RESEARCH COMMUNICATION

Correlation of Mast Cell Density, Tumor Angiogenesis, and Clinical Outcomes in Patients with Endometrioid Endometrial Cancer

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Abstract

Background: Tumor angiogenesis has been demonstrated in several kinds of neoplasms. There is evidence that mast cells can produce many different chemical mediators with angiogenic properties. Since their specific role in female genital tract cancer has not been well understood, this study was conducted to determine correlations between among mast cell density, tumor angiogenesis, and clinical outcomes in patients with endometrioid adenocarcinoma of endometrium. Methods: Histologically, four-micrometer-thick haematoxylin and eosin stained slides of hysterectomy specimens were evaluated. Microvessels were highlighted by CD31 immunostaining and mast cells were stained with 0.1% toluidine blue. All clinicopathological characteristics were reviewed to determine their possible correlation to microvessel density and number of mast cells. Results: A total of 46 patients who underwent a complete staging surgery were eligible for this study. The median age was 55 years (range, 32-70 years) and the median follow-up was 27.0 months (range 3.6-83.8). Microvessels appeared to correlate to some extent with parity and the mean count was likely to be higher in women with non-menopausal status (p=0.07), advanced FIGO stage (p=0.09), and lymph node metastasis (p=0.08). However, there was no significant correlation between microvessel counts, mast cell density, and disease recurrence. Conclusion: Our data suggest that the number of microvessel counts and mast cell density do not affect clinical progression or recurrence of endometrioid endometrial cancer.

Keywords: Mast cell density - microvessel count - tumor angiogenesis - endometrial cancer

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Introduction

Endometrial cancer is one of the most common gynecologic malignancies and is a significant cancer-related cause of death in women worldwide. The most common histopathology is endometrioid adenocarcinoma (Bokhman, 1983). A number of surgical and pathological parameters, including histological type, histological grade, stage, depth of myometrial invasion, vascular invasion, and cervical involvement have been found to have prognostic value in endometrial cancer (Prat J, 2004). Although surgery is the cornerstone of treatment, adjuvant radiotherapy is indicated to patients who have high risk for local relapse of disease. Approximately 10-20% of early-staged patients still have disease recurrence and finally die from endometrial cancer (Creutzberg et al., 2000). Therefore, other prognostic determinants need to be defined to provide more accurate information for patients who are at risk of disease recurrence or progression. The proliferation of new vessels around and within a tumor (tumor angiogenesis) is essential for the growth, invasion, and metastasis of solid tumors by providing nutrients, oxygen, and vascular route for hematogenous spread of cancer cells to distant sites. In addition, tumor cells are stimulated in a microenvironment of paracrine fashion by surface adhesion molecule, cytokines, and several growth factors produced by capillary endothelial cells: fibroblast growth factors, heparin-binding epithelial growth factor, granulocyte colony-stimulating factor, platelet-derived growth factor, and insulin-like growth factor I (Folkman, 1990; Folkman, 1995). Tumor cells and host immune cells such as tumor-infiltrating lymphocytes, macrophages and mast cells, cooperate with endothelial cells in promoting angiogenesis. Mast cells can produce many kinds of chemical mediators with angiogenic properties and to regulate endothelial cell proliferation and function. Stem cell factor, vascular endothelial growth factor, epidermal growth factor, basic fibroblast growth factor, and platelet-derived growth factor induce chemotactic migration of mast cells to sites of neovascularization. Additionally, mast cell products such as tryptase also degrade connective tissue matrix to

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provide space for neovascular sprouts. Accumulation of mast cells can be found in many angiogenesis-dependent conditions, including tumor growth, rheumatoid arthritis, ovulation, wound healing, and tissue repair (Park, 2000; Ribatti et al., 2001; Hiromatsu and Toda, 2003; Ribatti et al., 2004). However, the significance of mast cell density in pathogenesis of endometrial cancer still has been less well defined. The purpose of this study was to determine the correlation between the mast cell density, tumor angiogenesis, clinico-pathological characteristics, and recurrence of disease in patients with endometrioid adenocarcinoma of endometrium.

Materials and Methods

The histology and medical records of the patients with first diagnosis of endometrial carcinoma at the Department of Obstetrics and Gynecology, Chiang Mai University Hospital between 1999 and 2004 were retrieved for the study after Research Ethics Committee approval. Eligible patients included: (a) patients who underwent a complete staging surgery (total abdominal hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing or ascites fluid collection, bilateral pelvic lymphadenectomy and paraaortic lymph node biopsy) according to the 1988 FIGO staging classification. (b) patients with pure endometrioid adenocarcinoma on endometrial histology. (c) patients with available paraffin-embedded tumor samples for histology review. All documents summarizing clinical information, pathological characteristics, and follow-up records of 46 eligible patients were reviewed. Recurrences were classified as local if they were detected in pelvis, or vagina and as distant if they were detected in extrapelvic locations.

