downregulated with their differentiation. The aim of this study was to investigate the hormone dependency of tenascin expression in (a) the transplantable EnDA endometrial tumor model with or without estrogen deprivation (ovariectomy) of the animals, (b) DMBA-induced rat mammary tumors with or without a hormonal treatment of the animals [ovariectomy, antiestrogen (tamoxifen) or antiprogestin (ZK 98299) treatment] and (c) in the rat prostate of untreated or androgen deprived animals (orchiectomy, flutamide-, casodex- or cyproterone acetate (CPA)-treatment). 1. Estrogen withdrawal by ovariectomy did not affect tenascin expression in transplantable EnDA endometrial adenocarcinoma, meaning the entire extracellular space of the stromal mesenchyme was decorated by tenascin immunoreactivity. 2. In untreated DMBA-induced rat mammary tumors almost the entire extracellular space of the stroma was stained by tenascin immunoreactivity. Ovariectomy and antiestrogen treatment did not affect tenascin expression. In contrast, antiprogestin treatment induced terminal differentiation of mammary tumor cells and in parallel downregulated tenascin expression. 3. In the normal rat prostate no tenascin was detectable by immunocytochemistry. However, following androgen deprivation we found tenascin expression in the stroma of the prostate. The most prominent expression was observable after CPA-treatment, possibly due to its progestagenic potency. In conclusion, the hormones and antihormones tested show no direct effect on the stromal expression of tenascin. However, proliferative activity and a low degree of differentiation of the epithelium induces tenascin expression, whereas epithelial differentiation apparently shuts down tenascin expression. Preliminary *in vitro* studies suggest that paracrine acting growth factors trigger the hormonal regulation of tenascin expression.

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INTRODUCTION

Tenascin/hexabrachion protein is a significant extracellular Matrix (ECM) glycoprotein with a restricted temporal expression pattern and a restricted tissue distribution [1, 2]. So far tenascin has exclusively been localized in mesenchymal tissue compartments, predominantly during fetal development and oncogenesis [3] and only in low amounts in normal adult tissues. We are interested in hormonal aspects of tenascin expression. Earlier studies with breast and endometrial tissue of humans and rodents suggested both a correlation of tenascin expression with the hormonal status of the tissue and the proliferative activity of the tissue [4–9]. In normal tissues of both organs tenascin expression was highest while the tissue was actively proliferating during the menstrual cycle and decreased while the differentiation of the tissue occurred. An even more pronounced expression of tenascin can be detected in proliferative diseases of the endometrial and breast tissue. Since tenascin expression increases following the process of carcinogenesis from normal tissue to precancerous proliferative lesions and carcinomas of

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the organ [4–9] it is tempting to assume that tenascin expression rather is under the control of the hormonally induced state of differentiation of the tissue than under direct hormonal control.

To further dissect the two possibilities of tenascin being a hormonally regulated gene or of tenascin being expressed as a function of hormonally alterated differentiation status of a hormone dependent tissue we examined three additional models, the proliferation or differentiation of which is under hormonal control: (a) the EnDA endometrial adenocarcinoma [10, 11], (b) the antiprogestin-induced terminal differentiation of DMBA-induced rat mammary tumors [12–14] and (c) the rat prostate with or without androgen ablation and resulting apoptosis, involution and dedifferentiation [15, 16].

MATERIALS AND METHODS

Antisera

A polyclonal antihuman tenascin antibody was used, kindly provided by Dr H. P. Erickson (Duke University, Durham, NC, U.S.A.). This antibody was raised in rabbits to tenascin purified from human foreskin fibroblast cultures [17].

Animals

Female DA/Han rats, female SD rats and male Han/Wistar rats (all from Central Institute for Laboratory Animal Breeding, Hannover, Germany) were used.

Hormonal treatment

EnDA endometrial adenocarcinoma. In two series of experiments of hormonal treatment of the EnDA tumor 40 female DA/Han rats were inoculated subcutaneously in the hind limb with a piece of EnDA tumor. 10 animals without treatment were used as untreated control group. 20 animals were treated by ovariectomy prior to tumor inoculation, 10 of them were substituted with estradiol and 10 intact animals received estradiol undecylate.

DMBA-induced mammary carcinoma of the SD-rat. Female Sprague–Dawley (SD) rats were given a single dose of 10 mg DMBA dissolved in 0.5 ml oil by gavage at the age of 50 days. The rats were examined for tumors by palpation once a week. Animals with at least one tumor with a tumor area of more than 150 mm^2 were randomly assigned to experimental groups. Tumor area was determined by caliper measurements and expressed as the product of the longest diameter and its perpendicular. Tamoxifen (5 or 10 mg/kg/day) and ZK 98.299 (3, 5 or 10 mg/kg/day) s.c. 6 times a week were dissolved in oil (10% benzylbenzoate) and administered daily (0, 1 ml/100 g body wt) for 4 weeks. Tumor area was measured weekly. At the start of therapy the tumor area of all tumors in one animal was termed 100%. The change of tumor area was calculated in terms of this value and is displayed for each animal. There was an approximately equal distribution of tumors of different latencies, numbers of tumors and total tumor areas amongst all experimental groups.

