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分担研究報告書

上皮性卵巣癌とくに明細胞腺癌と子宮内膜症の関連に関する研究

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研究要旨
（研究１）呉医療センターで 1992 年から 2002 年に治療を行った上皮性卵巣癌 91 例中 16 例（17.6%）に子宮内膜症の共存を認め、組織型別の共存頻度は漿液性腺癌 2.2%、粘液性腺癌 5.0%、明細胞腺癌 62.5%、類内膜腺癌 40.0%であった。類内膜腺癌ととくに明細胞腺癌で内膜症共存頻度が高く、ともに漿液性腺癌、粘液性腺癌に比べ有意に高頻度であった（$p<0.01$、$p<0.01$）。子宮内膜症が共存する卵巣癌は、46 歳から 55 歳の年齢層に頻度が高く、臨床進行期は I/II 期症例に内膜症の共存が有意に多く確認された（$p<0.01$）。
（研究２）呉医療センター（1992 年－2002 年、16 例）と広島大学病院（1982 年－1999 年、15 例）で治療を行った、他の組織型の混在のない卵巣明細胞腺癌 31 例中 17 例に子宮内膜症の共存を確認し、うち 8 例（8/17、47.5%）に子宮内膜症から癌への移行像が確認された。卵巣明細胞腺癌 31 例の内膜症共存の有無による臨床的事項を比較した結果、平均年齢は共存群（以下 E 群）で有意に低く（$p<0.05$）、臨床進行期については非共存群（以下 N 群）に III 期進行癌 2 例が見られたが、E 群 17 例はすべて I 期、II 期症例であった。卵巣明細胞腺癌 I、II 期 29 例の累積 5 年生存率は E 群と N 群の間に有意差は認められなかった。症例数が最も多かった Ic 期 18 例についても累積 5 年生存率は E 群と N 群の間に有意差は認められなかった。

A. 研究目的

近年卵巣明細胞腺癌の増加が指摘されているが、その原因として子宮内膜症の増加が関与しているか否かは不明である。卵巣明細胞腺癌は化学療法に抵抗性を示し、上皮性卵巣癌の中でもとくに予後不良な組織型であり、とりわけ日本では上皮性卵巣癌全体に占める頻度が高いことが知られている。もし子宮内膜症が悪性化し、しかもその多くが卵巣明細胞腺癌になるとすれば、子宮内膜症の外科的治療後あるいは保存的治療後の残存子宮内膜症をどのように治療し、長期管理していくかはきわめて重要な課題である。そこで上皮性卵巣癌とくに明細胞腺癌と子宮内膜症の関連を明らかにする目的で、上皮性卵巣癌症例について子宮内膜症共存の有無を病理学的に検索し、子宮内膜症共存卵巣癌の臨床的特徴について検討した。

B. 研究方法

（研究１）子宮内膜症と上皮性卵巣癌の関係を明らかにするために 1992 年から 2002 年に呉医療センターで治療を行った上皮性卵巣癌 91 例(漿液性腺癌 45 例、

粘液性腺癌 20 例、明細胞腺癌 16 例、類内膜腺癌 10 例）について、各組織型の子宮内膜症共存頻度を臨床背景因子別に検討した。

（研究２）上皮性卵巣癌のうち、とくにに子宮内膜症との関連が示唆されている明細胞腺癌について検討を行った。呉医療センター婦人科（1992 年－2002 年、16 例）と広島大学病院婦人科（1982 年－1999 年、15 例）で治療を行った、他の組織型の混在のない卵巣明細胞腺癌 31 例を対象とし、子宮内膜症が共存している E 群 17 例と共存していない N 群 14 例の 2 群に分けて臨床背景、経過、予後などを比較検討した。

本研究で用いた子宮内膜症共存の定義は、上皮性卵巣癌が存在する患側卵巣に病理組織学的に子宮内膜症所見が確認された症例を子宮内膜症共存例とした。また 2 群間における臨床背景因子の検討には Fisher’s test、平均値の差の検定には Mann-Whitney test を、生存率の解析は Kaplan-Meier 法、log-rank test を用いた。$p<0.05$ で有意差ありとした。

C. 研究結果

（研究１）表１に呉医療センターで治療を行った上皮性卵巣癌の各組織型別の子宮内膜症共存頻度を示した。上皮性卵巣癌 91 例中子宮内膜症の共存を確認した症例は 16 例（17.6%）であった。漿液性腺癌 45 例中 1 例（2.2%）、粘液性腺癌 20 例中 1 例（5.0%）、明細胞腺癌 16 例中 10 例（62.5%）、類内膜腺癌 10 例中 4 例（40.0%）であり、明細胞腺癌と類内膜腺癌の頻度が高く、ともに漿液性腺癌、粘液性腺癌に比べ有意に高頻度であった（$p<0.01$、$p<0.01$）。表２に上皮性卵巣癌 91 例の臨床背景因子別の子宮内膜症共存頻度を示した。年齢は 23 歳から 82 歳、平均年齢 53.9 歳で、子宮内膜症が共存する卵巣癌は、46 歳から 55 歳のいわゆる閉経前後 10 年間の年齢層に頻度が高い結果であった。50 歳未満と 50 歳以上では 14.3%と 19%で 50 歳以上の頻度が高かった。臨床進行期は、I/II 期症例に内膜症の共存が有意に多く確認された（$p<0.01$）。閉経前と閉経後で子宮内膜症共存卵巣癌の頻度に差はなかった。経産の有無では産婦に比べ未産婦に頻度が高い傾向にあったが、有意差はなかった。

（研究２）表３に呉医療センターと広島大学病院で治療を行った卵巣明細胞腺癌症例における子宮内膜症共存の有無を示した。卵巣明細胞腺癌 31 例中 17 例に子宮内膜症の共存を確認し、うち 8 例（8/17、47.5%）に子宮内膜症から癌への移行像が確認された。表４に明細胞腺癌 31 例の子宮内膜症共存の有無による臨床的背景を示した。平均年齢は共存群（以下 E 群）で有意に低く、年齢層は E 群では 46－55 歳の閉経前後の年齢層に多かった（$p<0.05$）。月経の状態で見ると有意差はないが、非共存群（以下 N 群）では閉経後に診断されることが多かった。初診時の主訴は、いずれの群も下腹部痛、下腹部緊満感が多く、また一方で、症状は無くがん検診で発見された症例 4 例（N 群 2 例、E 群 2 例）や卵巣チョコレート嚢胞のフォロー中に発見された症例 3 例（E 群 3 例）もあった。分娩回数については N 群と E 群 で差はなかった。臨床進行期については N 群に III 期進行癌 2 例が見られたが、E 群 17 例はすべて I 期、II 期症例であった。N 群に比し E 群で腹腔内細胞診陰性の割合が高く、腫瘍径も若干小さく、治療前の CA125 値も低い傾向にあったが、いずれにも有意差はなかった。卵巣明細胞腺癌 I、II 期 29 例について累積生存率を群別に検討した結果、5 年生存率は E 群 92.9%、N 群 64.3%であったが、E 群と N 群の間に有意差は認められなかった（図 1）。症例数が最も多かった Ic 期 18 例について累積生存率を群別に検討してみると 5 年生存率は E 群 100%、N 群 85.7%で、E 群と N 群の間に有意差は認

められなかった（図2）。

D. 考察

今回の研究で上皮性卵巣癌 91 例中 16 例（17.6%）に子宮内膜症の共存を認め、組織型別では漿液性腺癌 2.2%、粘液性腺癌 5.0%、明細胞腺癌 62.5%、類内膜腺癌 40.0%であった。やはり、類内膜腺癌ととくに明細胞腺癌で子宮内膜症との共存頻度が高い結果であり、このことは明細胞腺癌と類内膜腺癌の発癌機構に子宮内膜症が関与している可能性を強く示唆するものと考えられる。

