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Alpha-smooth muscle actin and other stromal markers in endometrial mucosa

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Abstract. Stromal-epithelial interactions as expressed by alpha-smooth muscle actin (alpha-SM actin) collagen type IV, fibronectin, laminin and tenascin were studied in normal and pathological endometrial mucosa. There was complete or incomplete cuffing of scattered endometrial glands by alpha-SM actin mostly in the basal layer of endometrial mucosa and in dilated or cystic glands. Individual adenocarcinomatous glands were not encircled by alpha-SM actin-positive cells. Collagen type IV and laminin were found surrounding all glands irrespective of the presence of periglandular alpha-SM actin. Fibronectin was diffusely present in the stroma. Tenascin was identified, albeit not exclusively, in a periglandular location similar to that of alpha-SM actin. We conclude that the periglandular cells staining for alpha-SM actin, which were negative for cytokeratins, are probably myofibroblasts (MFs). Since this phenomenon was most commonly observed in dilated and cystic glands, we suggest that stromal cells immediately surrounding these glands may be subjected to mechanical or other stress resulting, as in other situations of tissue remodelling, in the development of MFs. This may also explain the appearance of tenascin in the same location. Thus, our finding may represent a further example of the local modulation of stromal cell phenotype, possibly under the action of micro-environmental factors.

Key words: Stromal – epithelial interactions – Desmin – Cytoskeleton – Endometrial adenocarcinoma

Introduction

Stromal-epithelial interactions have been studied with immunohistochemical techniques in a variety of organs and have revealed evidence of stromal regulation of epithelial functions in normal and pathological conditions (Barcellos-Hoff et al. 1989; Donjacour and Cunha 1991; Haffen et al. 1987; Hodges 1982; Kratochwil 1986; Liotta et al. 1986, 1991; Montesano et al. 1991; Nakashini and Ishii 1989; Van den Hoff 1988). Studies of this nature, especially those involving fibroblastic stromal cells with smooth muscle cell differentiation in the female genital tract, have been few. They have included investigations of alpha-smooth muscle (alpha-SM) actin in stromal cells of normal and abnormal ovaries (Czernobilsky et al. 1989) and in the uterine cervix (Cintorino et al. 1991). In these studies it was shown that alpha-SM actin was particularly prominent in the stroma of epithelial tumours and could be considered to be a marker of stromal cell reaction to the development of neoplastic lesions. Similar results were obtained in stromal cells of breast lesions in which alpha-SM actin was prominent, not only in malignant tumours but also in certain benign conditions (Sappino et al. 1988).

The endometrial mucosa, which is composed of epithelial glandular structures intimately surrounded by mesenchymal stroma, has not been investigated in this way. The profound morphological changes in both glands and stroma, which this mucosa undergoes during the normal menstrual cycle and in various forms of neoplasia, warrants an investigation of its stromal-epithelial interactions as expressed by alpha-SM actin and other stromal markers.

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Materials and methods

Samples from 60 endometria and underlying muscular wall obtained from hysterectomies and 26 endometrial curettings were fixed in 4% phosphate-buffered formaldehyde solution for light microscopy, embedded in paraffin, cut at 3–5 μm and stained with haematoxylin and eosin, Gomori's reticulin and elastic-van Gieson strains.

In addition 15 cases, samples from hysterectomy specimens were snap-frozen in isopentane pre-cooled in liquid nitrogen and stored at −70° C for immunofluorescence staining.

The following primary antibodies were used: anti-alpha SM1, a monoclonal IgG_2 recognizing alpha-SM actin (Skalli and Gabbiani 1988), affinity-purified polyclonal rabbit IgG_2 against: (a) desmin (Kocher et al. 1984): (b) human von Willebrandt factor (Biomakor, Rehovot, Israël); (c) collagen type IV; (d) fibronectin; and (e) laminin (c–e, gifts from Dr. J.R. Couchman, University of Alabama, Birmingham, Ala., USA); (f) tenascin (gift from Dr. R. Chiquet-Ehrismann, Friedrich Miescher Institut, Basle, Switzerland). We also used the following antibodies against cytokeratins: (a) M-69054, broad spectrum against cytokeratins 1, 2, 5, 11, 12 and 15 (Oxoid Dotticon, Switzerland); (b) D5/16D4, against cytokeratins 5 and 6 (Boehringer, Mannheim, Germany); and (c) CK13.8 against cytokeratin 13 (Progen, Heidelberg, Germany).

