Original Article

Expression of CD117, CD34, and VEGF proteins in progression from endometrial hyperplasia to endometrioid carcinoma

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Abstract: Background: The risk of endometrial hyperplasia progressing into endometrioid carcinoma ranges from 1% for benign hyperplasia to 46.2% for endometrial intra-epithelial neoplasia. Differentiation between both types of hyperplasia is thus crucial for optimal management. The present study investigates the expression of the following immune-histochemical markers, for their potential roles in differentiating between both types of endometrial hyperplasia: as well as their expression in endometrial carcinoma: VEGF, CD34 and CD117. Methods: Tissue samples were obtained, fixed, processed, stained by hematoxylin and eosin for diagnosis, and then immunohistochemically stained using anti CD117, CD34, and VEGF antibodies. Results: In benign endometrial hyperplasia, the cells show weak expression to VEGF and CD34, and absent CD117. In endometrial intra-epithelial neoplasia, the cells show strong expression of VEGF and weak expression of CD34 and CD117. In case of endometrioid carcinoma, all cases showed strong reaction for VEGF and CD34, and moderate expression to CD117. Conclusion: Our data suggests a role for CD117, CD34, and VEGF in progression from hyperplasia to carcinoma.

Keywords: Endometrial hyperplasia, endometrioid carcinoma, CD117, CD34, VEGF

Introduction

Endometrial hyperplasia (EH) is a well-known precursor lesion to endometrioid carcinoma (EC) of the endometrium. It is the second most common gynecological malignancy world-wide [1]. It is presented with abnormal uterine bleeding, usually in the early postmenopausal years [2].

EH has been linked to continuous exposure of estrogen, unopposed with progesterone [3, 4]. The degree of risk increases in obese nulliparous postmenopausal females; especially if diabetic and hypertensive [5].

Pathologically, EH represents a heterogeneous group of lesions, characterized with an increase in the gland to stroma ratio compared to proliferative endometrium [6]. Progression of EH to EC is related to the degree of architectural complexity and cytological atypia. The latter is considered the principal histological determinant for malignant potential [7]. Moreover, aberrant expression of the VHL gene is associated with progression of EH to EC [8].

The pathological diagnosis and classification of EH has been through many changes and updates. Prior to 2014, the World Health Organization (WHO) classification system used cellular complexity, crowding of the endometrial gland, and the presence of cytological atypia to classify EH as simple or complex and with or without atypia [9]. This system suffered from significant interobserver variation [10]. Accordingly, the new WHO classification, accepted by the International Society of Gynecological Pathologists, divided EH into only two groups: benign hyperplasia and atypical hyperplasia/endometrial intraepithelial neoplasia (EIN) [11-13].
The risk of EH progressing into EC ranges from 1% for benign EH to 46.2% for EIN [14]. Differentiation between both types of EH is crucial for optimal management. The distinction between benign and atypical EH is based on certain microscopic diagnostic criteria determined from a hematoxylin and eosin (H&E) stained endometrial section. However, such histopathological assessment can lead to subjective inter-observer variation especially in the diagnosis of atypical EH. This necessitates the possible use of certain immunohistochemical (IHC) markers for further confirming the diagnosis and identifying ‘at risk’ EH patients for malignant progression [15]. Several markers have been proposed, as estrogen receptor (ER), progesterone receptor (PR), Cyclooxygenase-2 (COX-2), Phosphatase and tensin homologue (PTEN), P53, B-cell lymphoma 2 (Bcl-2), E-cadherin, and CD10 [16-21]. However, contradictory data about the usefulness of these makers have been reported [15]. The present study investigates the expression of the following IHC markers, for their potential roles in differentiating benign EH from EIN and EC, and thereby identify groups of interacting proteins that could be involved in the progression of EH to EC: vascular endothelial growth factor (VEGF), CD34, and CD117.