子宮内膜症との共存頻度が高かった卵巣明細胞腺癌について臨床的背景を検討した結果、子宮内膜症共存例は、非共存例に比べ年齢が有意に低く、46－55 歳のいわゆる閉経前後の年齢に好発していた。一般に子宮内膜症の発症頻度のピークは 40－44 歳時にあり、エストロゲン分泌が低下する 40 歳代後半になると頻度は減少してくるが、それに続いて子宮内膜症共存卵巣明細胞腺癌のピークが存在することになる。すなわち、閉経前後のエストロゲン分泌の急激な低下が子宮内膜症の癌化に関係している可能性が考えられる。子宮内膜症共存例の主症状はいわゆる月経痛などの子宮内膜症に典型的な痛みではなく持続性の痛みであった。内膜症のフォロー中に月経とは関係なく痛みが突然増強した場合や持続性の痛みが出現した場合、特に閉経前後に痛みが生じた場合は悪性化を疑ってみる必要があると思われる。子宮内膜症共存卵巣癌の発生に不妊がリスク因子として関与しているのか、または子宮内膜症の影響で不妊であるのかは明確ではないが、子宮内膜症共存卵巣癌と不妊との関連が予想される。臨床進行期の特徴については、子宮内膜症共存例のほとんどはI、II 期症例であった。この結果は現在の子宮内膜症悪性化の判定基準が病理所見により定義されていることに起因していると思われる。つまり進行癌では癌の浸潤増殖につれて子宮内膜症が癌組織に駆逐されてしまうために病期の進んだ症例では既存の子宮内膜症の確認が困難となり、このことが臨床進行期 I、II 期症例に子宮内膜症共存例が多く見られる理由のひとつと考えられる。このことは‘内膜症共存例の予後はよいか’との疑問にも関連してくる。今回、卵巣明細胞腺癌 I、II 期について子宮内膜症共存の有無で予後を比較したところ、症例数が少ないこともあるが、予後の差はなく、Ic 期に限定した場合でも予後の差は見出させなかった。子宮内膜症共存症例と非共存例で卵巣癌自体の性質が異なるために予後に差があることを証明するためには子宮内膜症共存卵巣癌の定義や各症例の背景因子を考慮し解析する必要があるが、現在のところそうした報告はなされていない。

E. 結論

上皮性卵巣癌 91 例中 16 例（17.6%）に子宮内膜症の共存を認め、各組織型別では明細胞腺癌（62.5%）と類内膜腺癌（40.0%）で共存頻度が高い結果であり、明細胞腺癌と類内膜腺癌の発癌機構に子宮内膜症が関与している可能性を強く示唆するものと考えられた。

卵巣明細胞腺癌 31 例中 17 例に子宮内膜症の共存を確認し、うち 8 例（8/17、47.5%）に子宮内膜症から癌への移行像が確認された。卵巣明細胞腺癌 I、II 期 29 例について子宮内膜症共存の有無で予後を比較した結果、予後の差はなく、Ic 期 18 例に限定した場合でも予後の差は見出させなかった。

F. 健康危害情報

特記すべきことなし

G. 研究発表

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2. 学会発表

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H. 知的財産権の出願・登録状況（予定含）

1. 特許取得
   なし
2. 実用新案登録
   なし
3. その他
   なし

表1　卵巣癌の各組織型別の子宮内膜症共存頻度

組織型				
漿液性腺癌 (n=45)	粘液性腺癌 (n=20)	明細胞腺癌 (n=16)	類内膜腺癌 (n=10)	計
2.2%*,** (1/45)	5.0%*,** (1/20)	62.5%* (10/16)	40.0%** (4/10)	17.6% (16/91)

*p=0.0001　**p=0.0023　Fisher's test　　(呉医療センター1992-2002)

表2　上皮性卵巣癌91例の臨床背景因子別子宮内膜症共存頻度

臨床背景	組織型				計 (n=91)
	漿液性腺癌 (n=45)	粘液性腺癌 (n=20)	明細胞腺癌 (n=16)	類内膜腺癌 (n=10)	
年齢（平均 53.9 歳、中央値 54 歳）					
-45	0/12	0/6	1/1	1/2	9.5% (2/21)
46-55	0/12	0/6	7/9	2/4	29.0% (9/31)
56-	1/21	1/8	2/6	1/4	12.8% (5/39)
臨床進行期					
I/II	1/15	0/14	10/16	2/5	26.0%* (13/50)
III/IV	0/30	1/6	0/0	2/5	7.3%* (3/41)
月経の状態					
閉経前	0/17	0/8	4/4	2/4	18.2% (6/33)
閉経後	1/28	1/12	6/12	2/6	17.2% (10/58)
経産の有無					
未産婦	0/5	0/4	4/4	1/3	31.3% (5/16)
経産婦	1/40	1/16	6/12	3/7	14.7% (11/75)

(子宮内膜症共存例/症例)、*p=0.0085　Fisher's test (呉医療センター1992-2002)

表3　明細胞腺癌と子宮内膜症の共存について

	症例数
N　群	14
E　群	17 (8)
計	31

N群：子宮内膜症非共存明細胞腺癌、E群：子宮内膜症共存明細胞腺癌

(　) 子宮内膜症から癌への移行像が確認された明細胞腺癌

(呉医療センター1992-2002、広島大学病院 1982-1999)

表4　明細胞腺癌31例の子宮内膜症共存の有無による臨床背景

臨床背景	N群(n=14)	E群(n=17)
平均年齢 *	56.2	50.9
年齢		
-45	1	2
46-55	6	12
56-	7	3
月経の状態		
閉経前	2	9
閉経後	12	8
主訴		
下腹部痛、緊満感	7	10
腫瘤触知	1	1
検診	2	5**
不正出血	1	0
その他	3	1
分娩回数		
0	4	6
1	4	6
2 以上	6	5
臨床進行期		
I期	8	15
Ia期	1	4
Ic期	7	11
II期	4	2
III期	2	0
IV期	0	0
腹腔内細胞診		
陽性	8	4
陰性	6	13
腫瘍径　(cm)	13.0±3.4	11.4±3.5
CA125値　(U / ml)	1517.6±3399.7	66.1±110.9

*p=0.023　Mann-Whitney Test、**5例中3例はチョコレート嚢胞フォロー中に癌化した（呉医療センター1992-2002、広島大学病院 1982-1999)

