The Prognostic Significance Of Angiogenesis in Epithelia Ovarian Carcinoma

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Özet

Amaç: Epitelyal over karsinomunda angiogenesisin prognostik değerini araştırmak.
Materyal ve Yöntem: 1990-1999 yılları arasında primer epitelyal over karsinomu tanısı almış 47 hasta (evre I-IV) çalışmaya dahil edilmiştir. " borderline" over tümörleri ve primer peritoneal karsinomalar çalışmaya dahil edilmemiştir. Bütün hastalarda FIGO kriterlerine göre cerrahi evreleme ve evreye göre tümör "debulking" gerçekleştirilmiştir. İlk tümör örneklerinden 5µm kalınlığında slaytlar hazırlanarak endotelyal hücreleri yüksek spesifite ile işaretleyen faktör VIII ile ilişkili antijene karşı antikor kullanılarak immünohistokimyasal olarak boyanmıştır. Slaytların X40 mikroskop alanında taranması ve en fazla damarlanmanın bulunduğu "hot spot"un seçiminden sonra X200'lük büyütmede mikrodamarlar sayılmıştır. Sonuçlar yaş, evre, grade, lenf nodu tutulum durumu, tümörün tek taraflı veya bilateral olarak overleri tutmuş olma durumu, asit miktarı, tümör büyüklüğü, preoperatif CA 125 düzeyleri, rezidü tümör ve sağkalım süreleri gibi klinikopatolojik faktörler ile karşılaştırılmıştır.
Bulgular: MVD sayısı ile klinikopatolojik faktörler arasında herhangi bir korelasyon saptanmamıştır.
Sonuç: Meme, prostat ve küçük hücreli olmayan akciğer kanseri gibi solid organ tümörlerinden farklı olarak angiogenesis, over karsinomunda prognostik değer taşımamaktadır.

Anahtar kelimeler: over kanseri, angiogenesis, faktör VIII ile ilişkili antijen, prognoz

Summary

Objective: We aimed to evaluate angiogenesis as a prognostic indicator in primary epithelial ovarian carcinoma.
Material and Method: 47 patients diagnosed as primary ovarian carcinoma (stages I-IV) between 1990-1999 were included in the study. Borderline ovarian tumors and primary peritoneal carcinomas were excluded. Extended surgical staging according to FIGO was performed in all patients. 5 µm thick sections were prepared from the formalin-fixed paraffin embedded tissues of the initial ovarian specimens and were stained using antibody to factor VIII-related antigen by streptavidin-biotin peroxidase technique. After selection of the most neovascularized area (hot-spot) in the low power field (40X), microvessels were counted using 200X magnification. Results were correlated with clinicopathological factors as age, histological type, FIGO stage, grade, lymph node involvement, ascite volume, CA 125 levels, tumor volume, tumor unilaterality or bilaterality, residue tumor and patient survival.
Results: We did not find any correlation between microvessel count and age, histological type, grade, FIGO stage, lymph node involvement, ascite volume, tumor volume, CA 125 levels, tumor unilaterality or bilaterality, residual tumor and patient survival.
Conclusion: Different from other solid organ tumors such as breast, prostate and non-small cell lung carcinoma angiogenesis is not a prognostic indicator in epithelial ovarian carcinoma.

Keywords: ovarian carcinoma, angiogenesis, factor VIII related antigen, prognosis

Introduction

Tumor growth and metastases are dependent on new blood vessel formation. Tumor growth beyond 1-2 mm is known to be strictly dependent on angiogenesis. For a tumor cell to metastasize a series of barriers must be overcome as well as it responds to several cytokines and growth factors. Angiogenesis is the critical step in metastatic process. For a tumor cell to metastasize, tumor cell must gain access to circulation, localize in the target organ, then induce angiogenesis in the target organ. The prognostic importance of tumor angiogenesis is first reported by Srivastava in cutaneous malignant melanoma (1). There have been increasing number of reports from that time and established data indicates the prognostic importance of angiogenesis in breast (2), prostate (3), non-small cell lung carcinoma (4), cervical carcinoma (5) and endometrial carcinoma (6). There is little knowledge regarding the association of angiogenesis with tumor growth and metastases in ovarian carcinoma. In this study we aimed to investigate angiogenesis as a prognostic factor in epithelial ovarian carcinoma and correlate angiogenesis with clinicopathological factors.

