Immunohistochemical expression of vimentin and secretory component antigens in endometrial hyperplasia and neoplasia

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Summary

Vimentin is an intermediate filament protein normally expressed in mesenchymal cells, but evidence is accumulating in the literature which suggests that the aberrant expression of vimentin in epithelial cancer cells might be related to local invasiveness and metastatic potential. Previous studies strongly support the implication of vimentin in the metastatic progression of breast and cervical lesions. The secretory component is isolated from human colostrum and is of help in more precise grading of endometrial carcinoma. In this study we examined vimentin and secretory component (SC) expression in adenomatous hyperplasia, atypical adenomatous hyperplasia and well-differentiated adenocarcinoma (cribriform pattern). The results showed decreased expression of vimentin and increased expression of the secretory component as the lesion progressed to malignancy.

Key words: Vimentin; Endometrial hyperplasia; Endometrial neoplasia.

Introduction

Vimentin is an intermediate filament protein normally expressed in cells of mesenchymal origin, such as fibroblasts [1]. Several earlier studies have neverthless described the presence of vimentin in epithelial cells in vitro [2, 3] and have led to the conclusion that this aberrant expression was induced by in vitro cultivation. However, data are now accumulating which suggest instead that vimentin expression in epithelial cells in vitro correlates with a high degree of transformation; it has been reported, for example, that highly invasive breast cancer cells lines express vimentin, in contrast with non-invasive cell lines [4, 5]. We have also reported such an association between vimentin expression and invasiveness in cervical cancer cell lines transfected by human papillomavirus type 33 (HPV-33) or HPV-33 together with the ras oncogene [6, 7].

A number of in vitro observations also support the concept that vimentin expression in epithelial cells might be related to tumor progression. Vimentin expression has indeed been reported in a variety of human epithelial tumors including renal, thyroid, ovarian, pulmonary, and prostatic carcinomas [8-17]. It has been extensively studied in breast cancer [18-26], where it was suggested that vimentin might have an important prognostic value. However, some controversy exists regarding the relationship between vimentin and invasiveness, at least concerning breast cancer progression. Indeed, Heatley *et al.* [24], found that vimentin could not clearly differentiate between benign and invasive breast lesions, although it was correlated with tumour grade and decreased survival in ductal carcinoma.

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Gilles and associates [6, 7, 27] have examined vimentin expression in cervical neoplasia and have found that vimentin is a useful marker for the transition of cervical intraepithelial neoplasia (CIN I, II, and III) to invasiveness in cervical carcinoma.

The secretory component antigen is also called polymeric immunoglobulin receptor (plg-R) and is highly specific when tested on human bone marrow cells and on human secretory epithelium. Previous studies have shown that well-differentiated endometrial carcinomas express the secretory component better than poorly differentiated tumours [28].

Materials and Methods

We studied 31 cases of adenomatous hyperplasia, 12 cases of atypical adenomatous hyperplasia and 39 cases of well-differentiated adenocarcinoma.

Source and preparation of neoplastic tissues

The samples were fixed in formalin and embedded in paraffin for immunohistochemical study.

Immunohistochemistry

Immunohistochemistry was performed with the various antibodies used on serial sections. Tissue sections (5 μm) were deparaffinized, rehydrated, and treated with 0.3 per cent hydrogen peroxide for 5 min to quench endogenous peroxidase activity. Non-specific binding was blocked with serum for 10 min. Slides were then incubated for 30 min with the monoclonal antibodies (1/40), namely mouse anti-human vimentin (3B4, Dako EPOS, Carpinteria, U.S.A.). Control slides were incubated for the same period with normal mouse serum. After several 10 min washes in PBS, samples were developed with the

peroxidase LSAB kit (labelled streptavidin-biotin method, Dako Carpinteria, U.S.A.), which allows the detection of the first antibody. The slides were briefly counterstained with Mayer's haematoxylin, mounted, and examined under an Olympus BX40 microscope.

The immunostained sections were examined with a x 40 objective and the distribution of vimentin and secretory component within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with vimentin and secretory component stainings, a 10 x 10 square calibrated grid was inserted into the eyepiece of an Olympus binocular microscope.

Five-to-ten fields were examined for each section, and at least 1,000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the vimentin and secretory component (SC) indices.

$$\text{Vimentin index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

$$\text{SC index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

The vimentin and SC indices ranged from 0-100%, with a mean of 18%. The mean index was evaluated in three ranges: low index (under 18%), grade I; moderate index (from18 to 50%), grade II; and high index (from 51 to 100%), grade III.

