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Tumor-associated macrophages correlate with vascular space invasion and myometrial invasion in endometrial carcinoma

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Abstract

Objective. This study was conducted to determine whether tumor-associated macrophages (TAMs) correlate with clinicopathological features in endometrioid adenocarcinoma.

Methods. 76 cases of endometrioid adenocarcinoma treated initially by hysterectomy with pelvic lymphadenectomy were retrospectively retrieved, and their histological features were evaluated. Immunohistochemical staining for CD68, CD34, and Ki-67 was performed on paraffin-embedded sections. TAMs were counted in two areas: in the invasive margin (margin TAMs) and in the tumor (intratumor TAMs).

Results. Margin TAMs were significantly associated with FIGO stage ($P=0.033$), histological grade ($P=0.008$), myometrial invasion ($P=0.0001$), pelvic lymph node metastasis ($P=0.027$), and vascular space invasion ($P=0.0001$). Intratumor TAMs were significantly associated with intratumor Ki-67 ($P=0.006$) and microvessel density ($P=0.020$). Patients with high margin TAMs (≥ 20) had significantly worse progression-free survival (PS) and overall survival (OS) than those with low margin TAMs (<20) (log rank test, $P=0.0031$ and $P=0.0085$, respectively). On multivariate analysis, high margin TAMs were significantly associated with vascular space invasion ($P=0.013$; HR, 6.05; 95% confidence interval [CI], 1.468–24.938) and myometrial invasion ($P=0.041$; HR, 4.03; 95% CI, 1.06–14.71). Vascular space invasion was only associated with PFS.

Conclusion. Although on univariate analysis TAMs are associated with other poor prognosticators, on a multivariate analysis, TAMs appear only to be associated with MI and VI. TAMs may play a significant role in the biology of tumor progression of endometrial adenocarcinoma, but do not appear to be independent prognostic indicators of patient's survival.

Keywords: Endometrioid adenocarcinoma; Tumor-associated macrophages; CD68; Immunohistochemistry; Vascular space invasion; Survival

Introduction

Invasion into surrounding tissues and intravasation into blood/lymphatic vessels are the most fundamental steps in tumor progression and metastasis, as well as the cause of death in patients with carcinoma [1]. The invasive ability of carcinoma is thought to be affected by several invasive molecules, which are produced not only by carcinoma cells but also by carcinoma stroma cells, such as fibroblasts and hematopoietic cells, including lymphocytes, dendritic cells, NK cells, and macrophages.

It has recently been reported that tumor-associated macrophages (TAMs) are associated with tumor angiogenesis and have prognostic significance in several malignant tumors, such as breast carcinoma [2,3], cervical carcinoma [4], follicular lymphoma [5], prostate carcinoma [6], renal carcinoma [7] and esophageal carcinoma [8].

On the basis of their activation state, macrophages can be distinguished as Type I and Type II cells [9–11]; differences in receptor expression, cytokine production, and functions define these two types of macrophages [10]. Type I macrophages (M1) are classified as cells capable of producing large amounts of

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pro-inflammatory cytokines and expressing high levels of MHC molecules; they are implicated in the killing of pathogens and tumor cells [10,11]. In contrast, Type II macrophages (M2) are classified as cells capable of moderating the inflammatory response, eliminating cell waste, and promoting angiogenesis and tissue remodeling [10,11]. The macrophages present in malignant tumors are referred to as TAMs, and they mainly belong to the M2 type [10]. TAMs release a number of potent proangiogenic cytokines, growth factors, and invasive factors. Through these factors, TAMs promote tumor invasion, growth, metastasis, and angiogenesis. TAMs are often found at the invasive front of advanced tumors; this suggests that tumors exploit the normal matrix remodeling capacities of macrophages, which enable them to invade surrounding tissues.

In endometrial carcinoma, several reports have demonstrated that TAMs play an important role in the promotion of angiogenesis [12–15] and are correlated with worse prognosis [12–14]. However, few reports have described the role that TAMs located in the tumor invasive margin have with respect to tumor invasion and vascular space invasion (VI).

The present study focused on the localization of TAMs, and the distribution of TAMs was classified into two areas (intratumor and invasive margin); the correlation of the localization of TAMs with clinicopathological factors and outcome was evaluated. The presence of TAMs in the invasive margin was significantly correlated with not only myometrial invasion (MI) but also with VI in endometrioid adenocarcinoma.