Material and Methods

47 patients diagnosed as primary ovarian carcinoma (stages I-IV) were included in the study who underwent surgical staging between years 1990-1999 at the Department of Obstetrics and Gynecology of Ankara University Medical School, Ankara. Borderline ovarian tumors and primary peritoneal carcinomas were excluded. Extended surgical staging according to International Federation of Gynecology and Obstetrics (FIGO,1986) (7) was performed in all patients. Total abdomi-

nal hysterectomy and bilateral salphingoopherectomy, pelvic and paraaortic lymph node sampling, omentectomy, peritoneal washings and multiple samplings and tumor debulking according to surgical stage were included in the surgical staging. Since the number of stage I and stage IV tumors was small, stage I tumors were evaluated along with stage II tumors and stage III tumors were evaluated with stage IV tumors. Original diagnoses were confirmed by another pathologist before beginning of the evaluation. 5μm thick sections were prepared from the formalin-fixed paraffin embedded tissues of the initial ovarian specimens and were stained using commercially available antibody to factor VIII-related antigen (factor VIII-RAg, Signet, Dedham MA, USA) that labels endothelial cells with high degree of specificity by streptavidin-biotin peroxidase technique. After scanning and selection of the most neovascularized area (hot-spot) in the low power field (40X), microvessels were counted using 200X magnification in three separate areas. Then the mean mivrovessel density (MVD) calculated. All brown-red stained single endothelial cells or cell clusters which were clearly separated from the adjacent vessels, tumor cells or connective tissue elements were considered and counted as microvessels. Existence of a lumen was not considered mandatory to define a red stained area as a microvessel. In addition dilated venules or bigger vessels with muscular walls were not taken into account and excluded from the counting. Results were correlated with clinicopathological factors such as age, histological type, stage, grade, lymph node involvement, ascite volume, tumor volume, preoperative CA 125 levels, tumor unilaterality or bilaterality, postoperative residue tumor and patient survival.

Results

Tumor samples of the 47 patients were evaluated. The mean age of the patients was 54.26±12,69. There were 31 serous papillary cyst adeno carcinoma, 8 mucinous carcinoma and 8 endometrioid carcinoma. According to the FIGO, 6 patients had stage I, 5 patients had stage II, 31 patients had stage III and 5 patients had stage IV disease. According to tumor grade, 15 patients were grade 1, 24 patients were grade II and 8 patients were grade III (*Table 1*). The mean microvessel density (MVD) was 104.9±33.1 ranging between 40 to 190. The mean preoperative serum CA 125 levels was 529.9±348.1 iu/ml ranging between 55 and 2190 iu/ml and ascite volume ranged between 0 and 10 lt. The mean tumor volume was 13.6±6.35 (3-40cm). Nine patients remained without residue tumor, 24 patients had residue tumor ≤ 2cm and 14 patients had residue tumor > 2 cm after surgical debulking.

Table 1: tumor type and grade according to tumor stage

	Stage I + II	Stage III +IV	Total
Tumor Type			
Serous	4	27	31
Mucinous	5	3	8
Endometrioid	2	6	8
Tumor Grade			
Grade I	7	8	15
Grade II	4	20	24
Grade III	1	7	8

Eighteen patients had lymph node involvement, whereas showed reactive changes.

41 patients with stage II-IV disease and 1 patient with stage I-grade III disease received at least 6 cycles of chemotherapy. Chemotherapy schemes included paclitaxel and cisplatin, carboplatin and cyclophosphamide, cyclophosphamide alone, cisplatin alone, carboplatin alone. There was not any age difference between patients having stage I-II and stage III-IV disease. Median survival of patients with stage I-II disease was 87 months while it was 65 months in patients with stage III-IV disease (p=0.05). According to type of tumor it was 96, 75, 60 months in serous, mucinous and endometrioid tumors respectively, without statistical significance (p>0.05).