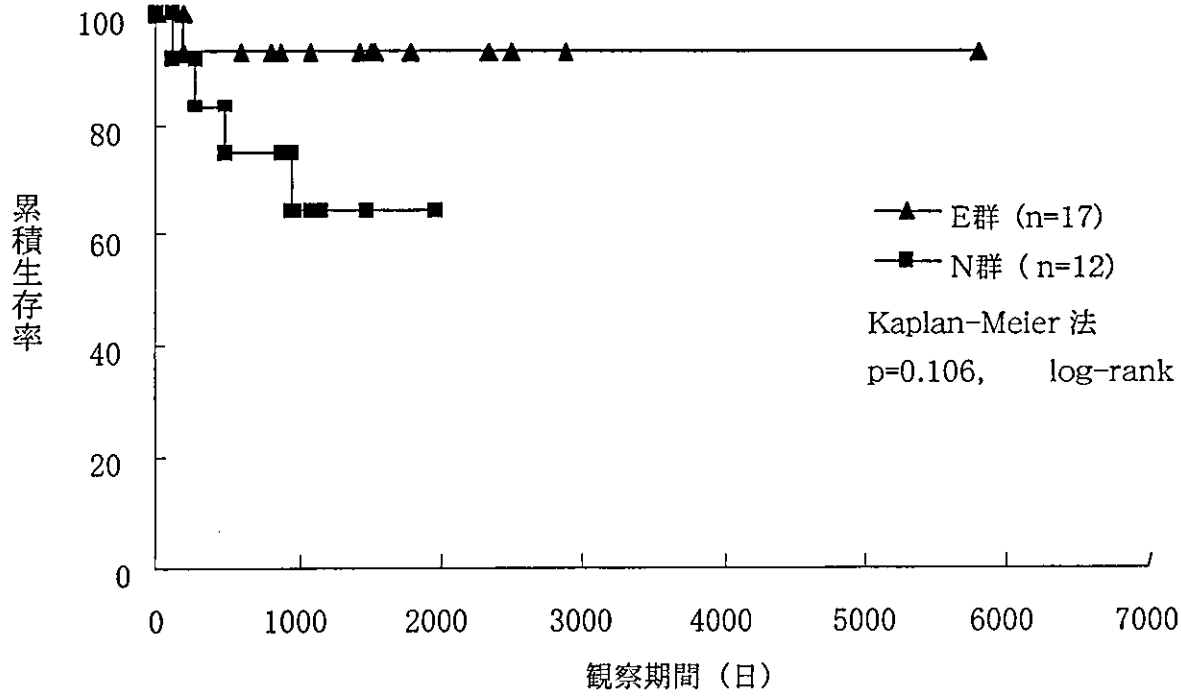

図１　明細胞腺癌Ⅰ、Ⅱ期生存曲線

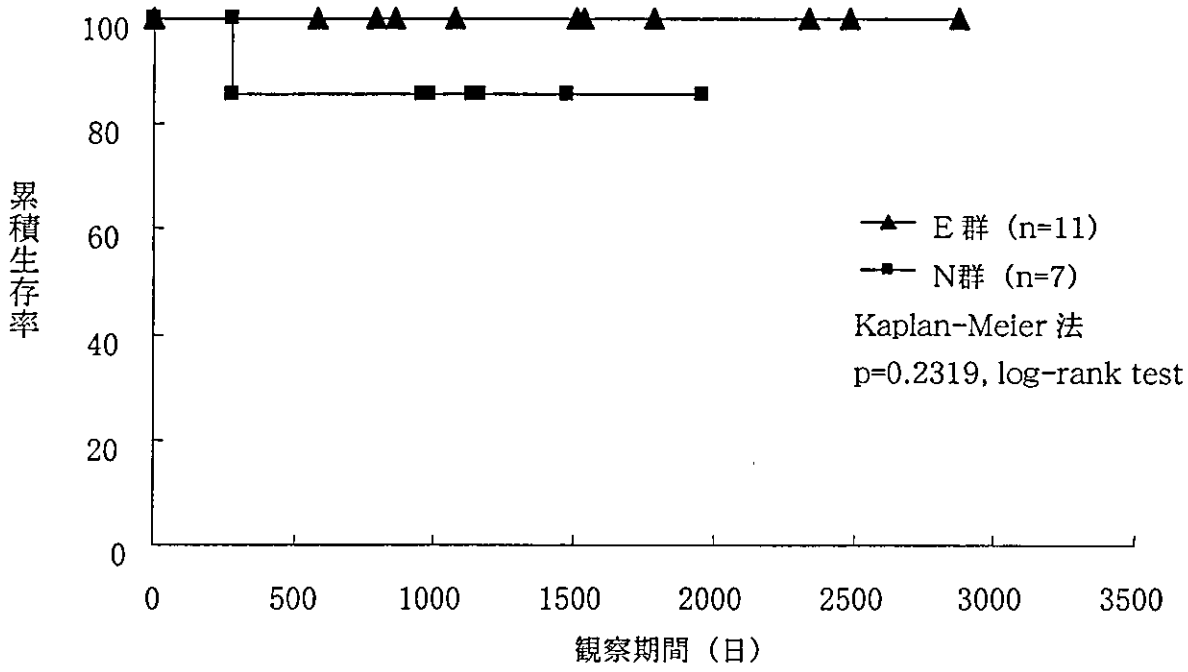

図２　明細胞腺癌 Ic 期生存曲線

研究成果の刊行に関する一覧表

書籍：該当なし

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発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Onda T, Yoshikawa H, Yasugi T, Taketani Y, et al.:	Secondary Cytoreductive Surgery for Recurrent Epithelial Ovarian Carcinoma; Proposal for Patients Selection.	British Journal Of Cancer			印刷中
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Onda T, Katsumata N, Yasugi T, Fujii T, Kamura T, Saito T, Yoshikawa H, et al.:	Cisplatin, Paclitaxel and Escalating Doses of Doxorubicin (TAP) in Advanced Ovarian Cancer: a Phase I Trial.	Japanese Journal Of Clinical Oncology	34(9)	540-546	2004
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Vascular Endothelial Growth Factor C and Vascular Endothelial Growth Factor Receptor 2 Are Related Closely to the Prognosis of Patients with Ovarian Carcinoma

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BACKGROUND. The vascular endothelial growth factor (VEGF) family and VEGF receptors (VEGFR) play an essential role in the angiogenesis of both pathologic and nonpathologic conditions. However, the prognostic significance of VEGF and VEGFR expression in ovarian carcinoma is unclear.

METHODS. The tissue expression levels of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 in 80 specimens of ovarian carcinoma were examined immnohistochemically. The results obtained were analyzed clinicopathologically.

RESULTS. VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were expressed both in tumor cells and in adjacent endothelial cells of blood and lymph vessels. The tissue expressions of VEGF-C and VEGFR-2 were correlated significantly with tumor extension, including peritoneal metastases outside the pelvic cavity ($P = 0.0010$ and $P = 0.0008$, respectively), lymph node metastases ($P = 0.0030$ and $P = 0.0018$, respectively), and positive ascitic cytology ($P = 0.025$ and $P = 0.0016$, respectively). Conversely, there was no significant correlation between VEGF-A and VEGFR-3 expression and clinicopathologic features of ovarian carcinoma. Logistic regression analysis revealed that the expressions of VEGF-C and VEGFR-2 also were independent risk factors for peritoneal and lymph node metastases. Survival curves determined by the Kaplan–Meier method and in univariate analysis demonstrated that high expression levels of VEGF-C and VEGFR-2 were associated with the 5-year survival rate. In multivariate analysis, high expression levels of VEGF-C and VEGFR-2 emerged as independent indicators for disease-specific survival.

CONCLUSIONS. High tissue expression of VEGF-C and VEGFR-2 reflects the aggressiveness of the spread of tumor in ovarian carcinoma. Thus, both have predictive value for identifying high-risk patients who have a poor prognosis. ***Cancer* 2004; 101:1364–74.** © *2004 American Cancer Society.*

KEYWORDS: vascular endothelial growth factor, ovarian carcinoma, immunohistochemistry, prognosis.

Neoplastic angiogenesis and lymphangiogenesis are essential for the growth of tumor tissue in both primary and metastatic sites. Vascular endothelial growth factor (VEGF) is now accepted as a powerful angiogenic agent in neoplastic tissues as well as in normal tissues. Under the influences of some cytokines and other growth factors, the VEGF family appears in tumor tissue and adjacent stroma, and it plays an essential role in the new proliferation of blood and lymph vessels.[1–3] Among the VEGF family, VEGF-A, VEGF-B, and VEGF-C induce new blood vessel proliferation, and VEGF-C and

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VEGF-D relate to lymphangiogenesis. These factors act with their own VEGF receptors (VEGFRs).[4–9]

Ovarian carcinoma has the poorest prognosis among malignancies in the gynecologic field, and surgical staging according to the International Federation of Gynecology and Obstetrics (FIGO) system is regarded as the most important prognostic factor.[10] When the tumor is confined to the ovary, a > 90% chance of 5-year survival is expected. If the disease extends to the peritoneal cavity, however, the prognosis is limited to ≤ 30%.[11] Histologic classification and grading also relate to the patient's prognosis.[12–16] Cytotoxic chemotherapy and maximal debulking are conventional prognostic methods of controlling ovarian carcinoma on which the treatment policy is based. Despite the current progress of cytotoxic chemotherapy and surgical techniques, the consequence of ovarian carcinoma is unchanged. Therefore, new management strategies against the disease are required. To evaluate the prognostic significance of the neoplastic angiogenic factors, tissue expression of VEGF-A, VEGF-C, and their receptors were examined in ovarian tumors.