**Material and methods**

**Patients**

Tissue samples from 40 females presented with perimenopausal bleeding and underwent diagnostic dilatation and curettage (D&C) in the Gynecology Departments of El-Zagazig University Hospital, and Suez Canal University Hospital, in Egypt. Two pathologists reviewed all the tissue samples to confirm the diagnosis. The histological grade of EC was assessed according to the latest WHO classification [11]. A full and informed patient consent was obtained from each patient.

### Table 1. Antibodies for immunocytochemistry.

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Lot</th>
<th>Working dilution</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>M7273</td>
<td>1:100</td>
</tr>
<tr>
<td>CD34</td>
<td>QBEnd-10</td>
<td>1:100</td>
</tr>
<tr>
<td>CD117</td>
<td>A4502</td>
<td>1:100</td>
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</table>

Histopathological evaluation: The samples were fixed with 10% formalin and embedded in paraffin. From each block, histological sections of 3 µm thickness were submitted, mounted to glass slides, stained by hematoxylin and eosin (H&E), and reviewed to confirm the diagnosis of benign EH, EIN, and EC and identify in the latter tumor grade and depth of invasion.

Immunohistochemical staining: Sections from the selected paraffin blocks were cut into 4 micrometers thick sections and mounted on poly-L-lysine glass slides for immunohistochemical staining. Primary antibodies (Table 1) were purchased from Dako (Glostrup, Denmark). This was followed by 60 min incubations with secondary antibodies (Envision+System-HRP Labelled Polymer, Dako) and visualization with the Liquid DAB+Substrate Chromogen System (Dako). All slides were lightly counterstained with hematoxylin for 30 s prior to dehydration and mounting.

Immunohistochemical scoring: The following semi-quantitative method to assess the staining intensity was used: a strong positive result was defined as strong or moderate immunoreactivity in 50% or more of cells (Score 2), a weak positive result was defined as weak immunoreactivity or staining of fewer than 50% of tumor cells (Score 1), and cells with no or focal minimal staining was scored as negative (Score 0).

Statistical analysis

Data were collected, coded, and entered into the computer statistical program. All statistical analysis was done using JMP9 software program (SAS Institute Inc, Cary, NC). Quantitative data were presented as mean and standard deviation. Qualitative data was presented in the form of numbers and percentages. Pearson’s Chi-square test was used to test the significance of the difference between groups in qualitative variables. Fisher’s exact-test was used instead, in cases that were >25% of cells had expected count <5. Mann Whitney U-test was used to test the significance of differences in quantitative variables between groups. For all statistical analysis level of significance was considered to be <0.05.
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Figure 1. Expression of VEGF, CD34, and CD117 in benign endometrial hyperplasia (EH), endometrial intraepithelial neoplasia (EIN), and endometrioid carcinoma (EC); type 1. The first row shows representative H&E figures for each lesion. In benign EH, endometrial glands (black arrows) are proliferative and lined with single layer of uniform columnar epithelial cells with no atypia (H&E, 10×). Few glandular cells show weak expression of VEGF at their luminal surfaces (Red arrows), which was scored as negative (IHC, 40×). Similarly, the expression of CD34 (Blue arrows) was weak and mostly at their luminal surfaces (IHC, 40×). In case of CD117 expression, no reaction was observed in glandular cells. In EIN, the H&E figure shows a focus of glandular complexity (Black arrows), with narrow stroma in between, in which the glandular epithelial cells show atypical morphology; stratified hyperchromatic nuclei with various degrees of pleomorphism (H&E, 10×; inset 40×). The expression of VEGF (Red arrows) was significantly stronger than in benign EH (IHC, 40×). CD34 expression (Blue arrows) in glandular cells showed weak positivity, similar to benign EH (IHC, 40×). CD117 (Arrow heads) showed weak focal apical staining which was scored as negative expression (IHC, 40×). In EC, the tumor (black arrow) is composed mostly of villo-glandular pattern of tumor cells arrangement, with few solid sheets (<5%), with mild degree of cellular and nuclear pleomorphism (H&E, 10×). The expression of VEGF (red arrow) and CD34 (Blue arrow) was diffuse strong cytoplasmic, in comparison to those of benign EH and EIN (IHC, 40×). CD117 (Arrow heads) showed moderate membranous reaction in more than 50% of glandular epithelial cells (IHC, 40×).
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Table 2. The immunohistochemical score for expression of VEGF, CD34 and CD117 in endometrial lesions. The average score from samples ± s.d. and p value are indicated.

