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ORIGINAL ARTICLE

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Immunophenotypic features of uterine stromal cells

CD34 expression in endocervical stroma

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Abstract CD34 is a myeloid progenitor cell antigen present in endothelial cells and some other mesenchymal cells, including perivascular and periadnexal dermal fibroblasts. It was evaluated immunohistochemically in uterine stromal tissue and in 4 aggressive angiomyxomas and 6 endometrial stromal sarcomas with potentially related and similar stromal tissues. The stromal cells in normal endocervix and endocervical polyps were strongly CD34 positive irrespective of the cycle phase, and negative for muscle actins. Ectocervical stroma was variably but generally less CD34 reactive. In the endometrium, the CD34 reactivity was limited to the stromal cells of the basal endometrium and was found only in 4 of 20 from proliferative endometria and 1 of 8 from secretory endometria. The uterine cervical and myometrial smooth muscle tissues showed CD34 positive cells only between the muscle bundles and around the vessels. In pelvic aggressive angiomyxomas and endometrial stromal sarcomas the tumour cells were CD34 negative and only the vascular endothelial cells were positive. Endothelial cell-specific antigen, CD31, was identified only in endothelial cells and was not present in the endocervical stroma. These results illustrate the particular immunohistochemical profile of endocervical stromal tissue, namely the strong CD34 expression. The CD34 reactivity of the endocervical tissues should be noted and not confused with neoplasms known to be strongly CD34 positive, such as angiosarcomas, Kaposi's sarcomas and some other spindle cell sarcomas.

Key words Immunohistochemistry · Endocervix · Uterine stromal tissues

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Introduction

CD34, a myeloid progenitor cell antigen present in approximately 1% of normal bone marrow cells representing the stem cell population, is also constitutively expressed in nearly all endothelial cells, although it is variably present in lymphatic endothelium [3, 11, 14]. It has also been used as a marker for vascular tumours, such as angiosarcoma and Kaposi's sarcoma, and for epithelioid sarcoma [1, 9, 12, 13]. In addition, CD34 is known to be present in some fibroblasts and related mesenchymal cells, such as dermal periadnexal and perivascular fibroblasts [6, 7]. Our preliminary observation of CD34 positivity in uterine cervical stroma led us to perform a systematic study to explore the distribution of this antigen in uterine cervical tissues from biological and diagnostic perspectives.

In this report, we describe the consistent and extensive CD34 reactivity of endocervical stromal tissues and discuss its significance.

Materials and methods

Tissue material

Formaldehyde-fixed and paraffin-embedded tissues obtained at hysterectomies and biopsies were studied. These included 29 cases of ecto- and endocervical and myometrial normal tissues and 20 endometria in proliferative phase and 8 endometria in secretory phase, along with 6 cases of adenomyosis. Eleven endocervical polyps were also studied, as were 6 low-grade uterine stromal sarcomas and 4 pelvic aggressive angiomyxomas potentially related to similar stromal tissues.

Antibodies and immunostainings

The primary antibody for CD34 was MY10 (Becton Dickinson, Mountain View, Calif., diluted 1:40). For comparative purposes, another mouse monoclonal CD 34 antibody, QBEND/10 (AMAC, Westbrook Me., diluted 1:150), was used in selected cases. The endothelial-cell-specific marker CD31 (PECAM-1) was used as a control (clone JC/70, Dakopatts, Carpinteria, Calif., dilution 1:50).

An enzyme digestion (crude preparation of 0.05% pepsin from Merck, Darmstadt, Germany, in HCl pH 2.0, for 30 min at +37°C) was used prior to the immunostaining for CD31 and CD34. Mouse monoclonal antibodies to alpha smooth muscle actin (Dakopatts 1:100), muscle actins (HHF-35 EnzoBiochem, New York, N.Y., 1:1600) and desmin (De-33,Dakopatts, dilution 1:50) were used to evaluate muscle cell participation in the stroma. The avidin biotin amplification and detection system was obtained from the Vector Elite-Kit (Vector Laboratories, Burlingame, Calif.). The secondary antibody was biotinylated horse antimouse immunoglobulin antiserum (1:200, followed by avidin combined in vitro with biotinylated-reacted horseradish peroxidase, each diluted 1:200). The colour was developed with diaminobenzidine (1 mg/ml), supplemented with hydrogen peroxide. Haematoxylin counterstain was used, and appropriate positive and negative controls were included in each run to monitor the sensitivity and specificity of the stainings.

Results

The results are summarized in Table 1.

In normal cervical tissues the endocervical spindle cell stroma showed generally strong CD34 immunoreactivity, which was more prominent in the superficial stromal round cells. The CD34-positive cells were negative for muscle cell markers, desmin and alpha smooth muscle actin, and larger actin-positive bundles of smooth muscle cells and the stroma in between could be identified as CD34-negative areas. The CD34-positive stromal cell zone ended abruptly where the alpha smooth muscle actin-positive smooth muscle layer started. The CD34 reactivity of the endocervical stromal tissues appeared not to be related to the phase of the cycle as determined from the histological appearance of the corresponding endometrium. CD31 immunostaining only labelled vascular endothelial cells, and the stromal round cells were negative. All epithelia were negative for both CD34 and CD31.

