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Original Article

Immunohistochemical expression of cancer stem cell related markers CD44 and CD133 in endometrial cancer

Samah S. Elbasateeny[a,*], Amira A. Salem[a], Walid A. Abdelsalam[b], Reham A. Salem[c]

[a] Pathology Department, Faculty of Medicine, Zagazig University, Egypt
[b] Gynecology and Obstetrics Department, Faculty of Medicine, Zagazig University, Egypt
[c] Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University, Egypt

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ABSTRACT

The goal of this study was to detect the presence of cancer stem cell markers CD44 and CD133 in immunohistochemically stained samples of endometrial cancer and correlate their expression with clinicopathological variables to identify the impact of CD44 or CD133 expression on tumor behavior and endometrial carcinogenesis. Marker expression was analyzed in 62 endometrial cancer samples (57 endometrioid carcinoma and 5 carcinosarcoma) and 15 proliferative endometrium samples. We detected CD133 and CD44 expression in 87.09% and 79.03% respectively of the studied endometrial cancers, and the expression was significantly different from the normal group. CD44 expression decreased with myometrial invasive depth and lymph-vascular space invasion (LVSI), and these inverse relationships were significant ($p = 0.034$, $p = 0.019$, respectively). CD133 was more expressed by early stage tumor (FIGO I-II) compared with those having FIGO III to IV stage disease ($p = 0.021$). The most notable conclusion of the present study is that CD44 and CD133 might participate in early-stage endometrial cancer carcinogenesis, and their overexpression may facilitate the early diagnosis of endometrial cancers. Analysis of our results supports the hypothesis that CD44 expression tends to decrease as the disease becomes invasive and progressive. So, we concluded that CD44 down-regulation might warn of a more aggressive course and may have a link with poorly prognosis carcinosarcomas. Further examination of the expression and function of CD44 and CD133 with a greater number of carcinosarcomas is warranted.

1. Introduction

Endometrial carcinoma is the most common invasive neoplasm in the female genital tract. Based on clinicopathological and molecular genetic features, it can be divided into two major groups referred to as type I and type II. The endometrioid subtype, which is the prototype of type I carcinoma, is associated with unopposed estrogenic stimulation, as well as endometrial hyperplasia [1]. Carcinosarcoma (malignant-mixed Müllerian tumor) is a biphasic neoplasm composed of distinctive and separate, but admixed, carcinomatous and sarcomatous elements. Recent immunohistochemical and molecular genetic studies support the hypothesis that both tumor components (epithelial- and mesenchymal-like elements) have a common clonal origin. Accordingly carcinosarcomas are currently thought to be "undifferentiated" or "metaplastic" carcinomas rather than uterine sarcomas [2]. Prognosis of endometrial carcinoma is dependent on some well accepted clinical and pathological parameters, including the histological type and grade of the tumor, the depth and pattern of myometrial invasion, the degree of disease extension beyond the uterine corpus, adnexal involvement and pelvic and para-aortic lymph node metastasis [1]. The risk of endometrial cancer recurrence has been well characterized and ranges from 7.7% to 63.3%, depending on the presence or absence of specific prognostic factors [3]. For these reasons, novel targeted therapies are currently being developed with the aim to achieve greater specificity for a selected population of cancer cells [4]. It has recently been reported that a small subpopulation of malignant tumors has a high proliferative potential and contributes to tumorigenesis, proposing the concept of cancer stem cells (CSCs) [5]. Cancer stem cells can be defined as a population of undifferentiated tumorigenic cells responsible for tumor initiation, maintenance, and spreading [6]. In accordance with the paradigm already established for hematopoietic stem cells CSC display unlimited proliferation potential, ability to self-renew, and capacity to generate a progeny of differentiated cells that constitute the major

* Corresponding author at: Pathology Department, Faculty of Medicine, Zagazig University, Egypt.
E-mail address: samah.elbasateeny@yahoo.com (S.S. Elbasateeny).

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tumor population [7]. The existence of CSCs was first reported in acute myeloid leukemia (AML) [8]. Thereafter, emerging evidence of the existence of CSCs has been demonstrated in a variety of solid tumors, including brain [9], breast [10], colon, prostate, ovary [11] and endometrium [12]. Many of these studies have used the surface expression of CD133, a 5-transmembrane glycoprotein, as a marker for the isolation of CSCs [13]. CD133 (human prominin-1) is a membrane glycoprotein with a putative function in plasma membrane organization. Current evidence suggests that CD133 is a reliable marker for the isolation of the endometrial CSC population [11]. In endometrial cancer CD133+ cells showed a higher proliferative capacity in vitro than the CD133− subset. Moreover, this population exhibited clonogenic growth activity in soft agar studies, forming colonies from a single cell, whereas the CD133− failed to do it. Interestingly, CD133 could be useful as a prognostic factor in endometrial carcinoma [14]. Besides CD133, there are other interesting proteins that may be expressed in CSC of solid tumors, such as endometrial carcinoma. Among these candidate markers is CD44 [14]. CD44 is a separate family of cell adhesion molecules that includes the standard (CD44s) and variant isoforms, which are products of alternative splicing [15,16]. CD44 has multiple biological functions, initially implicated in the process of invasion and metastasis, but now recognized as a marker for tumor initiating cells (CSCs) in different tissues. Interestingly, CD44 is expressed in the spheres that are generated from endometrial cancer cell lines [17].