Secondary antibodies for immunofluorescence microscopy were fluorescein isothiocyanate-labelled goat anti-rabbit IgG and Texas Red isothiocyanate-labelled goat anti-mouse iIgG (Nordic Immunological Laboratories, Tilbury, The Netherlands).

Immunoperoxidase staining was performed on formalin-fixed, paraffin-embedded material. Sections 4 μm thick were deparaffinized and treated with 0.3% hydrogen peroxide in methanol for 30 min. After washing in phosphate buffered saline (PBS) and treating with normal "blocking serum" for 20 min at room temperature, sections were incubated with primary antibodies for 30 min and with the diluted biotinylated secondary antibodies for 20 min at room temperature in a humid chamber. Bound antibodies were visualized using the avidin-biotin peroxidase complex (ABC) protocol (Hsu et al. 1981) and the Vectastain kit. For the localization of cytokeratins and desmin, the tissue sections were treated with 0.1% protease from *Streptomyces griseus* (type XIV, Sigma, St. Louis, Mo., USA) for 10–20 min at 37° C prior to the immunochemical staining.

For immunofluorescence staining cryostat sections 4 μm thick were fixed for 5 min in cold acetone (−20° C), air-dried for 2 h and exposed to primary and secondary antibodies as previously described (Franke et al. 1979). For double-labelled immunofluorescence microscopy, both primary antibodies were added simultaneously, as were secondary antibodies. After three rinsings in PBS, the sections were mounted in buffered polyvinyl alcohol.

Sections were examined with a photomicroscope (Zeiss, Oberkochen, Germany) equipped with epi-illumination using plan apochromate × 10/1.0 to 632/1.0 objectives and photographed on Kodak TMAX 400 (Kodak, Hemel Hempstead, UK) or Ilford PAND black and white films (Ilford, Basle, Switzerland).

Results

The diagnoses of the endometria obtained from 60 hysterectomy specimens were as follows: atrophic (8 cases), proliferative (25), secretory (7), simple (cystic) hyperplasia (4), complex (adenomatous, non-atypical) hyperplasia (4), chronic endometritis (2), and adenocarcinoma (10). In 12 of the above cases adenomyosis was also present. The patients' ages ranged from 30 to 93 years with a mean of 57 years.

In the curettings from 26 patients, the endometrial diagnoses were as follows: atrophic (3 cases), proliferative (13), secretory (7), complex hyperplasia (2), adenocarcinoma (1). The patients' ages ranged from 30 to 85 with a mean of 45 years. The endometrial adenocarcinomas were of the well and moderately differentiated endometrioid type.

The diagnosis of the endometria in the 15 hysterectomy cases from which samples were snap frozen were as follows: atrophic (4 cases), proliferative (7), secretory (3), chronic endometritis (1). The patients' ages ranged from 39 to 78 years with a mean of 58 years.

On haematoxylin and eosin stained sections the glands which were subsequently seen to present an alpha-SM actin-positive cuffing showed a concentric layer of stromal cells usually situated within a relatively hypocellular zone. The nuclei of these stromal cells were either spindly or ovoid (Fig. 1 a).

In the periglandular stroma which stained for alpha-SM actin, there appeared to be an increased concentration and sometimes an increased width of reticulin fibres which were otherwise diffusely present throughout the stroma (Fig. 1 b). The only positive elastin staining in the stroma was found within blood vessel walls.