In ectocervical stromal tissues, the CD34 reactivity was variable. In some cases, there was moderate CD34 reactivity approaching that seen in endocervix, but more commonly the reactivity was limited to the dendrite-like spindle cells. Endocervical polyps showed extensive stromal CD34 reactivity. The stroma showed CD31 reactivity only in the vascular endothelial cells.

Endometrial stroma was typically CD34 negative. However, in 4 of 20 proliferative phase endometria, there were CD34-positive cells in the basal aspect, often seen as a band-like basal zone pattern. A similar finding was seen in 1 of 8 secretory endometria. In the myometrium the CD34 reactivity was limited to perivascular fibroblasts and spindle cells surrounding muscle cell bundles. The stromal cells of adenomyosis were CD34 negative. Endometrial stromal sarcomas of low grade and malignant mixed müllerian tumours showed CD34 immunoreactivity only in the vascular endothelial cells, but the sarcomatous components were negative, which is reminiscent of the findings after immunostaining for CD31.

The 4 aggressive angiomyxomas showed CD34 reactivity limited to the vascular endothelial cells and to scattered perivascular fibroblasts, but the round tumour cells between the vessels were consistently CD34 negative, as after immunostaining for CD31. The stromal cells were almost uniformly positive for desmin, and alpha smooth muscle actin labelled small bundles of smooth muscle cells, whereas the round cells forming the principal non-vascular tumour cell component were negative.

Discussion

In this study, we evaluated the CD34 reactivity in uterine stromal tissues immunohistochemically. In general, endocervical stroma has been classified as fibrous tissue admixed with strands of smooth muscle fibres [4]. The most significant observation in this study was the consistent and striking CD34 reactivity of endocervical stromal tissue (especially superficially) and, to a lesser extent, of the ectocervical stroma and basal endometrium. The CD34-positive cells were negative for desmin and alpha smooth muscle actin, representing non-muscle cells apparently belonging to the fibroblastic population. Our ability to subclassify mesenchymal non-muscular fibroblast-like cells immunohistochemically is presently limited. CD34 reactivity seems to be a new marker characterizing a major subset of cervical fibroblast-like stromal cells. It is possible that the CD34-positive cells represent a functionally distinctive subset of stromal cells with an increased capability for regeneration and overgrowth, as seen in the CD34-positive endocervical polyps. In the endometrium the CD34-positive zone of deep basal stromal cells probably also represents a germinative layer for the regeneration of the endometrium. Certainly, the CD34 reactivity in endocervical and endometrial stromal

Table 1 The immunohistochemical presence of selected antigens in uterine stromal tissues, endocervical polyps, aggressive angiomyxomas and endometrial stromal sarcomas. The endothelial reactivity of CD34 and CD31 is excluded from the table

Tissue type	CD34	CD31	Alpha-SMA	Desmin
Ectocervix	28/28[a]	0/28	21/29	20/29
Endocervix	29/29	0/29	26/29	28/29
Endometrium	6/24[b]	0/28	4/23	0/27
Adenomyosis Stroma	0/6	0/6	0/6	0/6
Myometrium	8/23[a]	0/28	26/26	24/25
Endocervical polyp	9/11	0/11	5/11	7/11
Aggressive angiomyxoma	0/4	0/4	1/4[c]	4/4
Endometrial stromal sarcoma	0/6	0/6	2/6[c]	2/6[c]

[a] Reactivity limited mainly to scattered dendritic and perivascular spindle cells
[b] Basal endometrium
[c] Scattered cells positive

