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*Cellular and Molecular Biology*

# Immunolocalizations of VEGF, its receptors flt-1, KDR and TGF-ß's in epithelial ovarian tumors

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**Summary.** Objective: Angiogenesis is an essential factor for growth, differentiation, invasion and metastasis of tumors. In this study, we aimed to evaluate the immunolocalizations of vascular endothelial growth factor (VEGF), its receptors flt-1, KDR/flk-1, and transforming growth factor-beta's (TGF-ß) in epithelial ovarian tumors, utilizing indirect immunohistochemistry to understand the role of the angiogenic events in ovarian neoplasia. Methods: Tissue blocks from 40 patients who had ovarian pathology (borderline serous–mucinous tumor and malignant serous–mucinous adenocarcinoma of the ovary) were included in this study. All formalin-fixed, paraffin-embedded tissue sections were stained with hematoxylin-eosin or primary antibodies against VEGF, flt-1, KDR/flk-1, TGF-ß1, TGF-ß2 and TGF-ß3 using the avidin-biotin-peroxidase method. H-SCORE, a semi-quantitative grading system, was used to compare immunohistochemical staining intensities. Results: Positive VEGF immunoreactivity was concentrated in the epithelial and stromal parts of all the ovarian samples and the endothelial cells in the stroma were also stained. Increased immunoreactivity of VEGF was observed in malignant ovarian adenocarcinomas compared to the borderline tumors of the ovary. VEGF receptors, flt-1 and KDR/flk-1 immunoreactivities were detected not only in vascular endothelial cells, but also in tumor cells at malignant sites. Immunoreactivities of VEGF and its receptors were coexpressed in tumor cells of the ovarian carcinoma. While immunoreactivities of TGF-ß1 and TGF-ß2 were both overexpressed in malignant ovarian carcinomas, immunoreactivity of TGF-ß3 was still mild. Conclusion: Our results suggest that overexpression of VEGF, its receptors flt-1, KDR/flk-1 and TGF-ß interaction may play an important role in the ovarian cancer biology, with potential effects on tumor growth and angiogenesis. New therapeutic strategies using VEGF and TGF-ß antagonists could obtain an additional approach to the treatment ovarian carcinoma by inhibiting angiogenesis.

**Key words:** Ovarian carcinoma, VEGF, flt-1, KDR, TGF-ß

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## Introduction

Epithelial ovarian cancer is the fifth most common malignancy among women. However it has the highest gynecological malignancy fatality (Berek et al., 1996). Ovarian cancer growth is angiogenesis-dependent and an increase in the production of angiogenic growth factors is prognostically significant (Byrne et al., 2003). Angiogenesis is the process of new capillaries developing from preexisting vessels. It is induced by inflammation, healing wounds, immune reactions and neoplasia (Folkman and Klagsbrun, 1987; Patan et al., 2004). Tumor cells have been shown to secrete a variety of angiogenic factors and thereby inducing local formation of new blood capillaries (Folkman, 1986). Vascular endothelial, acidic and basic fibroblast and platelet-derived growth factors appear to be the most important promoters of angiogenesis. Other possible promoters include; transforming growth factor-ß, transforming growth factor-ß, folliculostellate-derived growth factor, angiotropin and tumour necrosis factor-ß (Folkman, 1986; Abulafia and Sherer, 2000).

Vascular endothelial growth factor (VEGF), a bifunctional glycoprotein, enhance vascular permeability and stimulate endothelial growth, which is a

chemoattractant for endothelial cells, inducer of capillary tube formation and which also is stimulated by hypoxia, cytokines and hormones (Folkman, 1986; Abulafia and Sherer, 2000; Gadducci et al., 2003; Tanir et al., 2003). VEGF induces endothelial cell proliferation, promotes cell migration and inhibits apoptosis. Human VEGF could be expressed in at least 5 isoforms, which have 206, 189, 165, 145 and 121 aminoacids, respectively (Sowter et al., 1997; Hazelton and Hamilton, 1999; Neufeld et al., 1999; Sonoda et al., 2003). These isoforms differ in their molecular mass and in biological properties such as their ability to bind to cell-surface heparan-sulfate proteoglycans. The various VEGF forms bind to two tyrosine-kinase receptors, VEGFR-1 (fms-like tyrosine kinase: flt-1) and VEGFR-2 (kinase domain-containing receptor: KDR/flk-1), which are expressed almost exclusively in endothelial cells (Boocock et al., 1995; Abu-Jawdeh et al., 1996; Orre and Rogers, 1999). Deregulated VEGF expression contributes to the development of ovarian tumors by promoting tumor angiogenesis and to the etiology of several additional diseases that are characterized by abnormal angiogenesis (Brown et al., 2000; Bamberger and Perrett, 2002; Wong et al., 2003).

