Gains and losses of glycoprotein CD44 and secretory component expression in endometrial hyperplasia and neoplasia

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Summary

CD44 is an adhesion molecule, which binds hyaluronic acid and participates in a number of cell-cell interactions, including lymphocyte homing. The CD44 antigen is expressed on approximately 90% of lymphocytes, monocytes, granulocytes, and, in lower amounts on thymocytes, fibroblasts, and erythrocytes. Platelets lack CD44. In non-haematopoietic tissues, CD44 is widely distributed. The secretory component is isolated from human colostrum and is of help in more precise grading of endometrial carcinoma. In this study we examined CD44 and secretory component expression in adenomatous hyperplasia, atypical adenomatous hyperplasia and well-differentiated adenocarcinoma (cribriform pattern). The results showed decreased expression of CD44 and increased expression of secretory component as the lesion progressed to malignancy.

Key words: Glygoprotein CD44; Endometrial hyperplasia; Endometrial neoplasia.

Introduction

The CD44 molecule belongs to a family of cellular adhesion molecules found on a wide range of normal and malignnat cells in epithelial, mesothelial and haemopoietic tissues. CD44 is a single gene with 20 exons, of which ten are normally expressed to encode the basic CD44 (H-CAM) molecule. The additional ten exons (v1 to v10) are only expressed by alternative splicing of the nuclear RNA. The expression of specific cell adhesion molecule CD44 splice variants has been shown to be associated with metastasis and poor prognosis in certain human malignancies, such as breast cancer. A complex pattern of CD44 variant expression in different tumors compared to the CD44 expression of the normal cell of origin has been reported [1, 2]. High levels of expression have been observed in many of the variant exons by breast carcinomas that arise from breast ductal epithelium which do not normally express CD44. Conversely, normal gastrointestinal epithelium expressed low levels of many of the CD44 variants and the derived colon cancers expressed low and variable levels of the variants. In addition, respiratory epithelium which expressed variants at high levels in normal cells expressed the same variants at similar levels in lung carcinomas. NCL-CD44v4, NCL-CD44v5 and NCL-CD44v6 will each be useful in the assessment of exon variant expression and their correlation with tumor characteristics. CD44 is involved in cell-cell and cell-extracellular matrix adhesive interactions. It participates in fundamental biological processes including cell traffic, lymphocyte homing, hematopoiesis, inflammation, wound healing, embryonal development and apoptosis [3-6Ë]. It is also implicated in tumor pathology, playing a role in tumor cell differentiation, invasion and metestasis. In humans, up-regulation of CD44 was observed in non-Hodgkin's lymphomas and colonic adenocarcinomas associated with poor prognosis [7, 8]. In renal cell carcinomas the prognostic significance of CD44 expression was equivocal [9]. In endometrial and urothelial carcinomas down-regulation of CD44 expression was observed whereas in squamous cell carcinomas both down-regulation and unchanged expression have been reported compared to normal tissue counterparts [10-13]. Initial studies indicate involvement of CD44 also in breast cancer development [14, 15].

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 tumors [16].

One major issue in endometrial pathology addresses the problem of distinguishing between benign and early neoplastic proliferative lesions (adenomatous hyperplasia, atypical adenomatous hyperplasia, and well-differentiated adenocarcinoma). Further molecular histological investigations are needed to elucidate functional differences between these lesions underlying the morphological characteristics seen under light microscope.

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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 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 percent 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 CD44 (DAKO) and secretory component (SC) (DAKO). 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), 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 CD44 and SC within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with CD44 and SC 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 1000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the CD44 and SC indices.

$$\text{CD44 index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

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

The 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 CD44 and SC antigens were manifested as fine red cytoplasmic granularity and/or surface membrane expression (Figures 1, 2).

CD44 was expressed in 20 of 31 of adenomatous hyperplasias (AHs) (64.5%), in four of 12 atypical adenomatous hyperlasias (AAHs) (33.3%), and in ten of 39 well-differentiated adenocarcinomas (25.6%). Of 20 positive AHs nine were scored as CD44 grade II and 11 as CD44 grade III. Of four positive AAHs one was scored as CD44 grade I, and three as CD44 grade II. Of ten positive carcinomas one was scored as CD44 grade I, seven as grade II, and two as grade III.