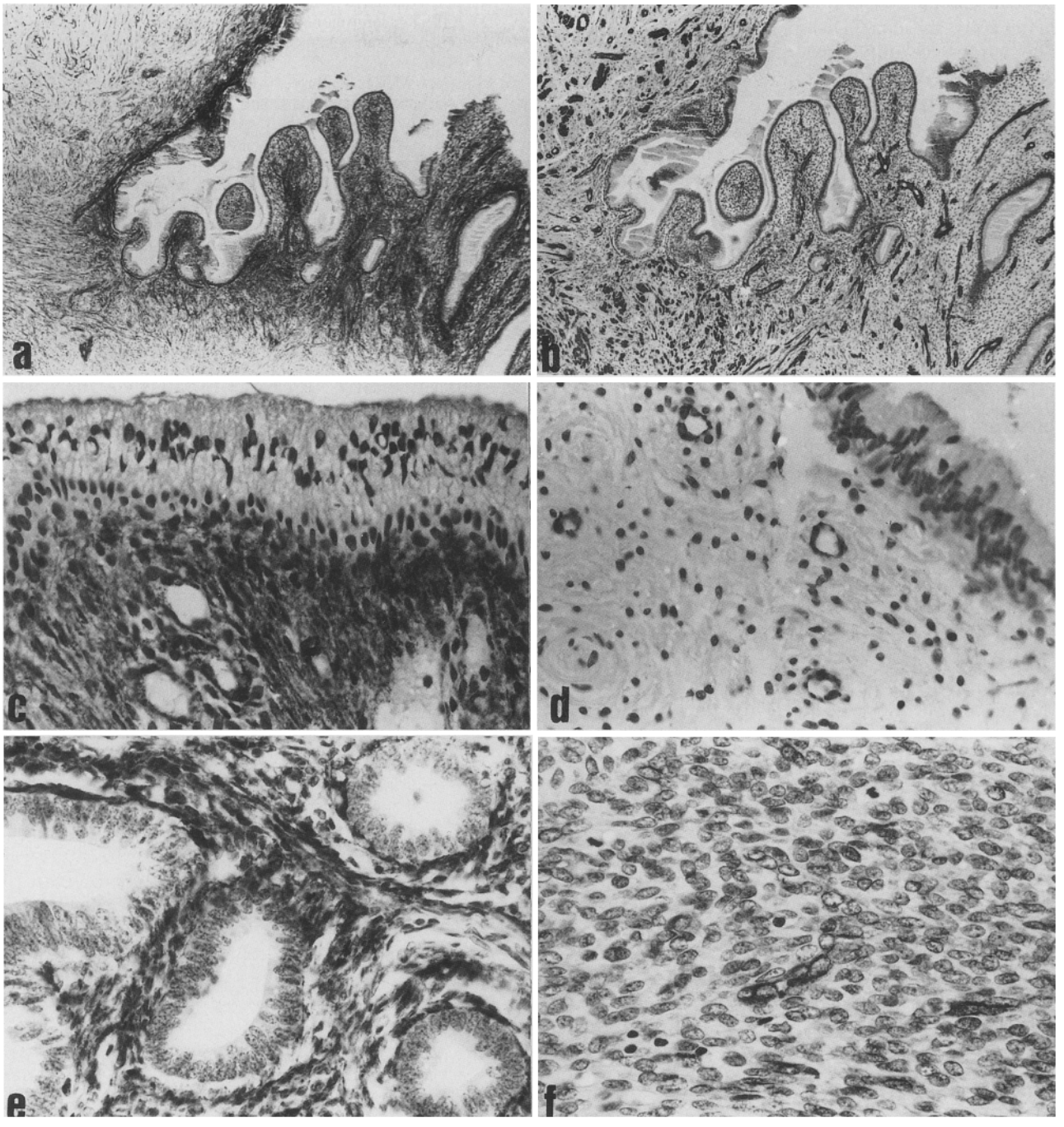

Fig. 1a The endocervical stromal cells are strongly CD34-positive. Note that the CD34-positive zone ends abruptly where the smooth muscle layer begins. **b** The endocervical stromal cells are negative for alpha smooth muscle actin, but deep stromal smooth muscle bundles, as well as pericytes, are positive, highlighting the vessels. **c** High magnification of CD34-positive stromal cells. **d** CD31 reactivity is only seen in vascular endothelial cells of the endocervical stroma. **e** The basal endometrial stroma shows CD34 immunoreactivity. **f** Endometrial stromal sarcoma is CD34 negative, except for vascular endothelial positivity

tissues is not limited to a subset of dendritic stromal cells, as is the case in the dermis and apparently also in the ectocervix [7, 13].

It has been suggested that the cutaneous perivascular and periadnexal CD34-positive stroma originate from CD34-positive circulating stem cells, which may give rise to the CD34-positive stromal cells [7]. Considering the widespread CD34 positivity of uterine stromal tissues, such an explanation seems unlikely for the cervix. It appears more probable that CD34 reactivity is an inherent property of subsets of fibroblasts such as those of uterine stromal tissues. The cervical stromal cells differ immunophenotypically from endothelial cells in being negative for CD31, an antigen specific for endothelial cells [8]. The CD34 reactivity of normal endocervical and other uterine stromal tissues should be evaluated in a context where small biopsies are subjected to immunohistochemical studies for CD34 reactivity in tumours such as angiosarcoma and Kaposi's sarcoma [1, 9, 12].

These endothelial cell tumours, like non-neoplastic endothelial cells, are also CD31 positive [2, 5, 6].

The aggressive angiomyxoma is a specific tumour type occurring in the pelvis and perineal soft tissues [10]. It can be characterized as a proliferation of the loose myxoid stromal tissues. Our results show that it differs from the endocervical stroma in being CD34 negative. Similarly, endocervical stromal sarcomas appear to be phenotypically different from the CD34-positive fibroblasts.

In conclusion, with this study we have demonstrated the unique nature of endocervical stromal tissue as a consistently CD34-positive cellular stromal tissue, illustrating that subsets of fibroblast stem cells may express CD34.

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