Histologically, four-micrometer-thick haematoxylin and eosin stained slides of the hysterectomy specimens were evaluated for tumor grade and stage, according to FIGO classification. Microvessels were highlighted by CD31 (1/300 dilution; Dako) immunostain, using standard indirect streptavidin-biotin technique. Mast cells were stained by 0.1% toluidine blue in distilled water. After microscopy scanning, four areas of highest density were photographed under a Nikon Eclipse E600W microscope (Nikon, Melville, NY) equipped with a digital still Nikon camera, and DXM1200F software. The number of microvessels were counted by computerized system. The final digitized image was analyzed and the highest density of microvessels and mast cells per 1mm^2 were present respectively.

Statistical analysis

Microvessel and mast cell counts were compared for the significance of changes within each clinicopathological parameters by means of Mann-Whitney U test or Kruskal-Wallis test (when appropriate). Correlations between counts were assessed with Pearson's (r) coefficient. SPSS 11.5 for Windows was used for the analyses. P value <0.05 was considered statistically significant.

Table 1. Tissue Density of Microvessels and Mast Cells Compared with Clinicopathological Characteristics

Variables	No.	Microvessel count (per 1 mm^2)		Mast cell count (per 1 mm^2)	
		Mean ± S.D.	p	Mean ± S.D.	p
Age (years)			0.37		0.84
≤ 50	16	37.06 ± 13.17		12.37 ± 12.66	
> 50	30	32.80 ± 11.71		11.20 ± 9.66	
Menopause			0.07		0.94
Yes	30	31.60 ± 10.74		11.27 ± 10.01	
No	16	39.31 ± 13.68		12.25 ± 12.14	
Parity			0.02		0.06
≤ 2	23	30.13 ± 11.18		10.17 ± 12.66	
> 2	23	38.43 ± 12.11		13.04 ± 8.28	
Body mass index (kg/m^2)			0.95		0.97
< 25	20	34.10 ± 12.44		11.85 ± 12.04	
≥ 25	10	34.20 ± 12.89		11.80 ± 11.45	
FIGO stage (1988)			0.09		0.12
I-II	25	31.72 ± 11.81		9.56 ± 10.04	
III-IV	21	37.33 ± 12.38		14.05 ± 11.13	
Tumor grade			0.64		0.63
I	21	35.62 ± 12.06		10.90 ± 10.89	
II	16	32.88 ± 13.36		12.31 ± 9.92	
III	8	33.88 ± 12.56		10.25 ± 12.17	
Depth of myometrial invasion			0.48		0.66
≤ 1/2	23	33.17 ± 11.91		10.65 ± 9.16	
> 1/2	23	35.39 ± 12.78		12.57 ± 12.13	
Cervical invasion			0.53		0.83
Absent	26	34.23 ± 14.08		11.85 ± 11.12	
Prersent	20	34.35 ± 9.78		11.30 ± 10.33	
Lymph node metastasis			0.08		0.75
Absent	33	32.55 ± 12.27		10.79 ± 9.27	
Prersent	13	38.69 ± 11.54		13.69 ± 13.83	
Lymph-vascularspace invasion			0.53		0.22
Absent	23	32.74 ± 11.60		9.43 ± 9.17	
Prersent	23	35.83 ± 12.97		13.78 ± 11.79	
Recurrence			0.37		0.91
Absent	35	35.37 ± 12.42		11.06 ± 9.32	
Present	8	31.63 ± 12.60		13.13 ± 15.23	

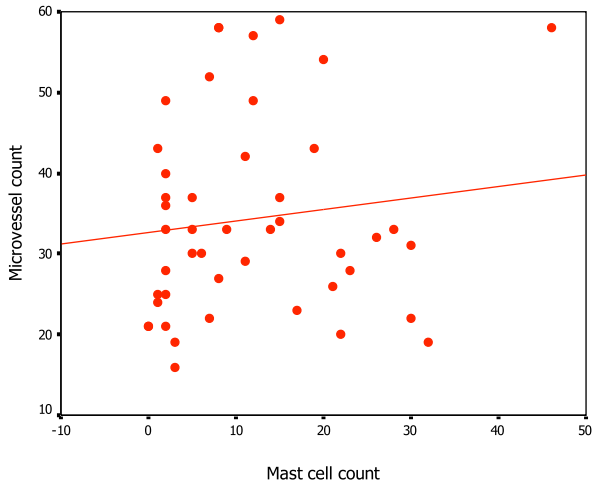

Figure 1. The Pearson's (r) Correlation Coefficient Showing a Non-Significant Correlation between Mast Cell and Microvessel Counts (r=0.124; p=0.41)