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 component 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

Endometrial hyperplasias form a morphologic continuum of abnormal epithelial and stromal proliferations ranging from focal glandular crowding or simple hyperplasia to well-differentiated adenocarcinoma. It encompasses a variety of patterns, some of which are characterized by varying degrees of cellular atypia. For many years, pathologists have been concerned about the malignant potential of the various types of endometrial hyperplasia. Much of the confusion in the literature has resulted from different uses of the terms "adenomatous hyperplasia", "atypical hyperplasia" and "carcinoma in

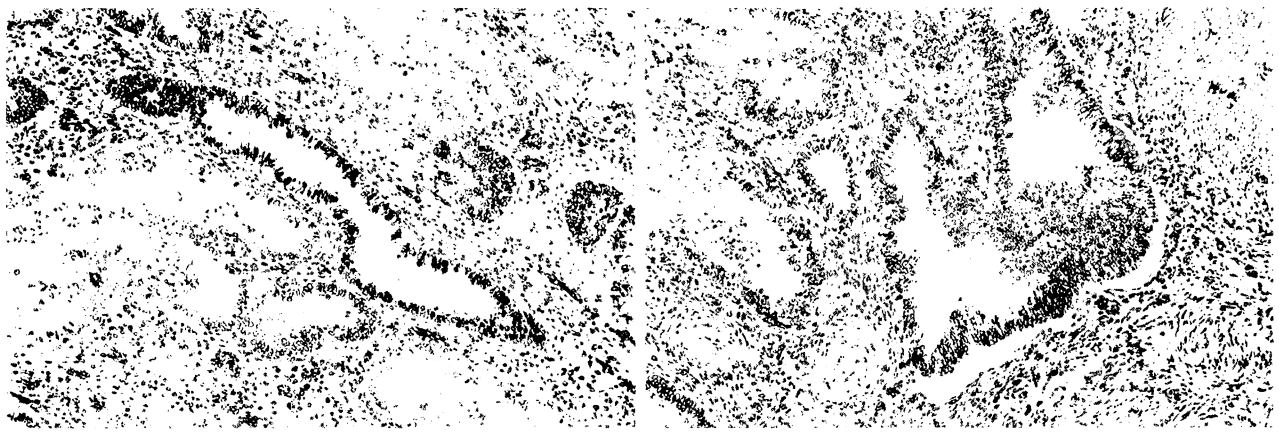

Figure 1. — Adenomatous hyperplasia of the endometrium. CD44 expression x100.
Figure 2. — Atypical adenomatous hyperplasia of the endometrium. CD44 expression x100.

situ" to describe lesions in the borderline area. Also, there has been an absence of follow-up data. Recently, ultrastructural and DNA studies have indicated that cytologic atypia represents a more serious change than structural abnormalities from the viewpoint of risk of developing carcinoma [17-19].

In 1977, it was first recommended that the architectural and cytologic abnormalities be evaluated separately to learn more about their own malignant potential. The following working classification was proposed: cystic hyperplasia, architectural atypia (mild to severe), and cytologic atypia (mild to severe) [17].

Cystic hyperplasia is characterized by large dilated glands with rounded profiles lined by nonproliferating or minimally proliferating epithelium, separated by an abundant and cellular stroma. The increased amounts of stroma account for the cystic dilatation of the glands. There is also dilatation and thrombosis of sinusoids and focal necrosis.

The complex forms of hyperplasia exhibit a more distinct proliferation pattern; the gland outlines are irregular because of outpouchings and papillary infoldings of the epithelium. In those cases with predominantly architectural abnormalities (formerly"adenomatous hyperplasia"), the glands are lined by columnar epithelial cells with large nuclei often exhibiting stratification, but polarity is generally maintained. The stroma is dense, cellular, and compact. There are numerous mitoses in both glands and stroma. In higher grades of hyperplasia (formerly "adenomatous hyperplasia") striking architectural abnormalities are commonly associated with varying degrees of cellular atypia and the cytologic features - large cells and large irregular nuclei and nucleoli - are more like those of adenocarcinoma. The cells that line the glands lose their polarity, and the cytoplasm often appears densely eosinophilic.