<table>
<thead>
<tr>
<th></th>
<th>Non-atypical (Benign) EH (n=20)</th>
<th>Atypical EH (EIN) (n=10)</th>
<th>Type I EC</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEGF</td>
<td>CD34</td>
<td>CD117</td>
<td>G1 (n=6)</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.2 (0.4)</td>
<td>0.2 (0.43)</td>
<td>0.2 (0.45)</td>
<td>1 (0.44)</td>
</tr>
<tr>
<td>CD34</td>
<td></td>
<td></td>
<td></td>
<td>0.4 (0.57)</td>
</tr>
<tr>
<td>CD117</td>
<td></td>
<td></td>
<td></td>
<td>1.8 (0.4)</td>
</tr>
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</table>

EH, endometrial hyperplasia; EIN, endometrial intra-epithelial neoplasia; EC, Endometrioid carcinoma; G, grade; VEGF, Vascular endothelial growth factor.

Results (Figure 1 and Table 2)

The age of patients from whom the specimens were taken ranged between 45-72 years with a mean age of 48.2 years, 52 years, and 69 years in benign EH, EIN, and EC respectively.

Among the 40 specimens, 20 samples were benign EH, 10 samples were EIN, and 10 samples were EC; 6 of which were grade 1 and the other 4 were grade 2.

Evaluation of the expression of VEGF in tissue samples revealed a statistical significant difference in its expression among the cases. All cases of EC showed strong positive diffuse cytoplasmic expression, with no significant difference between grade 1 or 2 tumors. In EIN, there was moderate to strong expression of VEGF, but in fewer than 50% of glandular cells, and thus was recorded as weak cytoplasmic positive, while in benign EH, it showed no expression. These findings suggest a role of acquisition of VEGF expression by endometrial glandular cells for progression from EH into EC.

Regarding CD34, there was a statistically significant difference in its expression between EC and the other lesions, with no differences in its expression between benign EH and EIN. 80% of cases of EC showed strong cytoplasmic expression, while its expression in benign EH showed weak to negative reactions. These findings indicate a possible role of CD34 in late stages of progression to EC, with no significant role in differentiating benign from EIN.

Regarding the expression of CD117, all cases of EC showed moderate membranous expression, with no significant difference between grade 1 or 2 tumors. In EIN, the expression of CD117 was observed in few glandular cells, at their apical luminal surfaces, and thus was recorded as negative expression, while in benign EH, it showed no expression. These findings suggest a role of acquisition of VEGF expression by endometrial glandular cells for progression from EH into EC.

Discussion

The progression of endometrial hyperplasia to carcinoma necessitates the search for certain markers for better diagnosis and understanding of such progression. Multiple non-epithelial surface and intracellular markers are associated with cancer progression [22]. We investigated the expression of CD117, VEGF, and CD34 in the progression of hyperplasia to carcinoma.