MATERIALS AND METHODS

Patients and Specimens

Clinical records and preserved specimens from 80 patients with ovarian carcinoma who underwent surgical treatment at Kurume University Hospital between 1997 and 2002 were examined consecutively. All patients submitted informed consent and agreed with the use of their tissues in this study. All patients underwent a staging laparotomy, including total hysterectomy, bilateral salpingooophorectomy, partial omentectomy, peritoneal cytology, and/or pelvic and paraaortic lymphadenectomy. No patient had received any preoperative treatment. The patients were staged in accordance with FIGO criteria and included 33 patients with Stage I disease, 5 patients with Stage II disease, 34 patients with Stage III disease, and 8 patients with Stage IV disease. Tumors were grouped according to the World Health Organization (WHO) histologic typing system and were graded according to the FIGO grading system.[17] Grading criteria offered by Silverberg et al.[15,16] also were applied. During the follow-up, which ranged from 11 weeks to 359 weeks (mean, 132.8 weeks), there were 34 disease recurrences and 24 disease-related deaths. The mean age of our patients at surgery was 54.4 years (range, ≈ 23–79 years). Surgically resected tissues from 10 tumors with low potential malignancy (LPM) and 22 benign cystadenomas also were examined for the purposes of comparison.

Immunohistochemistry

Formalin fixed, paraffin embedded serial sections (4 μm) that were mounted on 3-aminopropyltriethoxysilane-coated slides (Matsunami Glass Ind., Ltd. Japan) were deparaffinized in xylene alcohol and graded alcohol. Rabbit polyclonal anti-VEGF-C (Zymed Laboratories Inc., San Francisco, CA), anti-VEGF-A, anti-VEGFR-3 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and anti-VEGFR-2 (Upstate Cell Signaling Solutions, VA) antibodies were used at concentrations of 1.6 μg/mL, 2.0 μg/mL, 2.0 μg/mL, and 5.0 μg/mL, respectively. Mouse monoclonal anti-CD31 antibody (1:40 dilution, DAKO, Ltd., Copenhagen, Denmark) also was used. Immunohistochemistry was performed by using a catalyzed signal-amplification system (DAKO, Ely, United Kingdom) according to the manufacture's protocol. The sections were treated with 0.3% hydrogen peroxide (H_2O_2) in water for 10 minutes to quench the endogenous peroxidase activity within the tissue. Nonspecific binding sites were blocked with 20% heat-inactivated nonserum protein for 10 minutes at room temperature. The sections were incubated for 15 minutes in the presence of the primary antibody. Slides were then washed in phosphate buffered saline (PBS) containing 0.1% Tween 20 (PBS/Tween) for 15 minutes by changing the solution 3 times before the application of the secondary biotinylated antibody. The slide was incubated with secondary antibody for 15 minutes at room temperature before it was washed for 15 minutes in PBS/Tween, which was changed 3 times. The sections were then incubated for 15 minutes with avidin-biotinylated-horseradish peroxidase complex, and the reaction was visualized with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (Nichirei, Tokyo, Japan) as a chromogen in Tris-HCl buffer, pH 7.6, containing 0.03% H_2O_2. Hematoxylin was used to counterstain of the nuclei. For each antibody, a negative control study was performed by using normal rabbit serum instead of the primary antibody. For positive controls, formalin fixed, paraffin embedded sections of human placenta were stained for VEGF-A and VEGF-C, and sections of normal human umbilical cord were stained for VEGFR-2 and VEGFR-3 using the same procedure.

Microscopic Assessment of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 Expressions and Microvessel Density

Two pathologists (H.Y. and N.N.) independently evaluated and interpreted the results of immunostaining without knowledge of the clinical data for each patient. VEGF-A and VEGF-C were stained both in tumor cells and in adjacent endothelial cells from blood and lymph vessels. The staining results in tumor cells were

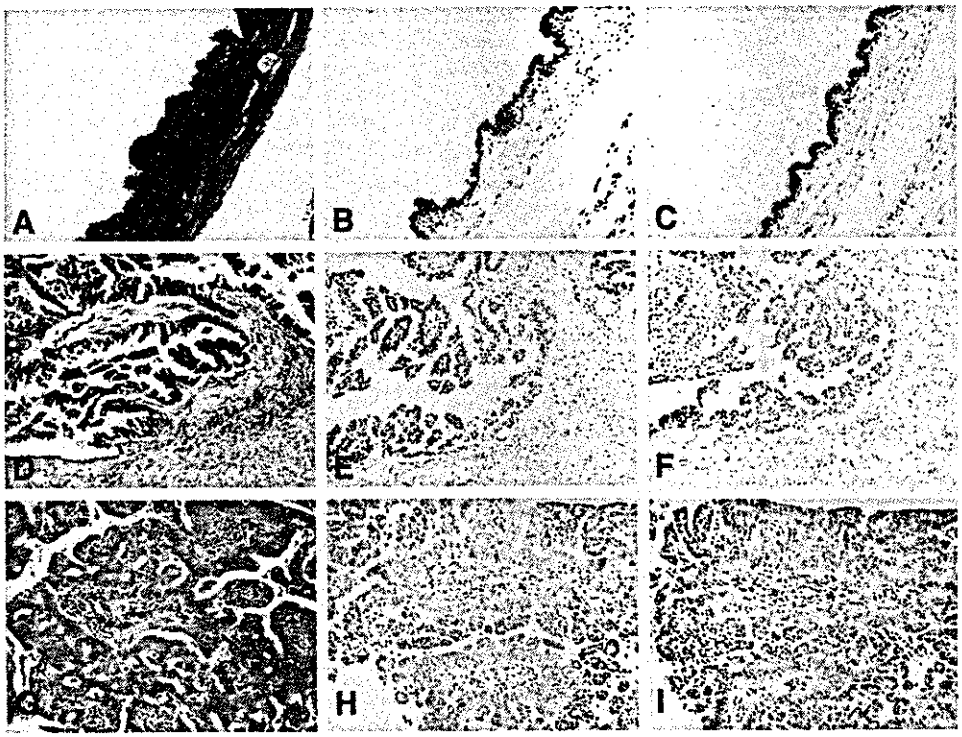

FIGURE 1. Immunohistochemical staining of vascular endothelial growth factor C (VEGF-C) and VEGF receptor 3 (VEGFR-3) in ovarian carcinoma. Samples of serous cyst adenoma (A), serous tumors with low potential malignancy (LPM) (D), and serous adenocarcinoma (G) were stained with hematoxylin and eosin. Serous cyst adenoma was negative for both VEGF-C (B) and VEGFR-3 (C). VEGF-C was positive in serous LPM (E) and in serous adenocarcinoma (H). VEGFR-3 was positive in serous LPM (F) and in serous adenocarcinoma (I).

classified into 3 levels: negative expression when immunostain-positive tumor cells accounted for < 10% of the tumor area on the section, low expression when the positive cells accounted for from 10% to < 50% of the tumor area, and high expression when the positive cells accounted for > 50% of the tumor area. We used such criteria, because the median proportion immunostain-positive tumor cells in the whole sample was ≈ 50%. Faint or equivocal immunoreactions were not regarded as positive. The specimens were considered positive to VEGF-A and/or VEGF-C when expression levels were ≥ 10%. VEGFR-2 and VEGFR-3 also were stained both in tumor cells and in adjacent endothelial cells from blood and lymph vessels. VEGFR-2 and VEGFR-3 staining in endothelial cells was considered positive when at ≥ 5% of endothelial cells in the area were strongly immunoreactive, as seen in the positive control cells. Microvessel density (MVD) was assessed by counting CD31-positive microvessels under × 200 magnification in a grid area of 0.16 mm^2 according to the criteria of Weidner.[18] Five areas of high vascular density (hot spots) were selected, and microvessels were counted on each section.

Statistical Analysis

All statistical calculations were performed by using Stat View software (version 5; SAS Institute Inc., Cary, NC). The significance of correlations between the expression of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3; MVD; and clinicopathologic factors was evaluated by using univariate analysis (chi-square test) and logistic regression analysis. Survival rates were calculated by using the Kaplan–Meier method, and the statistical significance of differences in the cumulative survival curves between the groups was evaluated by using the log-rank test. Multivariate survival analysis was conducted by using the Cox proportional hazard method. Other statistical analyses were carried out with the Mann–Whitney U test. Results were deemed significant at $P < 0.05$.