The most striking finding in the immunoperoxidase stained slides was the complete or incomplete cuffing of scattered endometrial glands by alpha-SM actin-positive spindle cells appearing in one or several layers (Fig. 1 c). This was present in 29 endometria of the 60 uteri and in 7 endometria of the 26 endometrial curettings examined. These encircling cells were situated outside the epithelial basement membrane, and were negative for desmin and for von Willebrand factor. In most instances, these actin-positive cells were detected in the lower, basal layer of the endometrial mucosa and in dilated or cystic glands, although occasionally they were also observed in non-dilated glands and in the more superficial mucosa. The glands involved were found in atrophic, proliferative or hyperplastic endometria. In secretory endometria, the cuffing by alpha-SM actin was mostly seen in the basal, inactive layer or in single non-secretory glands. Individual adenocarcinomatous glands were not encircled by alpha-SM actin positive cells. On the other hand, some groups of adenocarcinomatous glands were surrounded by an alpha-SM actin-positive cell layer. No periglandular alpha-SM actin-positive cell layer was identified in glands of adenomyosis.

The endometrial blood vessels throughout the mucosa stained with alpha-SM actin and with von Willebrand factor. The latter also stained capillaries. The vasculature was most prominent and widespread in secretory endometrium where, in addition to stromal, small blood vessels, delicate capillary-type vessels surrounded individual glands.

Alpha-SM actin and desmin stained bundles of smooth muscle cells which penetrated into the mucosa from the underlying myometrium and were mostly present in the basal third of the mucosa, although occasionally they penetrated into more superficial layers in between the glands.

Positive staining for alpha-SM actin was also detected in isolated stromal cells at different levels of the mucosa in 14 of the 60 uteri and in 7 of the 26 curettings examined (Fig. 1 d). These did not stain for desmin or for von Willebrand factor. There was no significant correlation between this observation and the histological diagnosis of the specimen. The alpha-SM actin-positive periglandular cells did not stain with any of the cytokeratin antibodies used.

In the frozen material examined by immunofluorescence the presence of alpha-SM actin around endometrial glands could be detected in 4 out of the 15 cases in the same locations as seen in the specimens obtained