CD117 protein is a tyrosine kinase receptor which is encoded by the proto-oncogene c-kit, located on chromosome 4 [23]. Over activation of CD117 is the primary mutation seen many malignant neoplasms; GIST, mastocytosis, AML, and melanoma [24]. It is also expressed in many other solid tumors with a trend towards worse prognosis in tumors with high CD117 expression [25]. We found that CD117 expression increased significantly from benign EH to EC. Similar results were recorded in many other types of tumors; like prostatic adenocarcinoma cells which shows CD34 high/CD117 high phenotype, when compared to benign prostatic lesions [26]. Moreover, in high grade prostatic tumors, CD117 positivity was higher, compared to low grade tumors and that xenograft tumors expressing CD117 were larger and have more angiogenesis [22]. In addition, studies on phyllodes tumor of the breast as well as ovarian serous carcinoma revealed that CD117 expression was associated with higher tumor grade and poor prognosis [27-31]. A study on melano-
cytic tumors found that intradermal nevi were negative for CD117, while dysplastic nevi were 100% positive and malignant melanoma was 83.3% positive [32]. In endometrium, some studies found that CD117 expression was significant in benign endometrium, with more intense staining in hyperplastic and proliferative endometrium, as well as in endometrial carcinoma [23, 33] and that there was decreased expression of CD117 with progression from hyperplasia to carcinoma [23]. These results, albeit different from our findings, could highlight the complexity of CD117 signaling in endometrial tumorigenesis, and that it could be acted on in a time-dependent manner. Thus, further extensive studies are needed to elucidate its role.

CD34 is a transmembrane phosphoglycoprotein, first identified on hematopoietic stem and progenitor cells, and is expressed also by mesenchymal stem cells, muscle satellite cells, corneal keratocytes, interstitial cells, epithelial progenitors, and vascular endothelial progenitors [34, 35]. The present data shows that there was a statistically significant difference in CD34 expression between EC and the other lesions, with no differences in its expression between benign EH and EIN. 80% of cases of EC showed strong cytoplasmic expression, while its expression in benign EH and EIN showed weak to negative reaction. These findings indicate a possible role of CD34 in late stages of progression to EC, with no significant role in differentiating benign from atypical EH. To our knowledge, this is the first report of identifying CD34 protein expression in glandular cells in endometrial lesions. Some studies showed that CD34 expression in vessels in endometrial carcinoma was significantly higher than in normal proliferative endometrium and its expression increased in higher grades endometrial carcinoma [36-38]. Our findings are similar to the results of other studies in other tumors, like hepatocellular carcinoma, which show positive CD34 staining, in comparison to hepatic adenoma and focal nodular hyperplasia, which show incomplete positive staining and to normal or cirrhotic liver, which show negative expression [39]. Similar results were found in colorectal adenomas and carcinomas [40, 41], head and neck squamous cell carcinoma [42, 43], malignant melanoma [44, 45], oral and esophageal squamous cell carcinoma [46], prostatic carcinoma [47], and NUT midline carcinoma [48]. Similarly, CD34 expression was significantly higher in malignant compared to benign surface epithelial tumors of ovary and in high grade compared to low grade tumors [49].

VEGF is a potent pro-angiogenic factor and a key mediator of neovascularization, a process which is involved in the pathogenesis of malignant tumors, as its overexpression in tumor cells stimulate tumor growth and metastasis in many cancers [50]. The current work revealed that all cases of EC showed strong positive diffuse cytoplasmic expression of VEGF, with no significant difference between grade 1 or 2 tumors. In EIN, there was weak cytoplasmic positive staining, while in benign EH, the expression was negative. These findings suggest a role of VEGF in the progression of EH into EC. Similar results were obtained by previous studies which reported significantly increased expression of VEGF, with progression from normal endometrium to simple endometrial hyperplasia without atypia to complex hyperplasia without atypia to complex hyperplasia with atypia [50, 51]. Moreover, high expression of VEGF correlates with higher grade and stage of endometrial cancer [52]. Studies in other cancers revealed similar results, as ovarian serous carcinoma [53], granulosa cell tumor [54], breast invasive ductal carcinoma [55], gastric adenocarcinoma [56, 57], colorectal adenocarcinoma [58], and prostatic carcinoma [59].

Summary and conclusion

In conclusion, our findings demonstrated that the expression of CD117, CD34, and VEGF were significantly increased when progressing from EH to EC, indicating that acquisition of these proteins could be involved in progression from EH to EIN and then to EC.

Acknowledgements

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The study was approved by The Ethics Committee of Suez Canal University (Research approval number #357). Informed consent was
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obtained from patients. Information of each patient was anonymized prior to analysis.

Disclosure of conflict of interest

None.

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