Antiandrogen treatment of male Wistar rats. As experimental conditions of androgen ablation surgical and pharmacological procedures were choosen. In a first set of experiments intact rats of a body weight of approx. 200 g (Wistar, Schering AG) were either orchiectomized by the scrotal route or pharmacologically castrated: the latter animals were treated with flutamide (1 mg/animal/day), casodex (in doses of 1, 3 and 10 mg/animal/day) or CPA (3 mg/animal/day).

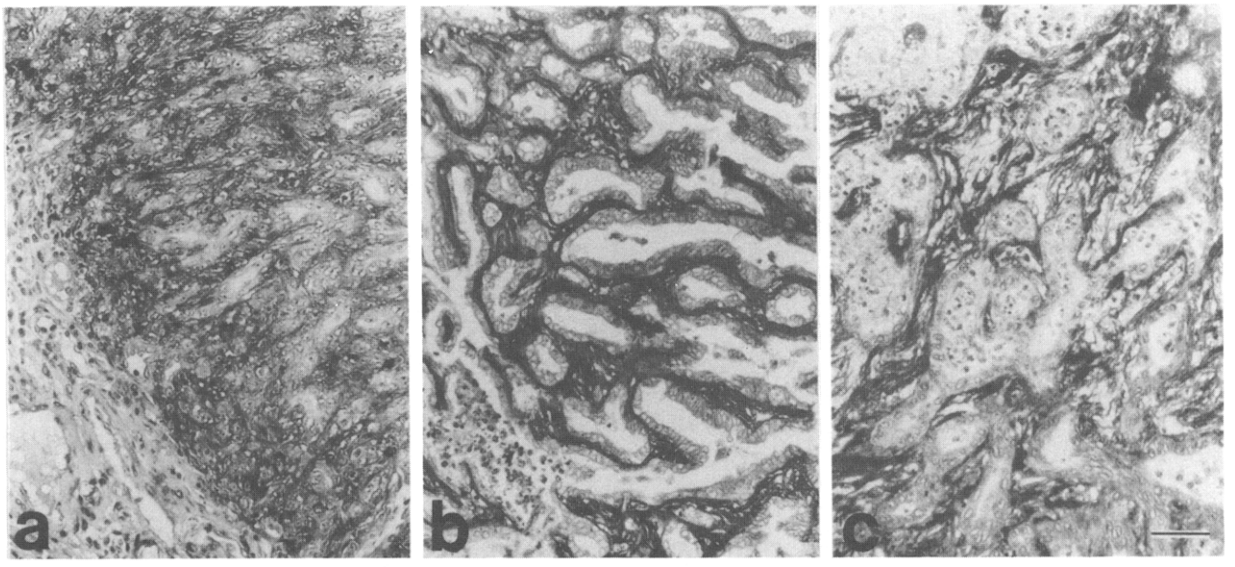

Fig. 1. Tenascin in rat endometrial adenocarcinoma: 5 μm thick paraffin sections of the EnDA enmodmetrial tumor were stained for tenascin immunoreactivity as described in Materials and Methods. Staining product in an untreated control tumor (a), in a tumor grown in an ovariectomized animal without estradiol substitution (b) and in a tumor grown in an ovariectomized animal with estradiol substitution (c) is shown. Calibration bars represent 0.1 mm.

All compounds were synthesized at Schering, dissolved and were given subcutaneously over a period of 14 days in Castor oil and benzyl benzoate (80:20). These antiandrogens were used since they cover the range of equivalent antiandrogenic effectiveness in the classical antiandrogenic rat test model (see below). In a second set of experiments the expression of tenascin was analyzed in ventral areas of prostates in the classical antiandrogen test model (Neumann and Steinbeck 1974). Therefore, rats were orchiectomized, substituted with testosterone proprionate (TP; 0, 1 mg/animal/day) either alone or in combination with CPA (3 mg/animal/day) or casodex. After a treatment period of 7 days animals were sacrificed, the ventral prostates were dissected and fixed.

Immunohistochemistry

After deparaffination 5 μm thick paraffin sections were treated for 30 min with 1% pronase at room temperature and thereafter incubated for 1 h at room temperature with the antihuman-tenascin antibody diluted 1:500 in RPMI medium containing 10% human serum. Thereafter sections were rinsed for 10 min with Tris buffered saline (TBS) with three changes of buffer. For detection of tenascin, sections were incubated with a goat antirabbit IgG antibody