Materials and methods

Patients

The records of 84 patients with endometrial carcinoma who underwent primary surgery at Fukushima Medical University Hospital between January 1995 and December 2000 were reviewed retrospectively. Patients with non-endometrioid carcinoma (clear cell carcinoma and serous adenocarcinoma) and those who had received preoperative chemotherapy were excluded; thus, 76 cases of endometrioid adenocarcinoma that had undergone surgical resection were analyzed. All patients underwent abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. Paraaortic lymphadenectomy was performed in 8 patients due to the presence of enlarged lymph nodes. Patients who had certain pathological risk factors (deep myometrial invasion, cervical involvement, positive peritoneal cytology, and lymph node metastasis) were treated with 3 to 5 cycles of a cisplatin-based chemotherapeutic regimen after the initial surgery (39/76, 51.3%). The histological grade, stage, and subtype of the tumor were re-evaluated according to the International Federation of Obstetrics and Gynecology (FIGO) classification by two of the authors (SS and NN) [16]. We had estimated VI by the presence of tumor cells in vascular channels with lining endothelial cell using both of the hematoxylin eosin stain and elastica masson staining. Immunohistochemistry with CD34 was also performed for detecting VI. The follow-up period for the 76 patients ranged from 13 to 130 months (mean 82 months) after surgery.

Tissue processing and immunohistochemistry

For immunohistochemistry, representative tumor areas with the deepest myometrial invasion were selected. In cases without myometrial invasion, one slide showing a representative tumor was selected. VI was recorded if tumor nests were located within vascular spaces lined by endothelium.

Monoclonal mouse anti-human CD68 antibody (M 0814; DAKO A/S, Glostrup, Denmark; 1:100 dilution), CD34 antibody (413361; Nichirei, Tokyo, Japan; 1:50 dilution), and Ki-67 antibody (M 7240; DAKO A/S; 1:300) were used.

Four-micrometer-thick sections of formalin-fixed paraffin-embedded tissue samples taken from selected areas were cut with a microtome and dried at 37 °C on a silanized-slide (Mathunami; CA, Osaka, Japan). Samples were deparaffinized in xylene at room temperature for about 20 min and washed with a graded ethanol/water mixture and then with distilled water. The endogenous peroxidase in the sections was then inactivated in 1% H_2O_2 for 10 min. Samples for CD34 and Ki-67 were heated in citrate buffer (0.01 M, pH 6.0) in a 700 W microwave oven for 15 min for antigen retrieval. Samples for CD68 were subjected to enzymatic (Proteinase K) epitope retrieval for 5 min. Slides were treated with normal rabbit serum for 10 min to block non-specific binding. The section was then reacted with monoclonal antibodies at 4 °C for 14 h. The streptavidin–biotin peroxidase complex (SABC) method was used for the immunohistochemical steps. The immunoreaction was visualized with the staining medium for peroxidase containing 0.05% 3,3′-diaminobenzidine tetrachloride. For the negative controls, non-immunized mouse immunoglobulin-G was substained for primary antibody at the same concentration.

Evaluation of immunohistochemistry

TAMs

Using immunohistochemistry with CD68, we determined the number of $CD68^+$ cells that had infiltrated into cancer nests or stroma (intratumor TAMs) and the number that had become distributed along the tumor-myometrial junction (margin TAMs). To count the TAMs, each section was scanned at low (×40 and ×100) magnifications, and three representative areas were identified. Necrotic areas were excluded when calculating the number of intratumor TAMs. The TAMs were counted at ×200 magnification, and the average of the values of the three representative areas was used for statistical analysis.

Ki-67 labeling index

The sections were examined at low (×40 and ×100) magnifications to identify the area with the most intense staining, basically according to the method of the Weidner et al. [17]. Based on the number of cancer cells with nuclei stained

Table 1
Clinicopathological characteristics of the patients with endometrioid adenocarcinoma

Variable	Number of patients (%)
FIGO stage	
Ia	11(14.5)
Ib	26(34.2)
Ic	9(11.8)
IIa	6(7.9)
IIb	7(9.2)
IIIa	10(13.2)
IIIb	0
IIIc	7(9.2)
FIGO stage	
G1	55(72.4)
G2	14(18.4)
G3	7(9.2)
Myometrial invasion	
Absent	13(17.1)
<1/2	36(47.4)
≥1/2	27(35.5)
Pelvic lymph node metastasis	
Absent	69(90.8)
Present	7(9.2)
Vascular invasion	
Absent	52(68.4)
Present	24(31.6)

FIGO: Federation of Obstetrics and Gynecology.