The anti-factor VIII-related antigen stained vascular endothelial cells brown-red. The mean MVD count according to stage, tumor type and grade are presented in **Table 2**. The MVD count was not found to be correlated with stage (student's t test, p > 0.05), type or grade (univariant analysis, p>0.05) . In addition MVD counts of the primary tumors were not significantly related with patient age (Spearman rank correlation, r=-0.05, p>0.05), lymph node status (student's t test, p > 0.05), ascite volume (Spearman rank correlation, r=-0.18, p>0.05), tumor volume (Spearman rank correlation, r=-0.24, p>0.05), preoperative CA 125 levels (Spearman rank correlation, r= -0.1911, p>0.05), tumor unilaterality or bilaterality (student's t test, p>0.05), postoperative residual tumor (univariant analysis, p>0.05) and patient survival (r=0.1646. p>0.05) (Kaplan Meier survival analysis).

Discussion

Table 2: MVD according to tumor stage, type and grade

	MVD Mean ± SD
Tumor Stage	
Stage (I+II)	114.9 ± 37.8
Stage (III+IV)	101.7 ± 31.5
Tumor Grade	
Grade I	101.1 ± 42.4
Grade II	102,6 ± 26.8
Grade III	122.25 ± 27.8
Histologic Type	
Serous	102.3 ± 29.0
Mucinous	105.12 ± 51.2
Endometrioid	114.5 ± 28.7

Although there has been great advance in the field of chemotherapy, ovarian cancer still remains as the most lethal one among gynecologic malignancies. Prognostic factors capable of identifying early recurrences will be the paramount advance in the field of ovarian cancer therapy. Neovascularization is mandatory not only for a tumor cell to grow beyond a critical size, but is also crucial for a tumor cell to metastasize. It constitutes

an important part in the metastatic process, because it provides access for tumor cells to systemic circulation via microvessels. These newly occurred microvessels are known to have fragmented basal membranes which facilitates spread of tumor cells. After establishment of the metastatic focus it is also necessary for metastatic focus to continue its growth and further establishment of new metastatic foci.

Secretion of matrix degrading enzymes such as plasminogen activator and collagenases by endothelial cells facilitates the invasion of tumor cells in to the newly formed microvessels (8). It was also demonstrated that tumor cells could secrete angiogenic substances into the tumor stroma as shown in cervical intraepithelial neoplasia (9). Additionally, angiogenic substances secreted by host immune cells such as macrophages and mast cells contribute to induction of angiogensis (10,11).

There has been increasing number of reports about angiogenesis and its prognostic value in solid organ tumors recently. It has been reported that microvessel density is related with patient survival in some solid organ tumors (2,3,4). Weidner and associates demonstrated that MVD in the 200X field was an independent prognostic factor for metastases in invasive breast carcinoma (2). In contrast, in studies investigating ovarian carcinoma, angiogenesis was not found to be a new prognostic factor (12,13). Epithelial ovarian cancer spreads by local extension, intraperitoneal seeding, lymphatic involvement and infrequently hematogenously. However, angiogenic properties of ovarian carcinoma cell lines have been demonstrated (14). Nakanishi proposed that angiogenesis is an early event and might be induced differently depending on the tumor type in ovarian cancer (13). We did not confirm this in our study, as we found that angiogenesis was induced equally irrespective of tumor type (*Table 2*). Surprisingly, it was demonstrated in the study of Abulafia and associates on ovarian carcinoma that MVD count of umental metastases significantly correlated with patient survival in stage IIIB and stage IIIC diseases, whereas MVD of primary tumor did not such correlation (12). Nevertheless, it was also suggested that there is a clear cut difference in the MVD of benign and malignant ovarian tumors (15). Importantly it was demonstrated by the same investigators that in the benign group the capillaries tended to be concentrated in the stroma close to epithelium, whereas in malignant epithelial tumors of the ovary they found an increase in the number of microvessels which were distributed heterogeneously in the tumor stroma. This difference is under evaluation by many investigators who indicated that this increased number of blood vessels in malignant epithelial tumors of the ovary resulted in typical changes observed in sonographic blood studies (16,17).