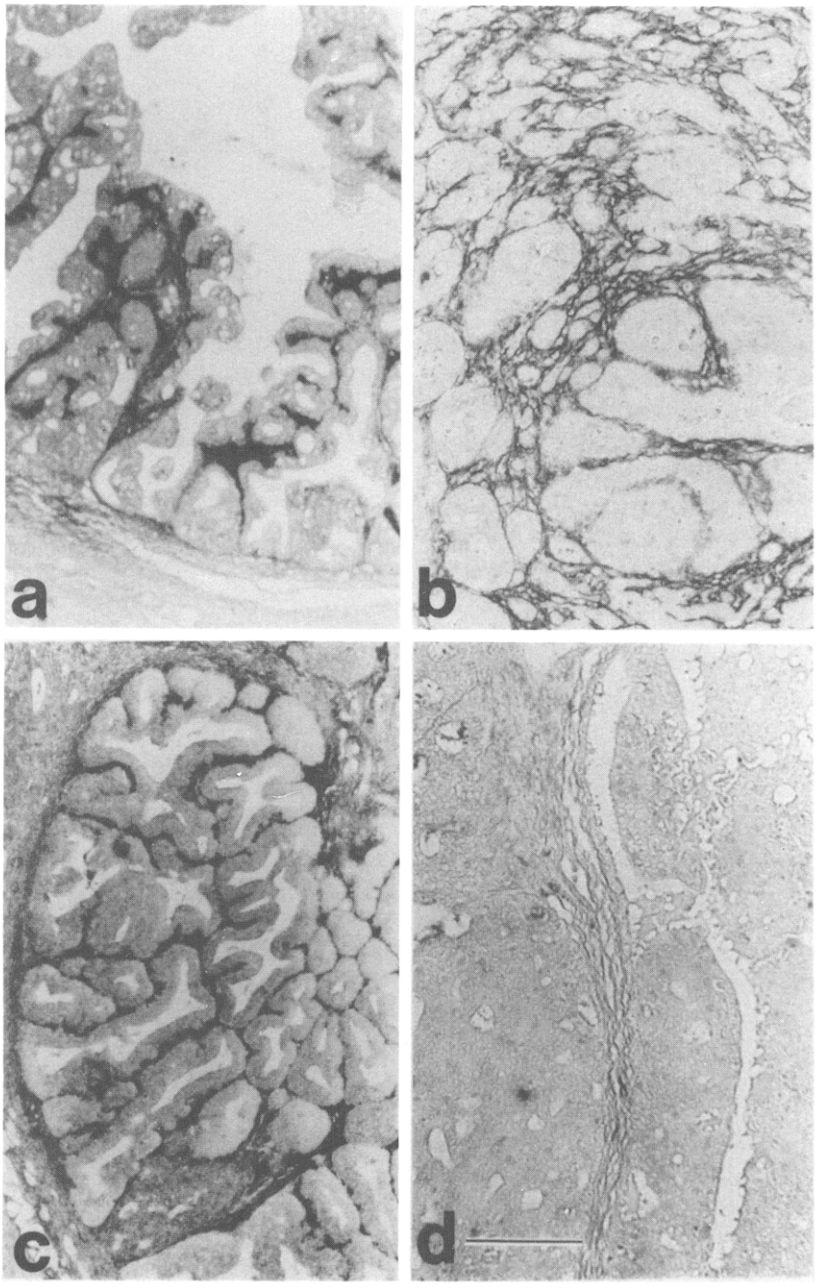

Fig. 2. Tenascin in DMBA-induced rat mammary carcinoma: 5 μm thick paraffin sections of DMBA-induced rat mammary tumors were stained for tenascin immunoreactivity as described in Materials and Methods. The immunolocalization for tenascin immunoreactivity is shown for an untreated control tumor (a), for a tumor grown in an ovariectomized animal (b), for a tumor treated with the antiestrogen tamoxifen (c) and for a tumor after treatment with the antiprogestin ZK 98 299 (d). Calibration bar represents 1 mm.

labeled with alkaline phosphatase (Dianova, Hamburg) diluted 1:500 with RPMI containing 10% human serum. After the next wash in TBS alkaline phosphatase was visualized as described in the manufacturers manual for the pico-blue detection kit (Stratagene, Heidelberg, Germany) or with the Neufuchsin method [19]. Depending on the color dye used to visualize alkaline phosphatase slides were subsequently counterstained with Azokarmin G or Hematoxylin and mounted. As a control parallel sections were treated with secondary reagents only to test for endogenous alkaline phosphatase.

For evaluation and dependent on the experiment, stained sections were examined by two or three independent investigators. Scoring comprised localization and distribution of the staining product (entire extracellular matrix of the stroma versus band-like distribution of the reaction product or association of immunoreactivity to collagenous fibers) and particularly staining intensity.

RESULTS

We examined hormonal influences on tenascin expression in three experimental models in which the proliferation and/or differentiation is under hormonal control: (1) the EnDA rat endometrial adenocarcinoma, (2) the DMBA-induced rat mammary tumor, and (3) the normal rat prostate.