Members of the transforming growth factor beta (TGF-ß) family are multifunctional cytokines with key roles in tissue morphogenesis, growth, angiogenesis and vasculogenesis (Pepper, 1997). There are three isotypes of TGF-ß (-ß1, -ß2 and -ß3) and their amino acid sequences display homologies on the order of 70-80%. They are abundant in mammalian reproductive tissues, where development and cyclic remodelling continue in post-natal and adult life (Ingman and Robertson, 2002). The most pronounced differences in the TGF-ß isoforms is, their spatially and temporally distinct expression of both the mRNAs and proteins in developing tissues, regenerating tissues and in pathologic responses, including ovarian carcinoma (Henriksen et al., 1995). Increased expressions of different TGF-ß isotypes have been associated with more aggressive tumor behavior and worse, prognosis in ovarian cancer (Bartlett et al., 1997). TGF-ß1 is the prevalent form and is found almost ubiquitously while the other isoforms are expressed in a more limited spectrum of cells and tissues. Rodriguez et al. suggest that TGF-ß1 and TGF-ß2 may enhance the invasiveness of ovarian cancers (Rodriguez et al., 2001).

The aim of this study was to evaluate the expression of angiogenesis-related factors VEGF, its receptors, flt-1 and KDR/flk-1 and three isotypes of TGF-ß, in borderline and malignant serous- mucinous ovarian carcinoma by using indirect immunohistochemistry.

## Materials and methods

The Ethics Committee at The Aegean Maternity Hospital approved our study protocol and all the patients involved granted their informed consent. Forty patients with borderline serous tumor, borderline mucinous tumor, mucinous adenocarcinoma and serous adenocarcinoma of the ovary (n=10 each) were treated at the Department of Gynecology and Obstetrics, Aegean Maternity Hospital (Izmir-Turkey), were used in the study. Patients age ranged from 42 to 65 years and the patient characteristics are demonstrated in Table 1. None of the patients had previous or syncronous cancer. All of the patients underwent laparotomy as initial treatment and specimens were obtained from the primary tumor on the ovary.

All specimens were fixed in 10% formalin during 24 h. Specimens were washed and soaked in a graded series of ethanol and cleaned in xylene. Then they were embedded in paraffin. Sections (5 µm thick) were cut and prepared for both histochemical and immuno-histochemical staining. The tissue blocks were characterized for the type of ovarian tumor, after histologic assessment of sections stained with hematoxylin and eosin (H.E.) at the Department of Pathology, Aegean Maternity Hospital. The criteria for the diagnosis of borderline tumors were epithelial proliferation with papillary formation and pseudostratification, nuclear atypia, increased mitotic activity and absence of true stromal invasion without tissue destruction.

For immunohistochemical staining, the samples were first incubated in 60°C overnight and then held in xylene for 30 min. After washing with a decreasing series of ethanol, the sections were washed with distilled water and phosphate buffered saline (PBS) for 10 min. Then they were held in 1% trypsin in tris buffer at 37°C for 15 min and washed with PBS. Sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% $H_2O_2$ for 15 min to inhibit endogenous peroxidase activity. Then sections were washed with PBS three times for 5 min each and incubated for 18 h at +4°C with primary antibodies; anti-TGF-ß1 (SC-146, rabbit Pab, Santa Cruz, California), anti-TGF-ß2 (SC-90, rabbit Pab, Santa Cruz, California), anti-TGF-ß3 (SC-82, rabbit Pab, Santa Cruz, California), anti-VEGF (SC-7269, mouse Mab, Santa Cruz, California), anti-flt-1 (RB-1527-R1, rabbit Pab, NeoMarkers, California), anti-KDR/flk-1 (RB-1526-R1, rabbit Pab, NeoMarkers, California). Afterwards, sections were washed three times for 5 min each with PBS, followed by incubation with biotinylated IgG and then with streptavidin-peroxidase conjugate (Histostain Plus kit Zymed 87-9999, San Francisco, CA). After washing with PBS three times for 5 min, these sections

**Table 1.** Demographic characteristics of the patients.

| N: 10 | Age | Parity |
|---|---|---|
| Borderline serous tumour (BLS) | 49±7.2 | 2.7±1.1 |
| Borderline musinous tumour (BLM) | 48±3.4 | 3.4±1.2 |
| Malignant serous adenocarcinoma (SAC) | 58±6.1 | 2.5±0.6 |
| Malignant mucinous adenocarcinoma (MAC) | 53±5.2 | 2.4±1.0 |

were colored with diaminobenzidine (DAB) to stain immunolabelling and then counter-stained with Mayer's hematoxylin. Sections were covered with entellan and were observed with a light microscopically with a BX 40 microscope (Olympus, Tokyo, Japan). Control samples were processed in an identical manner, in order to primary antibodies the same type of IgG was used. Two observers, blinded to clinical information, evaluated the staining scores independently. Staining intensity was graded semi-quantitatively using the HSCORE that was calculated with the following equation: HSCORE= Σ Pi (i+1), where i = intensity of staining with a value of (±), (+), (++) or (+++) (minimal, mild, moderate, or strong, respectively) and Pi is the percentage of epithelial cells stained with each intensity, varying between 0-100 %. Results were expressed as mean ± SE. Differences among groups were statistically analysed with one-way ANOVA where appropriate. A p value of $<0.05$ was considered significant.

