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Tumor budding and E-Cadherin expression in endometrial carcinoma: Are they prognostic factors in endometrial cancer?

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ABSTRACT

Objective. To evaluate the prognostic value of tumor budding (TB) in endometrioid (EEC) and non-endometrioid endometrial cancers (NEEC) and to determine its correlation with expression of E-cadherin.

Methods. Ninety-five patients with primary endometrial carcinoma were examined statistically. All patients were diagnosed, treated, and given follow-up care at Dokuz Eylul University Faculty of Medicine. Tumor budding detected by either H&E-stained sections and anticytokeratin-staining C11. The tissue block with the largest invasive front was chosen for budding counting and immunostaining. E-cadherin expression was examined by immunohistochemistry using the primary antibodies against to it.

Results. Tumor budding was low-grade in 73 and high-grade in 22 cases. E-cadherin expression loss was identified in 48 patients. The high-grade TB was significantly higher in patients with advanced stage and deep myometrial invasion ($p = 0.032$ and 0.018, respectively). E-Cadherin expression was significantly lower in NEECs than EECs ($p = 0.032$). The negative expression of E-cadherin was associated with advanced stage and poor differentiation ($p = 0.001$ and $p = 0.024$, respectively). We determined that tumor budding adversely correlated with the presence of E-cadherin expression but not statistically significant. Based on the results of multivariate analysis, TB has an independent impact on cumulative overall survival. We found no statistically significant difference between E-cadherin expression and survival.

Conclusions. TB is associated with undifferentiated tumor, advanced stage and decreased postoperative survival in endometrial cancer. It might be a valuable prognostic clinicopathologic factor which can be applicable in routine examination.

Introduction

Endometrial cancer is the most common malignancy of the female reproductive tract in developed countries and overall survival is relatively higher compared with other gynecologic malignancies [1]. It can be classified into two major clinicopathologic types; endometrioid carcinomas (EECs) and non-endometrioid endometrial carcinomas (NEECs), including papillary, serous or clear cell histology. The patients with NEECs have a worse prognosis due to increased tendency of these tumors to extend out of uterus at the time of diagnosis. In contrast, the majority of endometrioid endometrial carcinomas (EECs) are confined to the uterus and their prognosis are usually well [2].

Tumor budding is a histopathological feature that can be easily identified by using routine pathologic examination, which is observed in the front of invasive margin of the tumor. It is described as an isolated single cancer cell or microscopic small cell clusters composed of <5 cells found outside the invasive margin of a tumor [3]. It has been proposed as one of potential prognostic biological variables in various cancers. In previous studies, it has been determined that this pathologic entity is related to lymph node status, local recurrence and poor prognosis, particularly in colorectal carcinomas, as well as anal, lung and esophageal carcinomas [4–7]. However, there was no established precise standard criteria for the assessment of tumor budding. While some researchers have classified the tumor budding as none, mild, moderate and severe, the other ones as according to presence or absence, or marked and non-marked [7–9]. This entity appears to be associated with two main events in invasion and metastasis of tumor; abnormal cell differentiation and loss of cell–cell adhesion [10]. Cell to cell adhesion is mainly mediated by cadherins which are transmembrane glycoproteins that join adjacent epithelial cells using a calcium-dependent binding mechanism [11]. Also it has been found that the loss of cell to cell adhesion is associated with decreased E-cadherin expressions in epithelial tumor cells [12]. Although, there are different molecular alterations between two types of endometrial cancer, more aggressive behaviors of NEECs might be due to decreased intercellular cohesiveness in these tumors. Many studies showed that decreased expression of cadherin facilitated

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tumor invasion and metastasis in various tumors such as breast, ovarian and endometrial cancers and it also correlated with known adverse prognostic factors and lower overall survival in both types of endometrial cancer [12–15].

Although budding has been suggested as a useful prognostic marker in some tumors, to our knowledge, tumor budding in endometrial carcinoma has not been investigated. Therefore, the objective of the current study was to evaluate the prognostic value of tumor budding in EECs and NEECs and to determine its correlation with expression of E-cadherin as a well-known prognostic marker.

Materials and methods

Patients and specimens

A retrospective review of all cases of endometrial cancer presenting to Department of Gynecology and Obstetrics at the Dokuz Eylul University Hospital from January 1999 through December 2009 was carried out. Patients included in the study had undergone complete surgical staging with hysterectomy, bilateral salphingo-oophorectomy, cytology and lymph node dissection. The records of the 112 patients who underwent surgery in our institution were reviewed. Of these 112 patients, 5 had incomplete surgical staging and pathologic information for review and of the remaining 107 follow up care was documented in 98 patients post operatively. The precise tumor budding and E-cadherin was determined in 95 cases because appropriate sections were not taken. Those 95 cases formed the basis of our analysis.

Data were collected by the review of existing patient medical records. These included: patient demographics, stage, grade, histological type of cancer and follow up data on recurrence. The tumors were classified according to histological typing of female genital tract by World Health Organization, staged and graded according to the International Federation of Gynecology and Obstetrics (FIGO) systems [16,17]. 73 cases were classified as FIGO stage 1, 6 cases as stage 2 and 16 cases as stage 3. All patients were followed up until death or a median of 49 months (ranging 19 to 127 months). None of the patients had received preoperative chemotherapy, radiotherapy or hormone therapy. Standard of care follow up and treatment were completed with adjuvant radiation and chemotherapy based on prognostic evaluation. The patients with incomplete surgical staging, no pathologic information for review and no documented postoperative one year follow up were excluded.