Results

The sections were examined independently by two observers, and positive cellular staining for anti-vimentin and anti-secretory component antibodies were manifested as fine red cytoplasmic granularity and/or surface membrane expression (Figures 1, 2, 3, 4).

Vimentin was expressed in 21 of 31 adenomatous hyperplasias (AHs) (68%), in six of 12 atypical adenomatous hyperlasias (AAHs) (50%), and in 15 of 39 carcinomas (38%). Of 21 positive AHs nine were scored as vimentin grade II and 11 as vimentin grade III. Of six positive AAHs one was scored as vimentin grade I, three as vimentin grade II and two as vimentin grade III. Of 15 positive adenocarcinomas one was scored as vimentin grade I, seven as vimentin grade II, and seven as vimentin grade III.

The secretory component was expressed in nine of 31 adenomatous hyperplasias (AHs) (29%), in five of 12 atypical adenomatous hyperlasias (AAHs) (42%), and in 26 of 39 carcinomas (67%). Of nine positive AHs four were scored as secretory component grade II and five as secretory component grade III. Of five positive AAHs one was scored as secretory component grade I, three as secretory component grade II and one as secretory com-

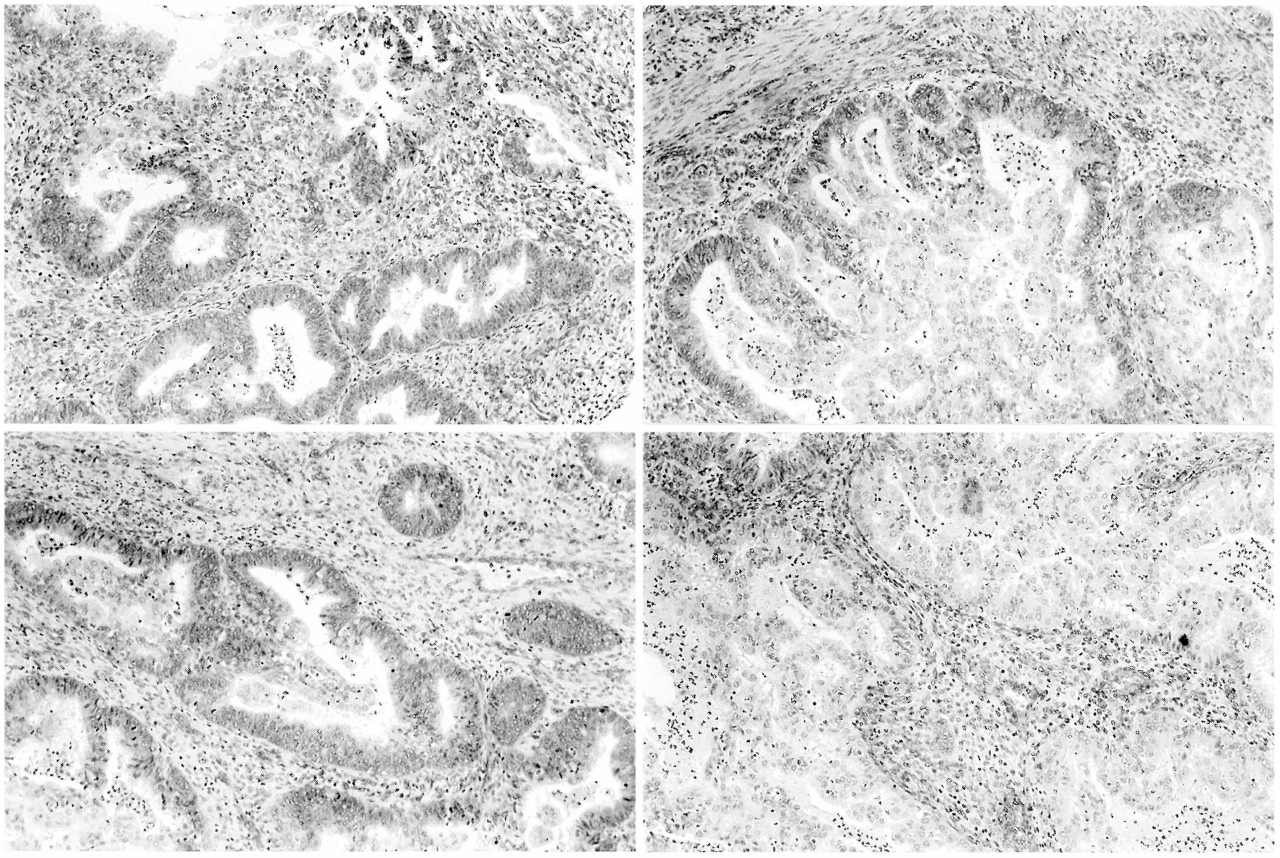

Figure 1. — Atypical adenomatous hyperplasia of the endometrium. Vimentin expression x100.
Figure 2. — Well-differentiated endometrial adenocarcinoma. Vimentin expression x100.
Figure 3. — Atypical adenomatous hyperplasia of the endometrium. Secretory component expression x100.
Figure 4. — Well-differentiated endometrial adenocarcinoma. Secretory component expression x100.

ponent grade III. Of 26 positive adenocarcinomas four were scored as secretory component grade I, 12 as secretory component grade II, and ten as secretory component grade III.

Discussion

The WHO classification for endometrial pathology, analogous to that proposed by thhe International Society of Gynecological Pathologists and the International Federation of Gynecology and Obstetrics [29], has been seriously challenged lately. The criteria for the differential diagnosis of benign, premalignant, and early malignant epithelial lesions of the endometrium given in the WHO fascicle on Histological Typing of Female Genital Tract Tumors [30] are difficult to sustain since they are associated with an unacceptably high inter- and intraobserver variation [31-35]. Recently, a European group of expert gynecological pathologists [31] reported a poor interobserver (54-73%) and intraobserver (68-73%) agreement in diagnostic curettage material using the four WHO diagnostic categories. Therefore, the question arises: why is it so difficult to define reliable and reproducible histological criteria in order to establish a correct diagnosis?

Firstly, the morphological changes of the endometrium during the normal menstrual cycle are extremely variable and greatly influenced by numerous factors such as age and hormone therapy, and consequently endometrial morphology may show a great number of overlapping features ranging from physiologic variability to true autonomous proliferation.