The new classification formulated by the WHO committee on endometrial tumors [20] breaks down endometrial hyperplasia into four subtypes: simple hyperplasia without atypia (SH), simple hyperplasia with atypia (SAH), complex hyperplasia without atypia (CH), and complex hyperplasia with atypia (CAH). The term atypia refers to cellular atypia, and the term complexity refers to severe architectural abnormality close to that seen in cases of well-differentiated adenocarcinoma [18].

Simple hyperplasia includes cystic hyperplasia and mild and moderate degrees of architectural abnormality. SH is not significantly precancerous. Similarly, CH is also not demonstrably precancerous. There is not enough follow-up information about SAH to indicate that it is precancerous; this lesion, therefore, deserves further investigation. Finally, when CAH is diagnosed in a biopsy specimen, a well-differentiated adenocarcinoma is discovered in the hysterectomy specimen in 15% to 20% of the cases, or the lesion will eventually be followed by carcinoma in approximately 30% of the patients [21, 22].

It is unquestionable that estrogen administration produces endometrial hyperplasia. Prolonged periods of anovulation with steady and unopposed estrogen secretion have a similar effect, even in young women [23, 24]. CAH apparently responds completely to the differentiating effect of progestins. Those lesions that do not respond to treatment (progestins, discontinued estrogen therapy, and D & C) are most likely to occur in women with polycystic ovarian disease, pronounced obesity, or both [18]. In obese women without polycystic ovarian disease, hyperplasia results from peripheral conversion of androstenedione to estrone in adipose tissue. The carcinomas that develop in this clinical setting are usually well differentiated, confined to the uterus, and associated with hyperplasia; they have an excellent prognosis [22].

In curettage specimens, well-differentiated adenocarcinoma is often difficult to distinguish from CAH because the histologic criteria for both lesions are highly subjective. Recently, criteria for making this differential diagnosis have been proposed by two groups of investigators [22, 25]. These criteria were validated by the findings in hysterectomy specimens, including the presence or absence of myometrial invasion, after a diagnosis of either CAH or well-differentiated adenocarcinoma had been made on biopsy specimens. The first group [25] underlined various architectural and cytologic abnormalities, some of which may be absent in individual cases, leaving the final decision to the overall evaluation of the lesion. Both pronounced architectural atypia and at least moderate cytologic abnormality were required for the diagnosis of adenocarcinoma [25]. In contrast, the second group of investigators proposed strict criteria emphasizing architecture, qualitative stromal changes, and quantitative features [22]. They used as their primary criterion for adenocarcinoma the presence or absence of stromal invasion, which is defined arbitrarily by the presence of at least one of the following features: 1) desmoplastic stromal response in the vicinity of infiltrating glands, 2) confluent of cribriform glandular pattern, 3) extensive papillary pattern and 4) replacement of stroma by squamous epithelium [22]. To qualify as invasion, the last three changes are required to occupy at least half (2.1 mm) of a low-power microscopic field 4.2 mm in diameter. Using these criteria, when stromal invasion was absent in the endometrial curettings (and a diagnosis had been made), invasive adenocarcinoma was present in the hysterectomy specimen in 17% of the cases. The carcinomas in these cases were well-differentiated and confined to the endometrium or were only superficially invasive [22]. In contrast, when stromal invasion was present in the curettings, residual carcinoma was identified in the uterus in 50% of the cases and, of these, one-third were moderately or poorly differentiated, and one-quarter deeply invaded the myometrium [22]. Although the criteria proposed in the first study [25] are imprecise and somewhat subjective, those of the second [22] are too strict.

Our aim was to map the CD44 and SC immunophenotypes of AH, AAH, and adenocarcinoma with special focus on their potential differential diagnostic value in early stages of endomatrial carcinogenesis. We found that there is a depletion of CD44 and a predominance of secretory component expression as endometrial hyperplasia progresses to endometrial carcinoma.

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