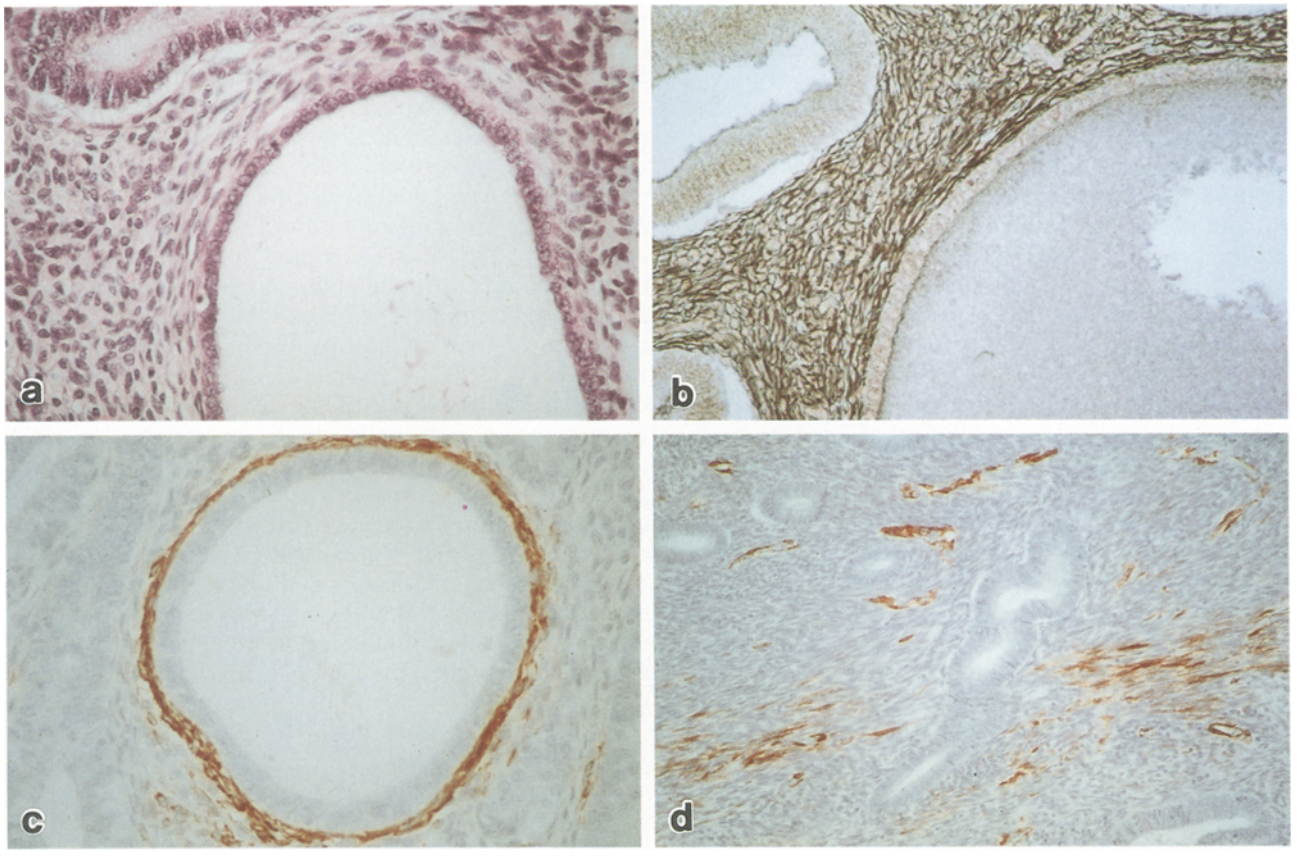

Fig. 1 a–d. Different aspects of endometrial glands. Cystically dilated endometrial gland showing several layers of spindle cells arranged concentrically (**a**). This zone appears to be less cellular than the surrounding stroma. Cystically dilated endometrial gland surrounded by an increased concentration of reticulin fibres (**b**). Dilated endometrial gland showing complete cuffing by α-smooth muscle (SM) actin-positive cells (**c**). Note absence of staining in surrounding glands. Endometrial mucosa with scattered α-SM actin-positive cells in stroma (**d**). **a** H & E, ×480; **b** Gomori's reticulin stain, ×480; **c** immunoperoxidase, ×480; **d** immunoperoxidase, ×240

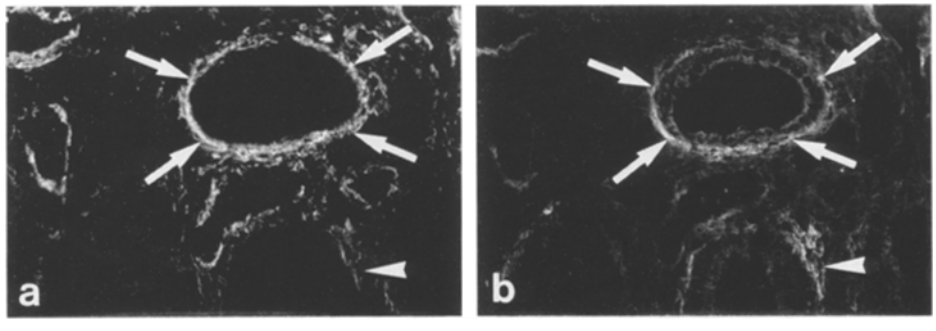

Fig. 2. Double immunofluorescence staining for α-SM actin (**a**) and tenascin (**b**) of a portion of uterine mucosa. α-SM actin is classically distributed in vessel walls but also around a slightly dilated gland where it is co-distributed with tenascin (*arrows*). Note that tenascin is also present in another gland (*arrowhead*) where the α-SM actin staining is absent. ×400

by the immunoperoxidase method. These 4 cases showed atrophic, proliferative, secretory and chronically inflamed endometria. Collagen type IV and laminin were found surrounding all glands irrespective of the presence of periglandular alpha-SM actin and in the walls of blood vessels. Fibronectin was diffusely present throughout the stroma. Tenascin was identified in a periglandular location in cases in which alpha-SM actin was also present (Fig. 2). In addition it was also occasionally present around glands lacking the alpha-SM actin mantle zone.