Secondly, morphology can vary greatly throughout the same endometrium: areas with a more or less normal appearance are found adjacent to simple cystic alterations and/or complex proliferations. The content of the endometrial stroma can also vary from normal to slightly increased or even to reduced, resulting in glands appearing more crowded and angular. The enigma seems to come in deciding which part is of diagnostic importance?

Furthermore, the epithelial cells of any one endometrium may show areas with only slight pseudostratification and normal gland-lining cells next to areas with pseudopapillary buds, increased proliferation, and polymorphism. The biological relevance of this variability is unclear.

Lastly, the definition and interpretation of atypicality seems to be particularly difficult to define in the endometrium; indeed, one is frequently faced with grade 1 endometrioid invasive adenocarcinomas that have minimal or absent nuclear atypia. This picture is even more complicated in patients undergoing concurrent hormonal treatment [36, 37].

There are no clear-cut criteria to differentiate between all these variations, making the weakness of the diagnoses of endometrium hyperplasia not a question of experience versus inexperience but rather a reflection of the lack of a sharp borderline between the different entities. However, this problem has not been considered sufficiently in the WHO classification system.

Endometrial cancer represents a heterogeneous group of neoplasms which is comprised of many distinct morphological variants. The most common variant is endometrioid cancer which represents the main hormone-dependent adenocarcinoma of the uterus. Many other morphological types are encountered such as mucinous, endocervical, adenosquamous and clear cell, as well as serous papillary adenocarcinoma which represents a non hormone-dependent tumor [38]. This morphological variety is explained in view of the mullerial derivation of these tumors and the ability of the mullerian epithelium to differentiate in many epithelial types. Intraglandular bridging without fibrous cores (cribriform pattern) and the random piling of cells into disorganized masses are frequent findings in carcinoma, and when these features are conspicuous we are unwilling to accept a lesion as atypical hyperplasia. We think these are the two most helpful morphologic features in distinguishing well-differentiated adenocarcinoma from atypical hyperplasia.

Atypical adenomatous hyperplasia gives rise to carcinoma with a frequency of at least 25%. The immunophenotypic profile of the proliferating endometrium may help in understanding the progress to carcinoma.

In our study we found that there is a loss of vimentin expression and a gain of secretory component expression as endometrial hyperplasia progresses to endometrial adenocarcinoma.

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