RESULTS

VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were expressed in the cytoplasm of both tumor cells and endothelial cells from the blood and lymph vessels in the stroma adjacent to tumor nests (Figs. 1, 2). The frequency of VEGF-C expression was found in 72.5% of adenocarcinomas (58 of 80 patients), in 40% of LPM tumors (4 of 10 patients), and in only 9.1% of benign cystadenomas (2 of 22 patients); whereas VEGFR-3 expression was found in 18.1% (4 of 22 patients), 50% (5 of 10 patients), and 72.5% (58 of 80 patients), respectively. Significant differences were observed in VEGF-C expression levels between benign cystadenomas and LPM tumors ($P < 0.04$; chi-square test), LPM tumors and adenocarcinomas ($P < 0.04$), and benign cystadenomas and adenocarcinomas ($P < 0.0001$) and in the VEGFR-3 expression levels between benign cys-

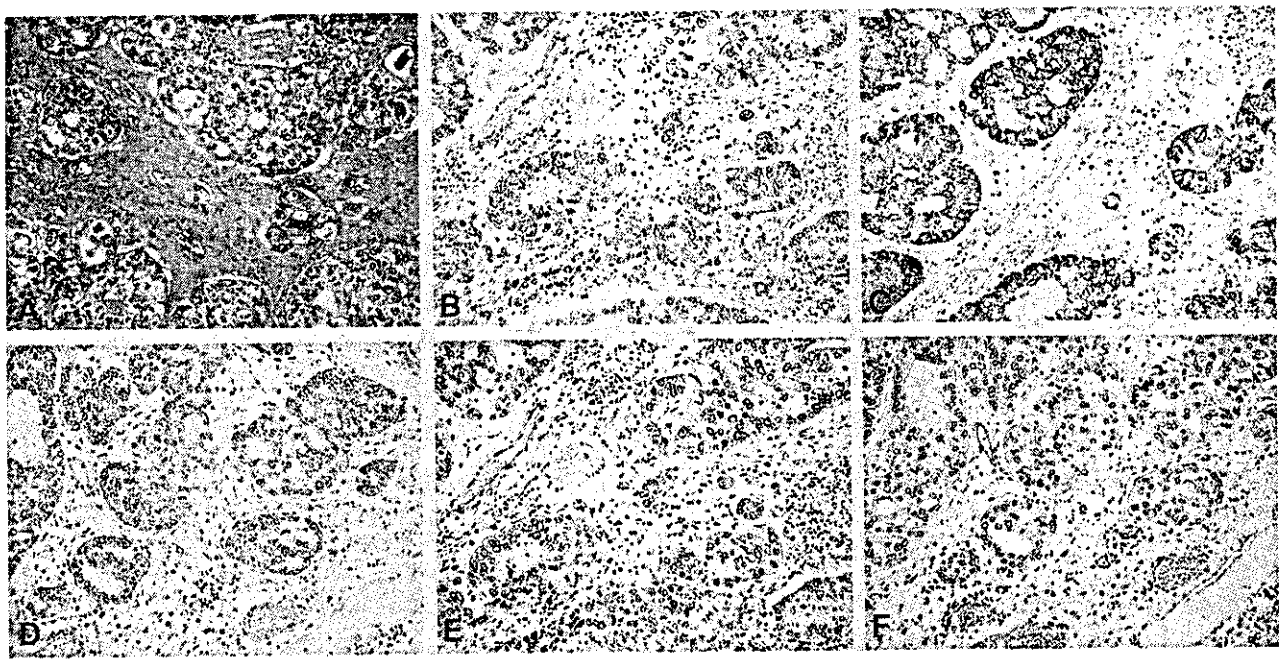

FIGURE 2. Immunohistochemical staining of vascular endothelial growth factor A (VEGF-A), VEGF-C, VEGF receptor 2 (VEGFR-2), and VEGFR-3 in ovarian carcinomas. A sample of clear cell adenocarcinoma (A) was stained with hematoxylin and eosin. Both tumor cells and adjacent endothelial cells were stained for VEGF-A (B), VEGF-C (C), VEGFR-2 (D), and VEGFR-3 (E). CD31 was stained in endothelial cells from blood and lymph vessels in the tumor stroma. (F).

tadenomas and adenocarcinomas ($P < 0.0001$). Expression levels of VEGF-C and VEGFR-3 increased along with the increase in malignant potential.

The histologic tumor types are listed in Table 1. Expression levels of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 were lower in mucinous carcinoma than in the other tumor types, but the differences were not significant.

Among the 80 tumors, 20 tumors were negative for VEGFR-2, and 60 tumors were positive for VEGFR-2. Twelve of 20 VEGFR-2-negative tumors (60.0%) were positive for VEGF-A, whereas 49 of 60 VEGFR-2-positive tumors (81.7%) also were positive for VEGF-A ($P < 0.05$; chi-square test). Regarding the relation between VEGFR-2 expression and VEGF-C expression, 8 of 20 VEGFR-2-negative tumors (40.0%) were positive for VEGF-C, and 50 of 60 VEGFR-2-positive tumors (83.3%) also were positive for VEGF-C ($P = 0.0002$). Conversely, VEGFR-3 expression was negative in 22 tumors and positive in 58 tumors, 12 of 22 VEGFR-3-negative tumors (54.5%) were positive for VEGF-C, and 46 of 58 VEGFR-3-positive tumors (79.3%) also were positive for VEGF-C ($P < 0.03$). These results showed that receptor-positive tumors have a strong propensity to exhibit the corresponding growth factor.

The correlations between clinicopathologic features and the expression of VEGF-A, VEGF-C, VEGFR-2, and VEGFR-3 are summarized in Table 2.

TABLE 1
Expression of Vascular Endothelial Growth Factor A (VEGF-A), VEGF-C, VEGF Receptor 2 (VEGFR-2), and VEGFR-3 in Ovarian Carcinomas

	No. of patients (%): Histologic type			
Expression	Serous ($n = 36$)	Mucinous ($n = 11$)	Endometrioid ($n = 18$)	Clear cell ($n = 15$)
VEGF-A				
Negative	7 (19.4)	5 (45.4)	3 (16.7)	5 (33.3)
Low	24 (66.7)	2 (18.2)	10 (55.5)	7 (46.7)
High	5 (13.9)	4 (36.4)	5 (27.8)	3 (20.0)
VEGF-C				
Negative	7 (19.4)	6 (54.5)	4 (22.2)	5 (33.3)
Low	18 (50.0)	2 (18.2)	3 (16.7)	4 (26.7)
High	11 (31.6)	3 (27.3)	11 (66.1)	6 (40.0)
VEGFR-2				
Negative	5 (13.9)	7 (63.6)	2 (11.1)	6 (40.0)
Low	17 (47.2)	2 (18.2)	7 (38.9)	5 (33.3)
High	14 (38.9)	2 (18.2)	9 (50.0)	4 (26.7)
VEGFR-3				
Negative	13 (36.2)	4 (36.4)	3 (16.7)	2 (13.3)
Low	17 (47.2)	6 (54.5)	9 (50.0)	6 (40.0)
High	6 (16.6)	1 (9.1)	6 (33.3)	7 (46.7)

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained.