In our study we did not find any correlation between angiogenesis and clinicopathological factors including survival. One possible explanation of this is that the geometrical make-up of these tumors is different from that of other solid organ tumors. For example, in breast carcinoma neovascularization occurs from different directions from the underlying stroma whereas blood supply of ovarian carcinoma arises just from one direction, the hilus. In other words, angiogenesis might vary depending on the distance from the origin of the vascular supply, which has not been studied yet. Secondly, ovarian tumors are so heterogenous that even in the same specimen there may be great differences resulting in substantial individual variation. Thirdly, different from other solid organs, ovarian tissue shows cyclic changes which could have important impacts in MVD formation and assessment. Additionally in normal nonneoplastic ovarian tissue it was demonstrated that human granulasa and theca lutein cells expresses vascular endothelial growth factor which is suggested to induce angiogenesis (18).

Although our data did not reveal angiogenesis as prognostic indicator in epithelial ovarian carcinoma, more comprehensive studies are needed to make a more clear-cut conclusion.

References

1. Srivastava A, Laidler P, Davies P, et al. The prognostic significance of tumor vascularity in intermediate thickness skin melanoma. Am J Pathol 1986; 133,419-423.
2. Weidner N, Semple JP, Welch WR. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. N Eng J Med 1991;324:1-8.
3. Weidner N, Carroll PR, Flax J. Tumor angiogenesis correlates with metastasis in invasive breast carcinoma. Am J Pathol 1993; 143:401-9.
4. Macchiarini P, Fontani G, Hardin MJ. Relation of neovascularity to metastasis of non-small cell lung cancer. Lancet 1992; 340 : 145-6.
5. Wiggins DL, Granai CO, Steinhoff MM. Tumor angiogenesis as a prognostic factor in cervical carcinoma. Gynecol Oncol 1993; 56 : 353-6.
6. Kirshner CV, Alanis-Amezcua JM, Martin VG. Angiogenesis in endometrial carcinoma: a new prognostic indicator. Am J Obstet Gynecol 1996; 174 : 1879-84.
7. International Federation of Gynecology and Obstetrics Cancer Committee. Staging announcement. Gynecol Oncol 1986; 26 : 383.
8. Moscatelli D, Gross JL, Rifkin DB. Angiogenic factors stimulate plasminogen activator and collagenase production by capillary endothelial cells. J Cell Biol 1981; 91 : 201a.
9. Smith-McCune KK, Weidner N, Bishop JM. Cervical intraephitelial neoplasia (CIN) is angiogenic. Proc Am Assoc Cancer Res 1993; 34 : 74.
10. Blood CH, Zetter BR. Tumor interactions with the vasculature. Angiogenesis and tumor metastases. Biochim Biophys Acta 1990; 1032 : 89-118.
11. Thornton S, Mueller S, Levine E. Human endothelial cells : use of heparin in cloning and long term serial cultivation. Science 1983; 222 : 623-625.
12. Ovadia Abulafia, William Triest, David Sherer. Angiogenesis in primary and metastatic epithelial ovarian carcinoma. Am J Obstet Gynecol 1997; 177 : 541-547.
13. Nakanishi Y, Kodama J, Yoshinouchi M, Keizo Tokumo, Shigehito Kamimura, Hiroyiku Okuda, Takafumi Kudo. The expression of vascular endothelial growth factor and transforming growth factor β associates with angiogenesis in epithelial ovarian cancer. Int J Gynecol Pathol 1997; 16 : 256-262
14. Ishiwata I, Ishiwata C, Soma M, et al. Tumor angiogenic activity of gynecologic tumor cell lines on the chorioallantoic membrane. Gynecol Oncol 1988; 29 : 87-93

15. Hermann Brustmann, Paul Riss, Susanna Naude. The relevance of angiogenesis in benign and malignant epithelial tumors of the ovary. A quantitative histologic study. Gynecol Oncol 1997; 67 : 20-26.
16. Kurjak A, Shalan H, Kupesic S, Predanic M, Zald I, Breyer B, Jukic S: Transvaginal color Doppler sonography in the assessment of pelvic tumor vascularity. Ultrasound Obstet Gynecol 1993; 3 : 137-154.
17. Maly Z, Riss P, Deutinger J. Localization of blood vessels and qualitative assessment of blood flow in ovarian tumors. Obstet Gynecol 1995; 85 : 33-36.
18. Kamat BR, Brown LF, Manseau SJ, Senger DR, Dvorak HF: Expression of vascular permeability factor/vascular endothelial growth factor by human granulosa and theca lutein cells. Role in corpus luteum development. Am J Pathol 1995; 146 : 157-165.