Discussion

The stromal cells staining for alpha-SM actin surrounding occasional endometrial glands probably belong to a subset of mesenchymal cells displaying features of smooth muscle differentiation, which have been called myofibroblasts (MF; Skalli and Gabbiani 1988). Since these periglandular cells were not a constant feature and were present in only isolated glands, this phenomenon cannot be related to growth and differentiation of epithelial cells as was proposed for colonic pericryptal

fibroblasts, in which these cells were present in all crypts (Sappino et al. 1989). Proliferation of myoepithelial cells which are known to be alpha-SM actin positive (Bussolati 1980) could be excluded by the absence of cytokeratins, using a broad spectrum antibody (Nathrath et al. 1982) as well as antibodies to cytokeratins 5, 6 (Nagle et al. 1986) and 13 in this periglandular zone.

Although no definite correlation could be established between the presence of the periglandular MFs and the menstrual cycle as reflected by the histological features of the endometrium, a certain relationship to oestrogen activity may be present, since the periglandular alpha-SM actin-positive cells were evident in basal, proliferative, cystically dilated and hyperplastic, but not in secretory glands. This suggests a modulation of these stromal cells by sex hormones as was reported in the case of uterine smooth muscle cells (Bo et al. 1968; Kawaguchi et al. 1985; Ross and Klebanoff 1967). However, in the ovary, no hormonal stimulation of alpha-SM actin-positive cells was evident (Czernobilsky et al. 1989).

Stromal stimulation by malignant neoplasms produces MFs in a variety of organs (for review see Schmitt-Gräff and Gabbiani 1992; Seemayer et al. 1979; Skalli and Gabbiani 1988), but cannot be implicated in the endometrial mucosa, since in endometrial adenocarcinoma no periglandular alpha-SM actin-containing cells surrounding individual glands were demonstrated, although such cells rarely encircled groups of malignant glandular structures. This resembles the observation of Sappino et al. (1989) concerning colonic pericryptal fibroblasts, since the latter were no longer recognizable in colonic adenocarcinoma.

The presence of scattered alpha-SM actin-positive cells within the endometrial stroma in some of the cases is consistent with what has been observed in normal stromal elements throughout the body (Sappino et al. 1990), including the female genital tract (Cintorino et al. 1991; Czernobilsky et al. 1989).

Our observation that in many instances the periglandular alpha-SM actin-positive cells appeared in dilated and/or cystic glands raises the possibility that the stromal cells immediately surrounding these glands are subjected to mechanical or other stress resulting, as in other situations of tissue remodelling, in the development of MFs (Gabbiani et al. 1971; Schürch et al. 1992; Skalli and Gabbiani 1988). The condensation of reticulin fibres which we found around these glands seems to support such a hypothesis. Periglandular tissue remodelling may also explain the appearance of tenascin, an extracellular matrix glycoprotein, in the same periglandular location as the alpha-SM actin, since an increase in tenascin has been observed in activated stromal cells in the adult (Mackie et al. 1988; Schalkwijk et al. 1991) in addition to its presence in normal embryonic tissue. The lack of a significant increase of fibronectin, which is a related extracellular matrix glycoprotein, in this periglandular location as well as in the skin model described by Schalkwijk et al. (1991), suggests that tenascin and fibronectin expression are subjected to different, distinct control mechanisms.

It is presently generally accepted that, in many instances, cell differentiation does not depend on genetically irreversible steps but is controlled by the balance of influences exerted by micro-environmental factors such as cytokines and extracellular matrix compounds (Aggarwal and Pocsik 1992; Ruoslahti 1989). This is particularly clear for stromal cells in general (Desmoulière et al., in press) and possibly for stromal cells of the endometrium, as the present findings tend to support. We do not know the factors playing a role in this modulation of periglandular stromal endometrial cells but the presence of tenascin has previously been suspected to be the cause of phenotypic fibroblastic modulations (Howeedy et al. 1990; Vollmer et al. 1990). The phenotype of stromal cells has been shown to be influenced by the presence of proteoglycans (Desmoulière et al. 1992) and of cytokines such γ-interferon (Desmoulière et al. 1992), tumour necrosis factor and granulocyte-monocyte-colony stimulating factor (for review see Sappino et al. 1990). Further studies will be needed in order to understand the factors playing a role in the phenomenon described here, but our findings may represent another example of the local modulation of stromal cell phenotype possibly under the action of micro-environmental factors.

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