TABLE 2
Relation between the Expression of Vascular Endothelial Growth Factor A (VEGF-A), VEGF-C, VEGF Receptor 2 (VEGFR-2), and VEGFR-3 and Clinicopathologic Factors in 80 Women with Ovarian Carcinoma

		No. of patients (%)			
Variable	Total	VEGF-A	VEGF-C	VEGFR-2	VEGFR-3
Peritoneal metastasis outside the pelvic cavity					
Not present	38	28 (73.7)	21 (55.3)	22 (57.9)	27 (71.1)
Present	42	33 (78.6)	37 (88.1)	38 (90.5)	31 (73.8)
P value[a]	—	0.61	0.0010	0.0008	0.78
Lymph node metastasis					
Not present	44	33 (75.0)	26 (59.1)	27 (61.4)	31 (70.5)
Present	36	28 (77.8)	32 (88.9)	33 (91.7)	27 (75.0)
P value[a]	—	0.77	0.0030	0.0018	0.65
Ascitic cytology in early stage					
Negative	11	6 (54.5)	3 (27.3)	2 (18.2)	6 (54.5)
Positive	27	22 (81.5)	18 (66.7)	20 (74.1)	21 (77.8)
P value[a]	—	0.087	0.025	0.0016	0.15
Age					
≤ 60 yrs	55	44 (80.0)	40 (72.7)	41 (74.5)	41 (74.5)
> 60 yrs	25	17 (68.0)	18 (72.0)	19 (76.0)	17 (68.0)
P value[a]	—	0.24	0.95	0.89	0.54
Silverberg grade					
1	12	10 (83.3)	9 (75.0)	8 (66.7)	9 (75.0)
2	49	37 (75.5)	35 (71.4)	35 (71.4)	36 (73.5)
3	19	14 (73.7)	14 (73.7)	17 (89.5)	13 (68.4)
P value[b]	—	0.63	0.99	0.17	0.70
FIGO grade					
1	23	18 (78.2)	16 (69.6)	15 (65.2)	17 (73.9)
2	33	24 (72.7)	23 (69.7)	25 (75.8)	23 (69.7)
3	24	19 (79.1)	19 (79.1)	20 (83.3)	18 (75.0)
P value[b]	—	0.94	0.49	0.18	0.93

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively: VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; FIGO: International Federation of Gynecology and Obstetrics.
[a] Chi-square test
[b] Mann–Whitney *U* test.

The frequency of peritoneal metastasis outside the pelvic cavity was significantly greater in patients with tumors that expressed VEGF-C ($P = 0.0010$) or VEGFR-2 ($P = 0.0008$), so did the frequency of lymph node metastasis in VEGF-C ($P = 0.0030$) and VEGFR-2 positive tumors ($P = 0.0018$). Positive results of ascitic cytology related to the expression of VEGF-C ($P = 0.025$) and VEGFR-2 ($P = 0.0016$). Conversely, tumors with peritoneal metastasis outside the pelvic cavity and lymph node metastases and tumors with positive ascitic cytology tended to have greater positivity in the expressions of VEGF-A and VEGFR-3, although the differences were not significant.

Age at surgery did not correlate to VEGF expression, although patients age ≤ 60 years tended to have higher rates of positive VEGF-A, VEGF-C, and VEGFR-3 expression. There was no significant difference between the expression of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3 and high FIGO or Silverberg grade.

VEGF expression levels also were examined as independent risk factors for peritoneal metastasis outside the pelvic cavity, lymph node metastasis, and positive ascitic cytology (Table 3). VEGFR-2 expression was related most closely to lymph node metastasis ($P = 0.027$; risk ratio, 5.11; 95% confidence interval [95%CI], 1.208–21.630), peritoneal metastasis outside the pelvic cavity ($P = 0.038$; risk ratio, 4.79; 95%CI, 1.087–21.079), and positive ascitic cytology ($P = 0.018$; risk ratio, 15.55; 95%CI, 1.592–151.884). VEGF-C expression also was an independent risk factor of peritoneal metastasis ($P = 0.026$; risk ratio, 4.82; 95%CI, 1.203–19.297) and lymph node metastasis ($P = 0.045$; risk ratio, 4.30; 95%CI, 1.031–17.957).

The disease-free and 5-year survival rates were determined using the Kaplan–Meier method (Table 4, Fig. 3). According to the staining level classification for VEGF-C expression, the 5-year survival rate was 90.9% (20 of 22 patients) for the negative-expression group, 81.5% (22 of 27 patients) for the low-expression group,

TABLE 3
Independent Risk Factors Associated with Metastasis

Variable	Coefficient	SE	Chi-square statistic	*P* value	RR	95% CI
Peritoneal metastasis outside the pelvic cavity						
VEGF-A	−1.03	0.77	1.83	0.18	0.36	0.079–1.592
VEGF-C	1.57	0.71	4.94	0.026	4.82	1.203–19.297
VEGFR-2	1.63	0.74	4.92	0.027	5.11	1.208–21.630
VEGFR-3	−0.57	0.67	0.73	0.39	0.56	0.151–2.100
MVD	0.034	0.014	5.54	0.019	1.04	1.006–1.064
Lymph node metastasis						
VEGF-A	−0.98	0.74	1.75	0.19	0.38	0.089–1.599
VEGF-C	1.46	0.73	4.01	0.045	4.30	1.031–17.957
VEGFR-2	1.57	0.76	4.29	0.038	4.79	1.087–21.079
VEGFR-3	−0.30	0.64	0.22	0.64	0.74	0.214–2.584
MVD	0.025	0.013	3.50	0.061	1.03	0.999–1.052
Ascitic cytology						
VEGF-A	0.64	1.03	0.39	0.53	1.91	0.254–14.306
VEGF-C	1.16	1.01	1.34	0.25	3.19	0.446–22.886
VEGFR-2	2.74	1.16	5.57	0.018	15.55	1.592–151.884
VEGFR-3	−0.33	1.06	0.098	0.75	0.72	0.090–5.701
MVD	−0.033	0.029	1.37	0.24	1.38	0.914–1.023

SE: standard error; RR: risk ratio; 95% CI: 95% confidence interval; VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; MVD: microvessel density.

and 45.2% (14 of 31 patients) for the high-expression group: The 5-year survival rate was significantly higher in patients with VEGF-C-negative tumors and in patients with low-expression tumors ($P = 0.0018$; log-rank test). Similarly, with regard to VEGFR-2 expression, the 5-year survival rate was 85.0% (17 of 20 patients) for the VEGFR-2-negative group, 83.9% (26 of 31 patients) for the low-expression group, and 44.8% (13 of 29 patients) for the high-expression group: The 5-year survival rate was significantly higher in patients with VEGFR-2-negative tumors and in patients with low-expression tumors ($P = 0.019$; log-rank test). Conversely, VEGF-A expression and VEGFR-3 expression were not related to the 5-year survival rate.

With regard to VEGFR-2 expression, the disease-free survival rate was 28.6% (8 of 29 patients) for the high-expression group, 67.7% (21 of 31 patients) for the low-expression group, and 85.0% (17 of 20 patients) for the negative-expression group. Recurrence rates increased along with increases in expression levels ($P = 0.0002$; log-rank test). With regard to the expression of VEGF-C and VEGFR-3, patients in the high-expression group tended to have higher recurrence rates, although there were no significant differences ($P = 0.25$ and $P = 0.054$, respectively; log-rank test). Patients who had tumors with high VEGF-A expression tended to have higher 5-year and disease-free survival rates.

Other factors that were included in the univariate analysis were peritoneal metastasis outside the pelvic cavity, lymph node metastasis, patient age at surgery, Silverberg grade, and FIGO grade. The presence or absence of peritoneal metastasis outside the pelvic cavity was related significantly to disease-free survival ($P = 0.042$; log-rank test) and 5-year survival ($P = 0.0013$; log-rank test). Lymph node metastasis also was related significantly to 5-year survival ($P = 0.0029$; log-rank test). Other factors did not show significant correlations.

A Cox regression analysis was used to examine the correlations between disease-specific and disease-free survival and VEGF-A expression, VEGF-C expression, VEGFR-2 expression, VEGFR-3 expression, FIGO stage, histology, age at surgery, FIGO grade, and MVD (Table 5). The results showed that the death rate in the high-VEGF-C-expression group was 5.0 times higher compared with the death rate in the low VEGF-C-expression group, and the death rate in the high VEGFR-2-expression group was 4.2 times higher compared with the death rate in the low VEGFR-2-expression group. The disease recurrence rate in the high VEGFR-2-expression group was 3.3 times higher compared with the disease recurrence rate in the low VEGFR-2-expression group.