This study was approved by the institutional review board at Dokuz Eylul University Faculty of Medicine.

Histopathological evaluation

All specimens were re-examined by senior pathologist (M.K.), who was unaware of the clinical outcomes, to confirm the diagnosis. The tissue block containing the deepest portion of the tumor penetration was selected and the invasive front which tumor budding was the most intensive was chosen for budding counting and immunostaining. Standard hematoxylin and eosin (H&E)-stained sections of representative formalin-fixed, paraffin-embedded tumor tissues were reviewed to confirm the histopathologic diagnosis and to determine presence or degree of budding by using a $\times 20$ objective lens by two investigators (M.K. and M.A.) reaching a consensus [6]. Budding was accepted as positive in the presence of small clusters (<5 cells and ≥ 1 cells) of dedifferentiated tumor cells at the invasive margin at any of the sections. After selecting the highest budding intensity section of the whole lesion, budding was classified as low-grade and high-grade according to "<5 budding foci/field" and "≥5 budding foci/field" respectively, as described previously by Roh et al. [6]. Tumor budding detected by either H&E-stained sections and anticytokeratin-staining C11 (S1 A, B,C, D). CD 34 was performed to exclude tumor emboli. Three months after the completion of the study, tumor budding was re-estimated by the senior pathologist (M.K.), intraobserver agreement was measured by the κ coefficient. Agreement was described as "moderate" for κ values of 0.41–0.60, "substantial" for values of 0.61–0.80 and "almost perfect" for values of 0.81–1.0 [18].

Immunohistochemistry

From the representative paraffin embedded section, 4 μm sections were taken onto poly-L lysin-coated slides from each representative archival paraffin-embedded tumor tissue for immunohistochemical staining. The standard streptavidin biotin immunoperoxidase method was performed using the primary antibodies against E-cadherin (1/50 dilution, Clone NCH-38, Dako, Denmark), Anticytokeratin, C11 (1:00 dilution ,MonoclonalMouse Anti-human, Biogenex,USA), CD34 monoclonal antibody (1:200 dilution; AB-1 QBEnd/10, Thermo,neomarkers, USA). Briefly, sections were deparaffinized in xylene, rehydrated in alcohol series, and immersed in distillated water. Endogenous peroxidase activity was blocked using a 0.3% solution of hydrogen peroxide in phosphate-buffered saline (0.01 mmol/L, pH 7.5) at room temperature for 15 min and rinsed with TRIS buffer. After antigen retrieval by heating in 10 mmol/L citrate buffer (pH 6.0) for 30 min, primary antibodies were applied for 60 min at room temperature and washed in TRIS buffer. Biotinylated secondary antibodies and streptavidin-peroxidase complex were added consecutively for 10 min at room temperature and washed in TRIS buffer. Peroxidase activity was visualized with 0.03% 3-3′ diaminobenzidine tetrahydrochloride applied for 7 min. After rinsing in deionized water and counterstaining in Mayer hematoxylin, the slides were dehydrated and mounted. Appropriate tissue sections as positive controls for each primary antibody were also stained simultaneously. As a negative control, sections were processed in the absence of primary antibody.

Assessment of the immunohistochemically stained slides was performed jointly by two pathologists (M.K. and M.A.) using a double objective microscope. A minimum of 25 fields were examined at $\times$ 10 magnification for each specimen by two pathologists. The results of immunohistochemistry were classified and analyzed based on the percentage of E-cadherin positive tumor cells according to criteria used in the previous study [12]. The different positive staining patterns for E-cadherin were identified: diffuse linear, when crisp membrane staining was seen in more than 75% of tumor cells in the absence of cytoplasmic staining; diffuse granular, when membrane and cytoplasmic staining was seen in 26–100% of the tumor cells; and focal granular, when membrane and cytoplasmic staining was seen in 5–25% of the tumor cells. Staining was considered negative when less than 4% of the tumor cells were positive, irrespective of pattern [12]. Then, the cases were grouped as negative and positive for statistical analysis. Benign endometrial and cervical epithelium invariably stained with the diffuse linear pattern. Tumor samples expressing E-cadherin in the diffuse linear pattern were considered to have a normal pattern of expression. The level of E-Cadherin staining in the remaining tumor samples decreased from diffuse granular to focal granular to negative. Structural localization, not the intensity of the reaction, was taken into consideration in the scoring system.

Statistical analysis

Budding was analyzed by two methods. First, budding was accepted as positive in the presence of budding in any proportion. Then, cases were grouped according to the presence of budding as low-grade and high-grade. Statistical analysis was conducted by Scientific Package for Social Sciences (SPSS, 11). The comparison of the groups and the relationship between E-cadherin expression immunohistochemical scores and tumor stage, histologic, nuclear grade were investigated by using non- parametric tests, such as a Chi-square test