Tenascin expression in the EnDA endometrial adenocarcinoma

The growth of this transplantable tumor model is under the control of estrogens. As described elsewhere [10] estrogen withdrawal by ovariectomy or antiestrogens resulted in a reduction of tumor weight. Substitution of ovariectomized animals with estradiol induced a tumor growth comparable to untreated control animals. However, none of the treatments exerted an effect on the gross morphology of the tumor (Table 1, Fig. 1). Examination of tenascin immunoreactivity in untreated control tumors [Fig. 1(a)], in tumors grown in ovariectomized animals with [Fig. 1(b)] or without [Fig. 1(c)] estradiol substitution led to an almost identical staining pattern with the staining product confined to the extracellular space of the stroma surrounding the tumor. Arbitrarily judgement of the staining intensity revealed slightly stronger staining intensities at sites where the tumor was in the process of invading neighbouring tissue compartments.

Tenascin expression in DMBA-induced rat mammary tumors

We examined the influence of ovariectomy, antiestrogen- (tamoxifen) and antiprogestin- (ZK 98299) treatment on tenascin expression in DMBA-induced rat mammary tumors. Each treatment protocol reduced tumor growth compared to untreated control tumors. As shown previously, the following consecutive order of tumor inhibitory potency was detectable: ovariectomy > antiprogestin treatment > antiestrogen treatment [12–14, 20, 21]. Despite the growth inhibitory potency of ovariectomy and tamoxifen treatment the gross morphology, if compared to untreated control groups remained unaffected by these two treatments (Table 1). In contrast, antiprogestin treatment induced and resulted in terminal differentiation of the epithelial tumor cells (Table 1; [13, 14, 21]).

Localization of tenascin immunoreactivity revealed an exclusive staining of the extracellular space of the stroma surrounding the tumor (Fig. 2). The reaction product decorated almost the entire extracellular space of the stroma in cases of untreated control tumors [Fig. 2(a)], in tumors grown in ovariectomized animals [Fig. 2(b)], and in tumors grown in tamoxifen treated animals [Fig. 2(c)]. Conversely, after antiprogestin treatment in tumors composed of terminally differentiated tumor cells we detected little if any staining product [Fig. 2(d)].

In the uterus of these animals antiprogestin treatment led to a permanent estrogenic effect in the uterus with an increase in the weight of the tissue. We

Table 1. Tenascin expression in three hormonal dependent growing experimental models

Tissue	Hormonal treatment	Differentiation	Tenascin expression
EnDA rat endometrial adenocarcinoma	None	Moderately differentiated tumor	High
	Ovariectomy	Moderately-poorly differentiated tumor	High
	Ovariectomy + estradiol	Moderately differentiated tumor	High
DMBA-induced rat mammary carcinoma	None	Moderately differentiated tumor	High
	Ovariectomy	Moderately differentiated tumor	High
	Antiestrogen (tamoxifen)	Moderately differentiated tumor	High
	Antiprogestin (ZK 98299)	Terminal differentiation of tumor cells	Down regulated
Normal rat prostate	None	Highly differentiated	Marginally
	Orchiectomy	Atrophy and dedifferentiation	Induced
	Casodex	Atrophy and dedifferentiation	Induced
	Flutamide	Atrophy and dedifferentiation	Induced
	Cyproterone acetate	Atrophy and dedifferentiation	Highly induced

Tenascin expression in the EnDA rat endometrial adenocarcinoma, the DMBA-induced rat mammary carcinoma and the normal rat prostate was examined by immunocytochemistry. The table summarizes the hormonal effects on the differentiation of the endometrial tumors (histological staging), the mammary tumors [13, 14, 20, 21], the involuting prostate [15, 16, 22] and on tenascin expression.

addressed the question whether or not this unopposed estrogenic stimulus affects tenascin expression. Neither the endometrium of untreated animals [Fig. 3(a)], nor the endometrium of antiprogestin treated animals [Fig. 3(b)] showed any detectable amounts of tenascin immunoreactivity. This finding was not completely unexpected since glands clearly showed some indications of secretory activity that would render them functionally differentiated.

Effects of androgen withdrawal on tenascin expression in the rat prostate

Androgen ablation by orchiectomy or by antiandrogen treatment resulted in a reduction of prostate weight. The relative potency of this effect that was variable from one treatment group to the other. None of the hormonal treatments was as effective as orchiectomy in reducing relative prostate weight. CPA and high doses of casodex exhibited an almost equal potency, whereas flutamide and low doses of casodex only marginally reduced the relative prostate weight. However, each treatment induced involution of the prostate and rendered the tissue less differentiated (Fig. 4).

With respect to tenascin immunocytochemistry, in the normal prostate we hardly ever detected any tenascin reaction product [Fig. 4(a)]. Orchiectomy [Fig. 4(b)], treatment with flutamide [Fig. 4(c) and treatment with casodex Fig. 4(d)] induced tenascin expression in the prostate with the reaction product confined to collagenous fibers within the stroma surrounding glands. However, in the above treatment groups staining of tenascin in the prostates of treated animals was variable both in respect to localization and quantity of the reaction product. Some prostates in these treatment groups showed only staining around some glands or some groups of glands, others almost around each individual gland.