The goal of this study was to detect the presence of cancer stem cell markers CD44 and CD133 in immunohistochemically stained samples of endometrial cancers and correlate their expression with clinicopathological variables to identify the impact of CD44 or CD133 expression on tumor behavior and endometrial carcinogenesis.

2. Material and methods

2.1. Patients and clinical data

A cross sectional study was conducted at the Departments of Pathology, Gynecology, and Oncology, Zagazig University Hospital, Egypt in the period from 2011 to 2014. Tissues were obtained from surgically resected specimens of 62 patients with endometrial cancer who underwent surgical staging. Pelvic lymphadenectomies were done only in 55 patients; the remaining 7 patients were disqualified from lymph node dissection due to poor status performance, advanced age, obesity, or other comorbidities. We obtained written informed consent from all patients for tumor tissue collection. Tumors were staged according to the 2010 FIGO (the International Federation of Gynecology and Obstetrics staging system). All tissue samples were formalin-fixed and paraffin-embedded. The corresponding hematoxylin–eosin slides were reviewed to determine the grade, the histotype, the depth of myometrial invasion (if present), and the presence or absence of lymphovascular invasion. 57 cases showed endometrioid histology, and only 5 cases were carcinosarcoma (metaplastic carcinoma). Clinicopathological parameters including the age, the macroscopic sizes, and the FIGO stage of all tumors were obtained from the patient's medical records. The control group consisted of 15 normal Proliferative phase endometrium tissue samples were obtained from patients who underwent hysterectomy for myoma uteri. Patients with endometrial hyperplasia, adenomyosis, or endometriosis were excluded.

2.2. Immunohistochemical staining

The sections (4–5 μm) obtained from representative tissue sample blocks were deparaffinised with xylene, rehydrated in graded alcohols, and placed in 0.5% hydrogen peroxide in methanol for 10 min to block endogeneous peroxidase activity. Antigen retrieval was carried out by incubation in 0.01 M citrate buffer (pH 6.0) for 5 min in a pressure cooker. The sections were exposed to the primary antibody for 60 min at room temperature. The standard strept avidin-biotin-peroxidase complex method was used for CD44 (mouse monoclonal antibody, Clone 156-3C11, catalog no. MS-668-P0Lab Vision, California, USA) and CD133 (mouse monoclonal antibody, clone 171-191, catalog no. 144305, Lab Vision, California, USA) by employing diaminobenzidine (DAB) as the chromogen. Human tonsil and renal tubular epithelial cells were used as a positive control for CD44 and CD133 respectively, while negative controls for both markers were obtained by omitting the primary antibody.

2.3. Evaluation of immunohistochemical staining

We calculated the "immunohistochemical score" (IHS) of CD44 and CD133 for each case. The scoring system used for both CD44 and CD133 was similar to previously published methods [18–20]. The extent of positively stained epithelial cells was estimated and classified on a four-point scale as follows: no staining = 0%, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, and 4 = 51–100%. The intensity of the immunoexpression was categorized into three groups: weak

Table 1
Clinicopathological characteristics of the studied 62 endometrial cancer patients.

Variable	*n*
Age at surgery (y)	
Mean ± SD	57.35 ± 7.799
Range	37–68
Mass size	
≤4	38
>4	24
Histopathology	
Endometrioid adenocarcinoma	57 (91.93%)
Carcinosarcoma	5 (8.06%)
Grading	
G1	27 (43.5%)
G2	21 (33.9%)
G3	14 (22.6%)
Depth of myometrial invasion	
No	6 (9.7%)
Superficial (<50%)	26 (41.9%)
Deep (≥50%)	30 (48.4%)
Involvement of the cervix	
No	36 (58.1%)
Yes	26 (41.9)
Adnexal/serosa involvement	
No	47 (75.8%)
Yes	15 (24.2%)
Distant metastases (lymph nodes not included)	
No	57 (91.9%)
Yes	5 (8.06%)
Lymph node metastases (in 55 lymphadenectomies)	
Negative	42 (76.4%)
Positive	13 (23.6%)
LVSI	
Negative	48 (77.4%)
Positive	14 (22.6%)
FIGO stage	
I	36 (58.1%)
II	6 (9.8%)
III	15 (24.2%)
IV	5 (8.1%)
Total cases	62 (100%)

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