Results

The median age of the patients was 55 years (range, 32-70 years). The mean body mass index (BMI) was 23.38±5.68kg/m^2. Among these patients, 65% were in menopausal status and 65% were aged beyond 50 years. Tumor stage and grade of the 46 patients were as follows: 25 (54%) were stage I-II and 21 (46%) were stage III-IV; 21 (46%) were grade I, 17 (37%) were grade II, and 8 (17%) were grade III. Deep myometrial invasion (>1/2) and cervical invasion were present in 23 (50%) and 20 (43%) out of 46 patients, respectively. The presence of lymph-vascular space invasion and lymph node metastasis were found in 23 (50%) and 13 (28%) of all 46 patients, respectively. The median follow-up was 27.0 months (range 3.6-83.8). Three patients were lost to follow-up after completion of their treatment. Of the 43 patients, 2 (5%) had local recurrence of disease, 6 (14%) had distant metatasis, and 1 (2%) had both local and distant metastasis. The median time to recurrence was 14.1 months (range 0-29.3). The clinicopathological characteristics of the patients and tissue density of microvessels and mast cells were described in Table 1. There were no significant differences of microvessel counts and mast cell counts among the following factors: age, menopausal status, BMI, FIGO stage, tumor grade, depth of myometrial invasion, cervical invasion, lymph node metastasis, lymph-vascular space invasion, and the recurrence of disease. Microvessel counts were only found significantly correlated with the number of parity. The mean microvessel count was higher in women with parity more than 2 when comparing with those who had less parity (38.4±12.1 vs. 30.1±11.2; p=0.02). Our data demonstrate that the mean microvessel count was higher, but not statistically significant, in women with non-menopausal status (p=0.07), advanced FIGO stage (p=0.09), and lymph node metastasis (p=0.08). The mean mast cell count was likely to be higher in women with parity more than 2 (13.0±8.3 vs. 10.2±12.7; p=0.06). Correlations between microvessel counts and mast cell counts assessed with Pearson's (r) coefficient did not demonstrate statistical significance (Figure 1).

Discussion

The role of angiogenesis, as an indicator of the aggressiveness of the tumor, has been demonstrated in several kinds of neoplasms such as cancer of the breast (Weidner et al., 1991; Bosari et al., 1992), prostate (Weidner et al., 1993), lung (Macchiarini et al., 1992), skin (Srivastava et al., 1988), larynx (Beatrice et al., 1998), colon and rectum (Acikalin et al., 2005), oral cavity (Williams et al., 1994), and bladder (Bochner et al., 1995). Most tumors become symptomatic and clinically detectable only after neovascularization. It should be emphasized, however, that the switch to the angiogenic phenotype does not always result in a rapidly proliferating tumor. Certain tumors, such as adrenal adenoma, do not have growth that corresponds to their high rate of angiogenic activity (Folkman, 1995). Certain thin melanomas may actually regress at the time of neovascularization, possibly because of the inhibitory effect of endothelium-derived interleukin-6 (Rak et al., 1995). Moreover, in distant metastases angiogenesis may be suppressed by circulating inhibitors from a primary tumor and may become apparent only after its removal (O'Reilly et al., 1994; Folkman, 1995; Holmgren et al., 1995).

In endometrial lesions, the differences in microvessel and mast cell density between endometrial hyperplasia and endometrial adenocarcioma were identified and seemed to correlate with severity of disease and tumor progression (Ribatti et al., 2005). On the contrary, our study found no relationship between microvessel count and mast cell density in endometrioid adenocarcinoma of endometrium. However, the mean microvessel count was significantly higher in women with parity more than 2 compare with those who had less parity. This may be due to the effects of direct or indirect hormone stimulation during previous pregnancies. Furthermore, we found that the mean microvessel count was higher, but not statistically significant, in women with non-menopausal status (p=0.07) and the mean mast cell count was also higher in women with parity more than 2 (p=0.06). The increase in mast cell density has not been associated always with bad or good prognosis in human or mice (Ozdemir, 2006a). Mast cells can be found decreasing significantly in some endometrial lesions such as atypical hyperplasia, endometrial sarcoma, and carcinoma (D'Souza, 1994). Furthermore, there was no significant correlation between mast cell density and angiogenesis in some mice studies, and in human cancers and diseases (eg, lung cancer and systemic mast cell diseases) (Ozdemir, 2006b). Mast cells in the tumor progression is difficult to evaluate. The specific role of mast cells either in benign or malignant disease of female genital tract has not been well understood. Mori et al., (1997) found fewer mast cells in the outer half of the myometrium and the cervix, but the proportion of mast cells that contain both tryptase and chymase (TC-MCs) was substantially higher than those contain only tryptase (T-MCs) within these areas. In contrast, the endometrium contained significantly fewer mast cells, but proportionally more T-MCs. There was no change in the number of mast cells between the proliferative and secretory phases of the menstrual

cycle; however, there was a significantly lower number in all areas after menopause. The latter was similar to our findings. Furthermore, most of the mast cells were observed in close association with uterine smooth muscle cells, as well as in the vicinity of fibroblasts and collagen which may suggest that they play an important role in the reconstruction of uterine tissues during the menstrual cycle (Mori et al., 1997).

In conclusion, the data from our study suggested that there was no significant correlation between microvessel counts and mast cell density in endometrioid adenocarcinoma of the uterus. The number of microvessel counts and mast cell density did not affect the clinical progression or recurrence of disease. Although the mean microvessel count was significantly higher in women with parity more than 2, this may be due to the hormonal effect of previous pregnancies. However, we cannot make any definitive conclusions because of the limited number of cases in this study. Further control studies are needed to explore the role of mast cells and microvessel formation by comparison of those with normal, benign, and malignant endometrial lesions.

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