CD31 was expressed in endothelial cells from blood and lymph vessels in the tumor stroma (Fig. 2F). Figure 4 summarizes the correlations between MVD and peritoneal metastasis outside the pelvic cavity ($P = 0.0054$), lymph node metastasis ($P = 0.027$), VEGF-A expression ($P = 0.0051$), and VEGF-C expression ($P = 0.042$; Mann–

TABLE 4
Univariate Analysis of Patients who Attained Disease-Free Survival or 5-Year Survival

		Disease-free survival		5-year survival	
Factor	No. of patients	No. (%)	*P* value	No. (%)	*P* value
Age					
≤ 60 yrs	55	28 (50.9)	0.16	39 (70.9)	0.62
> 60 yrs	25	18 (72.0)	—	17 (68.0)	—
Peritoneal metastasis outside the pelvic cavity					
Not present	38	27 (71.1)	0.042	35 (92.1)	0.0013
Present	42	19 (45.2)	—	21 (50.0)	—
Lymph node metastasis					
Not present	44	28 (63.6)	0.25	38 (86.4)	0.0029
Present	36	18 (50.0)	—	18 (50.0)	—
VEGF-A expression					
Negative	20	11 (55.0)	0.47	13 (65.0)	0.46
Low	43	24 (55.8)	—	30 (69.8)	—
High	17	11 (64.7)	—	13 (76.5)	—
VEGF-C expression					
Negative	22	14 (63.6)	0.25	20 (90.9)	0.0018
Low	27	17 (62.9)	—	22 (81.5)	—
High	31	15 (48.4)	—	14 (45.2)	—
VEGFR-2 expression					
Negative	20	17 (85.0)	0.0002	17 (85.0)	0.019
Low	31	21 (67.7)	—	26 (83.9)	—
High	29	8 (28.6)	—	13 (44.8)	—
VEGFR-3 expression					
Negative	22	18 (81.8)	0.054	16 (72.7)	0.99
Low	38	20 (52.6)	—	27 (71.1)	—
High	20	8 (40.0)	—	13 (65.0)	—
Silverberg grade					
1	12	9 (75.0)	0.24	10 (83.3)	0.055
2	49	29 (59.2)	—	32 (65.3)	—
3	19	8 (42.1)	—	14 (73.7)	—
FIGO grade					
1	23	15 (65.2)	0.84	18 (78.3)	0.17
2	33	20 (60.6)	—	20 (60.6)	—
3	24	11 (45.8)	—	18 (75.0)	—

VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained; FIGO: International Federation of Gynecology and Obstetrics.

Whitney *U* test). Conversely, MVD was not an independent risk factors of peritoneal metastasis outside the pelvic cavity ($P = 0.014$; risk ratio, 1.04; 95%CI, 1.006–1.064) (Table 3). MVD showed no significant correlation with either the disease recurrence rate ($P = 0.84$) or the 5-year survival rate ($P = 0.36$).

DISCUSSION

The prognostic value of neoplastic angiogenic factors is controversial. In the gynecologic oncology field, Kaku et al. emphasize the prognostic significance of both angiogenic factors and MVD for patients with endometrial carcinoma[19] and cervical adenocarcinoma[20]; whereas there are contrary opinions with regard to this correlation in patients with ovarian carcinoma.[21–24] There also are differing reports regarding VEGF-A expression as a poor prognostic factor[23] or as a factor that has no effect on some histologic tumor types.[24] These controversies suggest that the growth of ovarian carcinoma does not depend only on VEGF-A but also on the presence of other factors that affect the proliferation and infiltration of epithelial cancer cells. In the current study, the role of VEGF-C, as the other angiogenic factor and the corresponding receptor, VEGFR-3, was observed; and the tissue expression of VEGFR-2, as the common receptor for both VEGF-A and VEFF-C, also was examined.

The expression levels of VEGF-C and VEGFR-3 increased along with the progression of malignant potential from benign cystadenoma, to LPM tumor, and to adenocarcinoma. This finding agreed with the report of Yokoyama et al.[25] The expression of VEGF-C,

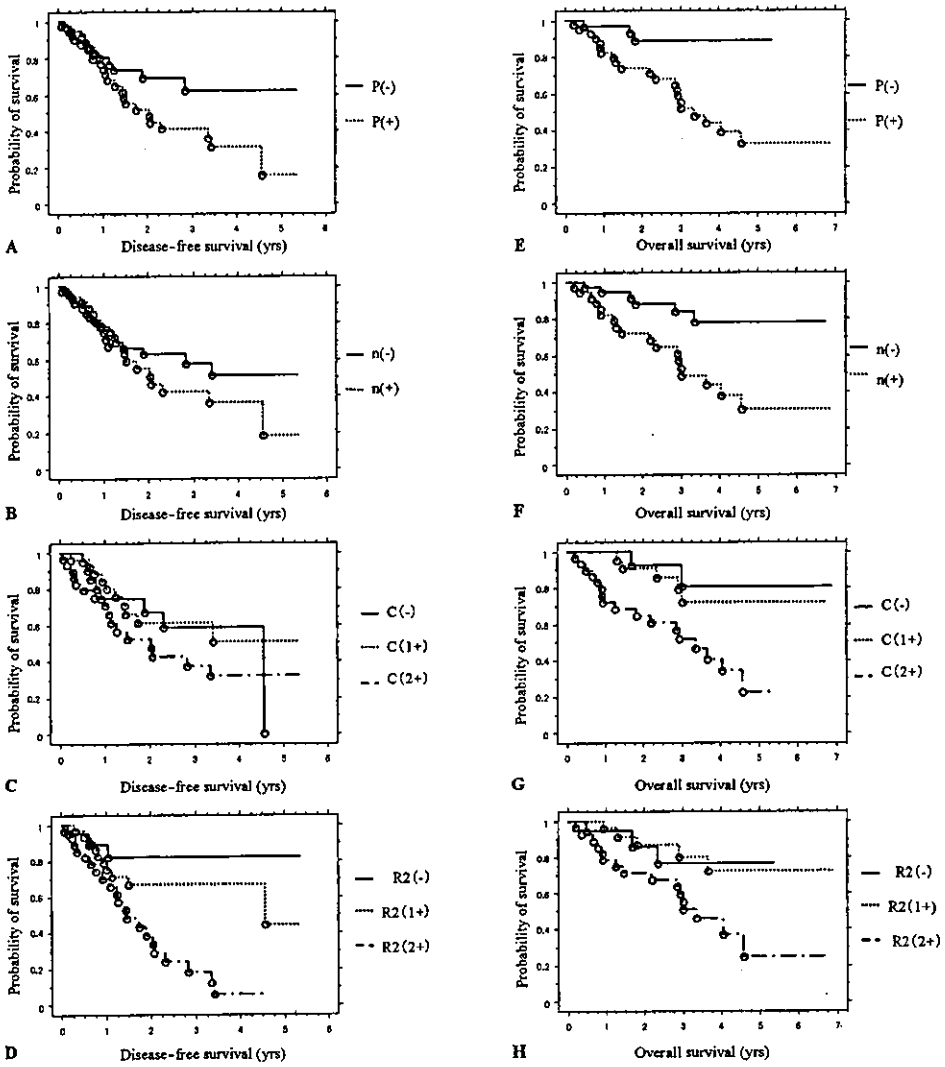

FIGURE 3. Kaplan–Meier analysis of overall survival and disease-free survival of patients after surgery for ovarian carcinoma according to the presence or absence of peritoneal metastasis (P) outside the pelvis (A,E), lymph node metastasis (n) (B,F), vascular endothelial growth factor C (C) expression (C,G), and VEGF receptor 2 (R2) expression (D,H).

VEGFR-3, VEGF-A, and VEGFR-2 was not related to the histologic type of ovarian carcinoma (Table 1). However, this result should be interpreted with caution, because the number of patients in each group was small.

In angiogenesis and lymphangiogenesis, it is known that VEGFR-1 and VEGFR-2 act as receptors for VEGF-A and that VEGFR-2 and VEGFR-3 act as receptors for VEGF-C.[4–9] The current study in patients with ovarian carcinoma also showed a significant correlation between expression of the two angiogenesis factors and their corresponding receptors and expression of the two angiogenesis factors and MVD (Fig. 4). These findings suggest that VEGF-A and VEGF-C act on their corresponding receptors and initiate angiogenesis in ovarian carcinoma.