The most uniform staining pattern, with the reaction product around most of the glands in combination with the highest staining intensity was seen in prostates of animals treated with cyproterone acetate [Fig. 4(e and f)]. In summary, these experiments showed that androgen ablation induces tenascin expression in the prostate. Additionally, our experiments demonstrate that both localization of the staining product and the staining intensity are dependent on the treatment applied.

DISCUSSION

Earlier investigations of our own group and by others indicated that the expression of the extracellular matrix glycoprotein tenascin to some degree correlates with the hormonal status of glandular tissues [4–9], strongly correlates with the proliferative activity of glandular tissues and in particular, inversely correlates with the differentiation of the tissue. With the study presented here we attempted to discriminate between two theoretically existing hormonal control mechanisms of tenascin expression, (1) tenascin being a hormone dependent gene, and (2) tenascin expression reflecting secondary effects following hormonal stimulation of proliferation and/or differentiation. For this purpose we examined tenascin expression by immunocytochemistry in three experimental models: (a) the EnDA

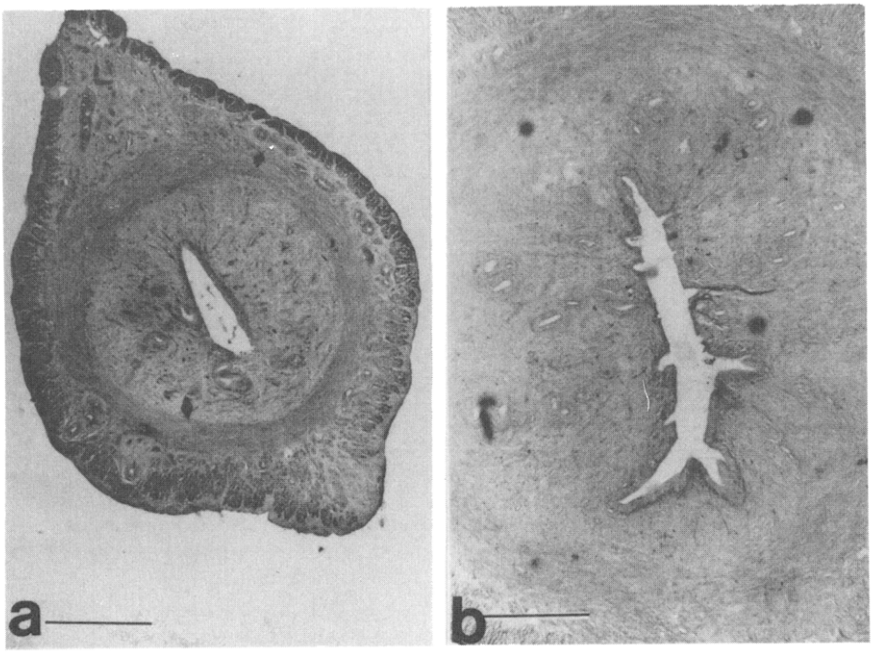

Fig. 3. Antiprogestin effects on tenascin in the uterus of animals bearing DMBA-induced mammary carcinoma: 5 μm thick paraffin sections of the uterus derived from animals bearing DMBA-induced mammary tumors were stained for tenascin immunoreactivity as described in Materials and Methods. The treatment lead to a permanent estrogenic effect in the uterus with an increase in the size and the weight of the tissue. Neither the endometrium of untreated animals (a), nor the endometrium of antiprogestin treated animals (b) showed any detectable amounts of tenascin immunoreactivity. Calibration bars represent 1 mm.

rat endometrial adenocarcinoma, (b) the DMBA-induced rat mammary tumor and (c) the normal rat prostate. We were particularly interested to find out how hormonal treatments would affect tenascin expression.

The most clearcut effects of hormonal involvement on tenascin expression were detectable in DMBA-induced rat mammary tumors. Antiprogestin-induced terminal differentiation of tumor cells was associated with a gradual reduction of tenascin expression which terminated with an almost complete disappearance of the staining product in some apparently secretory active areas of the tumor. Reduction of tumor growth alone without alteration of the differentiation status of the tumor tissue had no effect on tenascin expression. It would be interesting to know how a complete block of mitosis would interfere with tenascin expression.

In line with our findings in DMBA-induced rat mammary tumors are the findings in the EnDA endometrial adenocarcinoma. Estrogen withdrawal by ovariectomy resulted in a strong reduction of the tumor growth but had no effect on tenascin expression. Additionally, substitution of castrated or intact animals with estradiol undecylate had no potentiating effect on tenascin expression. Conversely, as a side effect in our treatment studies of DMBA-induced rat mammary carcinomas we observed that antiprogestin treatment transformed the endometrium into a highly differentiated, secretorily active tissue, rather than into a proliferating one [14]. Therefore, a significant expression of