It has been reported that VEGF-A and VEGF-C are expressed not only in tumor cells but also in endothelial cells from blood and lymph vessels and on fibrous connective tissues from tumor stroma.[26–28] In the current study, we found that their receptors also are expressed both in tumor cells and in endothelial cells from blood and lymph vessels. This indicates that there are both paracrine and autocrine mechanisms: VEGF-A and VEGF-C produced by tumor cells act on their corresponding receptors on endothelial cells through a paracrine mechanism, promote angiogenesis and lymphangiogenesis, and may affect hematogenous and lymphogenous metastases; the factors also may act on receptors on the tumor cells through an autocrine mechanism and promote tumor proliferation (Tables 2, 3).

In the FIGO grading system, in which tumor histology was graded according to the ratio of solid components, there were no significant differences of any other factor. According to the Silverberg grade, which is the overall evaluation of architectural pattern, nu-

TABLE 5
Multivariate Cox Regression Analysis for Disease-Free Survival or Carcinoma-Specific Survival

	Disease-free survival			Carcinoma-specific survival		
Variable	OR	95% CI	*P* value	OR	95% CI	*P* value
Age						
≤ 60 yrs	1.21	0.380–3.833	0.75	0.74	0.181–3.007	0.67
> 60 yrs	1.00			1.00		
Histology						
Clear cell	1.29	0.396–5.423	0.73	6.01	0.920–39.232	0.061
Endometrioid	1.27	0.426–3.788	0.67	1.77	0.458–6.865	0.41
Mucinous	0.945	0.004–0.502	0.012	1.11	0.131–9.424	0.92
Serous	1.00			1.00		
Stage						
I/II	0.60	0.202–1.760	0.35	0.072	0.009–0.572	0.013
III/VI	1.00			1.00		
FIGO grade						
1	1.36	0.440–4.213	0.59	3.53	0.753–16.095	0.11
2	2.01	0.745–5.436	0.17	4.17	1.124–15.456	0.033
3	1.00			1.00		
VEGF-A expression						
Negative	7.16	1.719–29.826	0.0070	2.83	0.497–16.095	0.24
Low	2.03	0.631–6.543	0.23	4.06	0.872–18.887	0.074
High	1.00			1.00		
VEGF-C expression						
Negative	1.76	0.531–5.843	0.36	0.29	0.043–1.937	0.20
Low	0.73	0.256–2.103	0.56	0.20	0.051–0.812	0.035
High	1.00			1.00		
VEGFR-2 expression						
Negative	0.33	0.068–1.593)	0.17	2.96	0.462–18.992	0.25
Low	0.30	0.105–0.859	0.025	0.24	0.066–0.892	0.033
High	1.00			1.00		
VEGFR-3 expression						
Negative	0.22	0.044–1.130	0.84	1.02	0.203–5.094	0.98
Low	1.11	0.391–3.162	0.23	1.86	0.536–6.452	0.32
High	1.00			1.00		
MVD	1.01	0.992–1.036	0.84	1.01	0.987–1.038	0.36

OR: odds ratio; 95% CI: 95% confidence interval; FIGO: International Federation of Gynecology and Obstetrics; VEGF-A/VEGF-C: vascular endothelial growth factors A and C, respectively; VEGFR-2/VEGFR-3: VEGF receptors 2 and 3, respectively; negative: 0–10% of cells were stained; low: from 10% to < 50% of cells were stained; high: ≥ 50% of cells were stained; MVD: microvessel density.

clear pleomorphism, and mitotic activity,[15,16] there also were no significant differences for other factors. These results show that histologic level and tumor cell atypism are not related to angiogenesis or lymphangiogenesis.

Prognostic factors, such as peritoneal metastasis outside the pelvic cavity and lymph node metastasis, are determined by surgical staging. Examination of these prognostic factors and their correlation with VEGF-A, VEGF-C, VEGFR-2, VEGFR-3, and MVD revealed that VEGF-C and VEGFR-2 have significant effects on prognosis (Tables 2, 3).

Patients who had tumors with high expression of VEGF-C and VEGFR-2 had a significantly high death rate, which also indicates that these factors are related to patient prognosis. Yokoyama et al. examined the relation between VEGF-C expression and death rates and showed that VEGF-C was related to the 10-year survival rate.[25] Patients in the current study were followed for a much shorter period (mean, 132.8 weeks); however, high expression levels of VEGF-C and VEGFR-2 were related to death from disease. Conversely, no significant correlation was observed in the current study between negative expression or low expression levels of VEGF-A, VEGF-C, VEGFR-2, or VEGFR-3 and the recurrence rate or death rate: This may be attributable to our short follow-up.

It is known that the VEGF family and VEGFRs affect the prognosis of patients with adenocarcinoma that develops in the uterus[19] and ovaries[25,29] and in patients with gastric[30,31] and colorectal carcinomas,[32,33] breast carcinoma,[34,35] lung carcinoma,[36–38] head and neck squamous cell carcinoma,[39] Kaposi

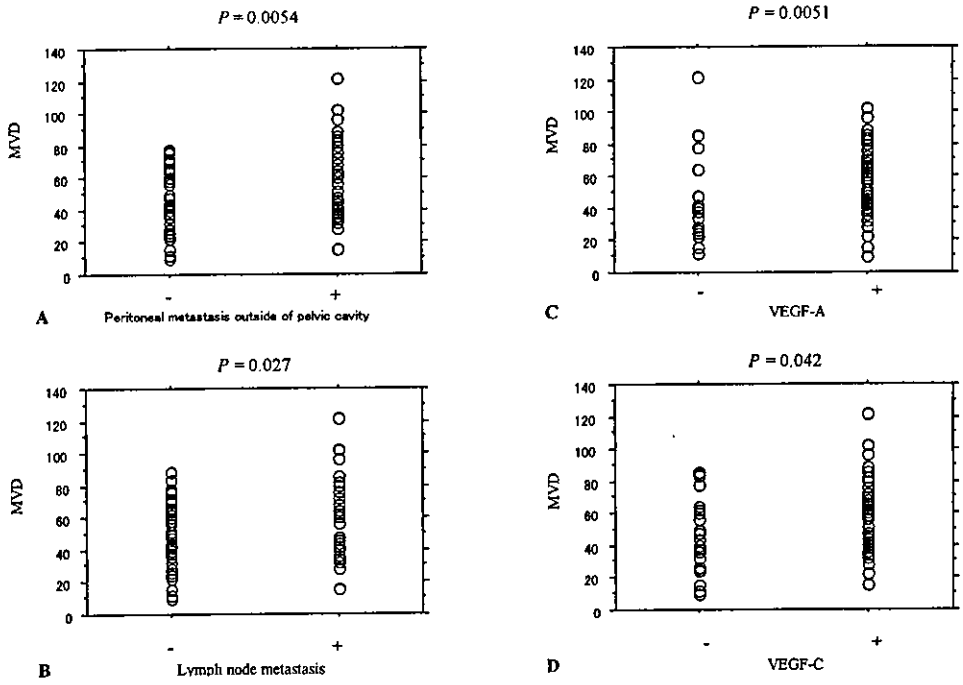

FIGURE 4. Plot of microvessel density (MVD) in relation to peritoneal metastasis outside the pelvis (A), lymph node metastasis (B), vascular endothelial growth factor A (VEGF-A) expression (C), and VEGF-C expression (D). The *P* value shown in each figure represents the significant level of correlation (Mann–Whitney *U* test).

sarcoma,[40] and malignant mesothelioma.[41] The relation between ovarian carcinoma and VEGF-C expression was examined in a previous study[25]; however, in the current study, we investigated the correlation between malignant potential and the expression of VEGFR-2 and VEGFR-3 (the receptor of VEGF-C) and MVD in patients with ovarian carcinoma for the first time. Our findings show that VEGF-C and VEGFR-2 expression levels relate to peritoneal metastasis and lymph node metastasis. The expression of VEGF-C and VEGFR-2 may be used to predict the metastatic spread of ovarian carcinoma cells and to identify patients prospectively who are at a high risk of a poor outcome. The suppression of VEGF-C and VEGFR-2 using angiogenesis suppressors or receptor inhibitors may suppress not only angiogenesis in tumor but also growth of the tumor itself.

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