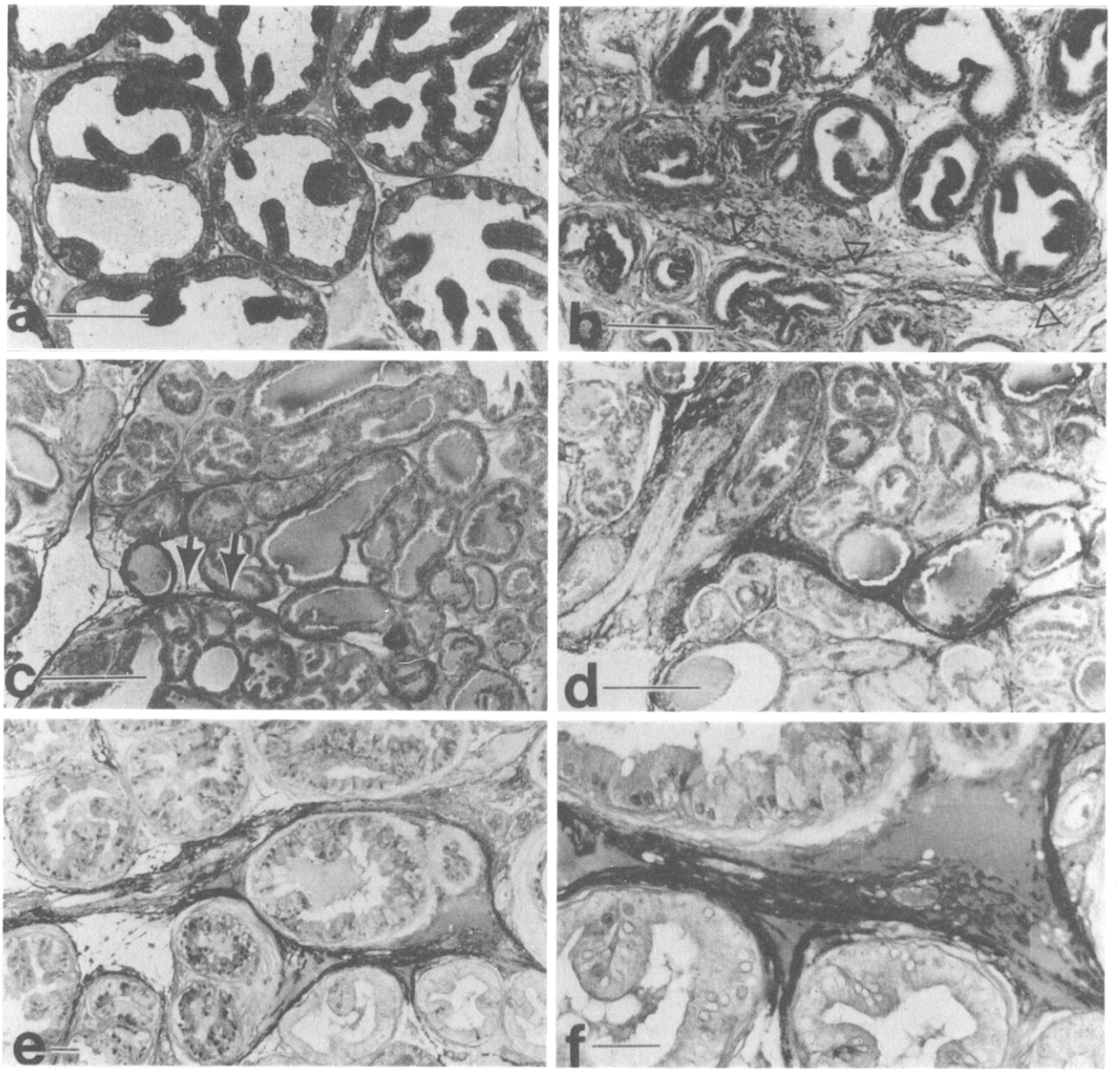

Fig. 4. Expression of tenascin in rat prostates: 5 μm thick paraffin sections of the rat prostate were stained for tenascin immunoreactivity as described in Materials and Methods. Androgen ablation lead to tenascin expression. We show tenascin immunoreactivity in an intact prostate (a), in a prostate after orchiectomy of the animal (b) and after treatment of the animals with the following antiandrogens: flutamide (c), casodex (d) and Cyproterone Acetate (e,f). Calibration bars represent 1 mm (a–d) and 0.1 mm (e,f), respectively.

tenascin in the uterus of antiprogestin treated animals could not be expected although the unopposed estrogenic effect led to an increase in uterine weight.

Tumors as highly proliferating tissues exhibited an abundant expression of tenascin. Conversely, in the highly differentiated normal prostate only a marginal amount of tenascin immunoreactivity was detectable. Androgen ablation in the prostate activates programmed cell death [22], induces temporally discrete biochemical events [16] which finally lead to the regression of the prostate gland leaving the tissue in a less differentiated or more or less undifferentiated status. In association with these processes tenascin is expressed and its expression apparently is independent of the mode of hormon ablation (orchiectomy or antihormon treatment).

In summary, our findings clearly favor the interpretation that hormonal effects on tenascin expression are not direct and tenascin therefore represents not a hormone dependent gene, which is in accordance with investigation of the 5′-region of the tenascin gene in which no hormone responsive element was detectable [23]. This interpretation is substantiated by findings in uterine derived tissue that neither estrogens nor antiestrogens had an effect on tenascin expression. In contrast tenascin expression is strongly coupled to processes of differentiation as shown for antiprogestin treatment of DMBA-induced rat mammary tumors, which in turn down regulated tenascin expression. Tenascin expression is additionally positively associated with processes of dedifferentiation as shown after androgen ablation in the rat prostate. Of course, in the experimental models studied differentiation or dedifferentiation are under hormonal control. In conclusion, a hormonal treatment will affect tenascin expression, whenever the hormonal treatment additionally affects the differentiation status of a glandular tissue.

Finally, possible molecular mechanisms underlying tenascin expression have to be discussed. As can be deduced from data in the literature it is most likely that paracrine acting growth factors [24, 25] and possibly other cellular interactions [26] participate in tenascin expression. These two models are particularly attractive to explain tenascin expression because in the examined experimental model systems the expression of growth factors like TGF-β and other growth factors could be under hormonal control [27–29].

Additionally, it is well established that epithelial/mesenchymal interactions are a prerequisite for hormones to control tissue function of the endometrium [30, 31] and of the prostate [32–34]. From this literature data one can deduce that the hormonal manipulations in our experimental models interfere with growth factor expression and/or cellular communication of epithelial and mesenchymal tissue compartments and only in the second line lead to induction or down regulation of tenascin expression.

In conclusion, it appears rather unlikely that tenascin is a hormone inducible gene. However, tenascin expression is strongly and inversely correlated to the differentiation status of glandular tissues and represents a very sensitive tool to monitor whether a hormonal treatment affects the differentiation of a glandular tissue.

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REFERENCES

1. Erickson H. P. and Lightner V. A.: Hexabrachion protein (tenascin, cytotactin, brachionectin) in connective tissues, embryonic brain, and tumors. In *Advances in Cell Biology* (Edited by K. R. Miller). JAI, London, Vol. 2 (1988) pp. 55–90.
2. Erickson H. P. and Bourdon M. A.: Tenascin: an extracellular matrix protein in specialized embryonic tissues and tumors. *A. Rev. Cell Biol.* 5 (1989) 71–92.
3. Chiquet-Ehrismann R., Mackie E. J., Pearson C. A. and Sakakura T.: Tenascin an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* 47 (1986) 131–139.
4. Ferguson J. E., Schor A. M., Howell A. and Ferguson M. W. J.: Tenascin distribution in the normal human breast is altered during the menstrual cycle and in carcinoma. *Differentiation* 42 (1990) 199–207.
5. Inaguma Y., Kusakabe M., Mackie E. J., Pearson C. A., Chiquet-Ehrismann R. and Sakakura T.: Epithelial induction of stromal tenascin in mouse mammary gland: from embryogenesis to carcinogenesis. *Devl Biol.* 128 (1988) 245–255.
6. Vollmer G., Siegal G. P., Chiquet-Ehrismann R., Lightner V. A., Arnholdt H. and Knuppen R.: Tenascin expression in the human endometrium and in endometrial adenocarcinomas. *Lab. Invest.* 62 (1990) 725–730.
7. Vollmer G., Deerberg F., Siegal G. P. and Knuppen R.: Altered tenascin expression during spontaneous endometrial carcinogenesis in the BDII/Han rat. *Virchow's Archs B Cell Path.* 60 (1991) 83–89.
8. Howeedy A. A., Virtanen I., Laitinen L., Gould N. S., Koukoulis G. K. and Gould V. E.: Differential distribution of tenascin in the normal, hyperplatic, and neoplastic breast. *Lab. Invest.* 63 (1990) 798–806.
9. Koukoulis G. K., Gould V. E., Bhattacharyya A., Gould J. E., Howeedy A. A. and Virtanen I.: Tenascin in normal, reactive, hyperplastic, and neoplastic tissues: biologic and pathologic implications. *Human Path.* 22 (1991) 636–643.
10. Horn D. W., Vollmer G., Deerberg F. and Schneider, M. R.: The EnDA endometrial adenocarcinoma: an estrogen sensitive, metastasizing in vivo tumor model of the rat. *J. Cancer Res. Clin. Oncol.* 119 (1993) 450–456.
11. Schütze N., Kraft V., Deerberg F., Winking H., Knuppen R. and Vollmer G.: Functions of estrogens and antiestrogens in the rat endometrial adenocarcinoma cell line RUCA-I. *Int. J. Cancer* 52 (1992) 941–949.
12. Schneider R. M., Michna H., Nishino Y. and El Eltreby M. F.: Antitumor activity of the progesterone antagonists ZK 98.299 and RU 38.486 in the hormone dependent MXT mammary tumor model of the mouse and the DMBA- and the NMU-induced mammary tumor models of the rat. *Eur. J. Cancer Clin. Oncol.* 25 (1989) 691–701.
13. Michna H., Schneider R. M., Nishino Y. and El Eltreby M. F.: Antitumor activity of the antiprogestins ZK 98.299 and RU 38.486 in the hormone dependent rat and mouse mammary tumors: mechanistic studies. *Breast Cancer Res. Treat.* 14 (1989a) 275–288.
14. Michna H., Schneider R. M., Nishino Y. and El Eltreby M. F.: The antitumor mechanism of progesterone antagonists is a receptor mediated antiproliferative effect by induction of terminal cell death. *J. Steroid Biochem.* 34 (1989b) 447–453.
15. Isaacs J. T.: Antagonistic effect of androgen on prostatic cell death. *The Prostate* 5 (1984) 545–557.
16. Bursch W., Kleine L. and Tenniswood M.: The biochemistry of cell death by apoptosis. Biochem. *Cell Biol.* 68 (1990) 1071–1074.

17. Lightner V. A., Gumkowsky F., Bigner D. D. and Erickson H. P.: Tenascin/Hexabrachion in human skin: biochemical identification and localization by light and electron microscopy. *J. Cell Biol.* **108** (1989) 2483–2493.
18. Neumann F. and Steinbeck H.: Antiandrogens. In Handbuch der experimentellen Pharmakologie (Edited by D. Eichler, A. Farah, H. Herken, and A. D. Welch). Springer Verlag, Berlin, Vol. XXXV (1974) p. 12.
19. Cordell J. L., Falini B., Erber W. N., Ghosh A. K., Abdulaziz Z., MacDonald S., Pulford K. A. F., Stein H. and Mason D. Y.: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and anti-alkaline phosphatase (APAAP complexes). *J. Histochem. Cytochem.* 32 (1984) 219–229.
20. Michna H., Nishino Y., Neef G., McGuire W. L. and Schneider M. R. Progesterone antagonists: tumor-inhibiting potential and mechanism of action. *J. Steroid Biochem. Molec. Biol* 41 (1992a) 339–348.
21. Michna H., Gehring S., Kuhnel W., Nishino Y. and Schneider M. R.: The antitumor potency of progesterone antagonists is due to their differentiation potential. *J. Steroid Biochem. Molec. Biol.* 43 (1992b) 203–210.
22. Kyprianou N. and Isaacs J. T.: Activation of programmed cell death in the rat ventral prostate after castration. *Endocrinology* **122** (1988) 552–562.
23. Jones F. S., Chalepakis G., Gruss P. and Edelman G. M.: Activation of the cytotactin promoter by the homeobox-containing gene Evx-1. *Proc. Natn. Acad. Sci. U.S.A.* **89** (1992) 2091–2095.
24. Pearson C. A., Pearson D., Shibahara S., Hofsteenge J. and Chiquet-Ehrismann R.: Tenascin: cDNA cloning and induction by TGF-beta. *EMBO Jl* 7 (1988) 2977–2982.
25. Chiquet-Ehrismann R., Kalla P. and Pearson C.A.: Participation of tenascin in reciprocal epithelial-mesenchymal interactions of MCF-7 cells and fibroblasts. *Cancer Res.* **49** (1989) 4322–4325.
26. Aufderheide E., Chiquet-Ehrismann R. and Ekblom P.: Epithelial-mesenchymal interactions in the developing kidney lead to expression of tenascin in the mesenchyme. *J. Cell Biol.* **105** (1987) 599–608.
27. Knabbe C., Lippman M. E., Wakefield L. M., Flanders K., Kassid A., Derynck R. and Dickson R. B.: Evidence that transforming growth factor-beta is a hormonally regulated growth factor in human breast cancer cells. *Cell* **48** (1987) 417–428.
28. Kyprianou N. and Isaacs J. T.: Expression of TGF-β in the rat ventral prostate during castration-induced programmed cell death. *Molec. Endocr.* 3 (1989) 1515–1522.
29. Lippman M. E. and Dickson R. B.: Mechanisms of normal and malignant breast epithelial growth regulation. *J. Steroid Biochem.* 34 (1989) 107–121.
30. McClellan M. C., West N. B. and Brenner R. M.: Immunocytochemical localization of estrogen receptors in the Macaque endometrium during the luteal-follicular transition. *Endocrinology* **119** (1986) 2467–2475.
31. McClellan M. C., Rankin S., West N. B. and Brenner R. M.: Estrogen receptors, progestin receptors and DNA synthesis in the Macaque endometrium during the luteal-follicular transition. *J. Steroid Biochem. Molec. Biol.* 37 (1990) 631–641.
32. Cunha G. R., Sekkingstad M. and Meloy B.A.: Heterospecific induction of prostatic development in tissue recombinants prepared with mouse, rat, rabbit and human tissue. *Differentiation* 24 (1983) 174–180.
33. Sugimura Y., Cunha G. R. and Bigsby R. M.: Androgenic induction of DNA synthesis in prostatic glands induced in the urothelium of testicular feminized (Tfm/Y) mice. *Prostate* 9 (1986) 217–225.
34. Chang S. M. and Chung L. W. K.: Interaction between prostatic fibroblast and epithelial cells in culture: role of androgen. *Endocrinology* 125 (1989) 2719–2727.