## Results

### *Histochemical evaluation*

Histological analyses of the samples of the ovarian tumors were determined in H.E staining sections (Fig. 1). The samples of the borderline serous tumor of the ovary revealed that the papillary pattern had an appearance similar to that of the epithelium lining of the fallopian tube (Fig. 1A). In these samples, complex papillary fronds were lined with columnar cells; the epithelium and the stroma were clearly separated by a basement membrane, indicating no stromal invasion (Fig. 1A). The samples of the borderline mucinous tumor of the ovary revealed that tumors were made up largely of endocervical-mucus secreting cells that resemble the endocervical glands (Fig. 1B). In malignant serous carcinomas, papillary and glandular structures were predominate, clusters and papillae of malignant cells were in direct contact with fibrous stroma, indicative of stromal invasion (Fig. 1C). In malignant mucinous carcinomas, irregular glandular spaces were lined with tall columnar cells with abundant mucinous cytoplasm, resembling endocervical cells (Fig. 1D). It was seen that although papillary proliferations were much less common in the mucinous tumors than in the serous tumors, such alterations were basic evidence of atypical proliferation.

### *Immunohistochemical evaluation*

Positive immunoreactivities of VEGF and its receptors flt-1, KDR/flk-1 were determined in the epithelial and stromal part of all the ovarian samples, in

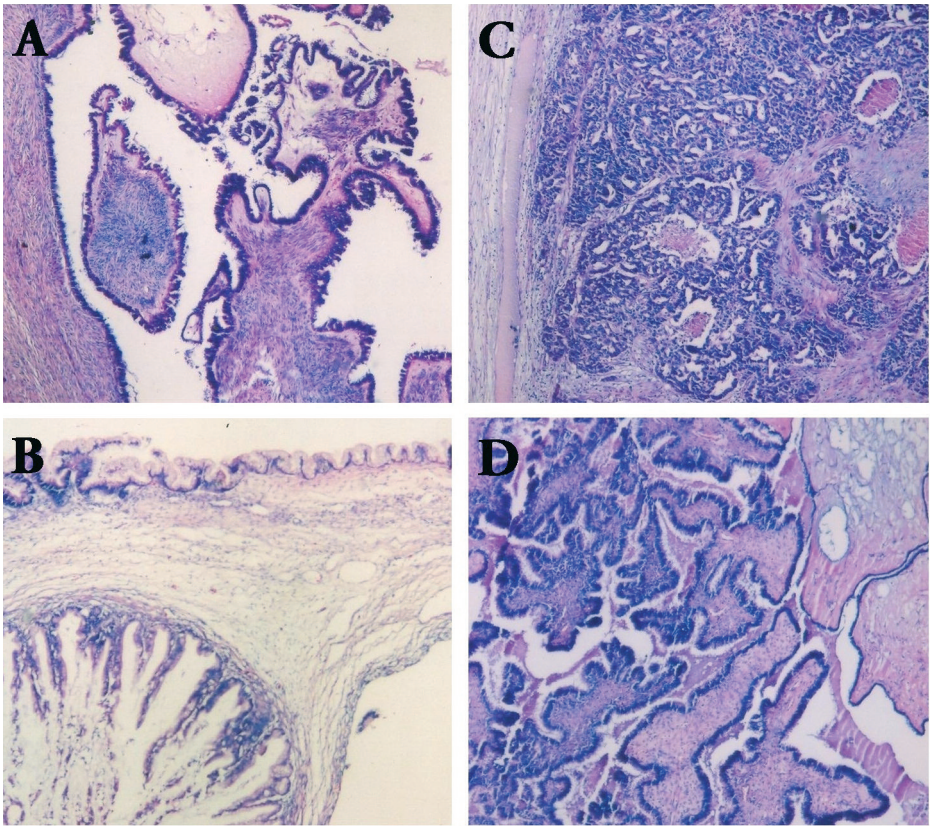


**Fig. 1.** Hematoxylin-Eosin (HE) stained sections of various ovarian neoplasia H.E. x40 (Original Magnification). **A.** Borderline serous tumor of the ovary: Complex papillary folds are lined with pseudostratified columnar cells. The epithelium and the stroma are clearly separated by basement membrane, indicating no stromal invasion. **B.** Borderline mucinous tumor of ovary. Tumors are made up largely of endocervical-mucus secreting cells. **C.** Malign serous adenocarcinoma of ovary: Clusters and papillae of malignant cells are in direct contact with fibrous stroma indicative of stromal invasion. **D.** Mucinous adenocarcinoma of the ovary: irregular glandular spaces are lined with a layer of tall columnar cells with abundant mucinous cytoplasm, resembling endocervical cells.

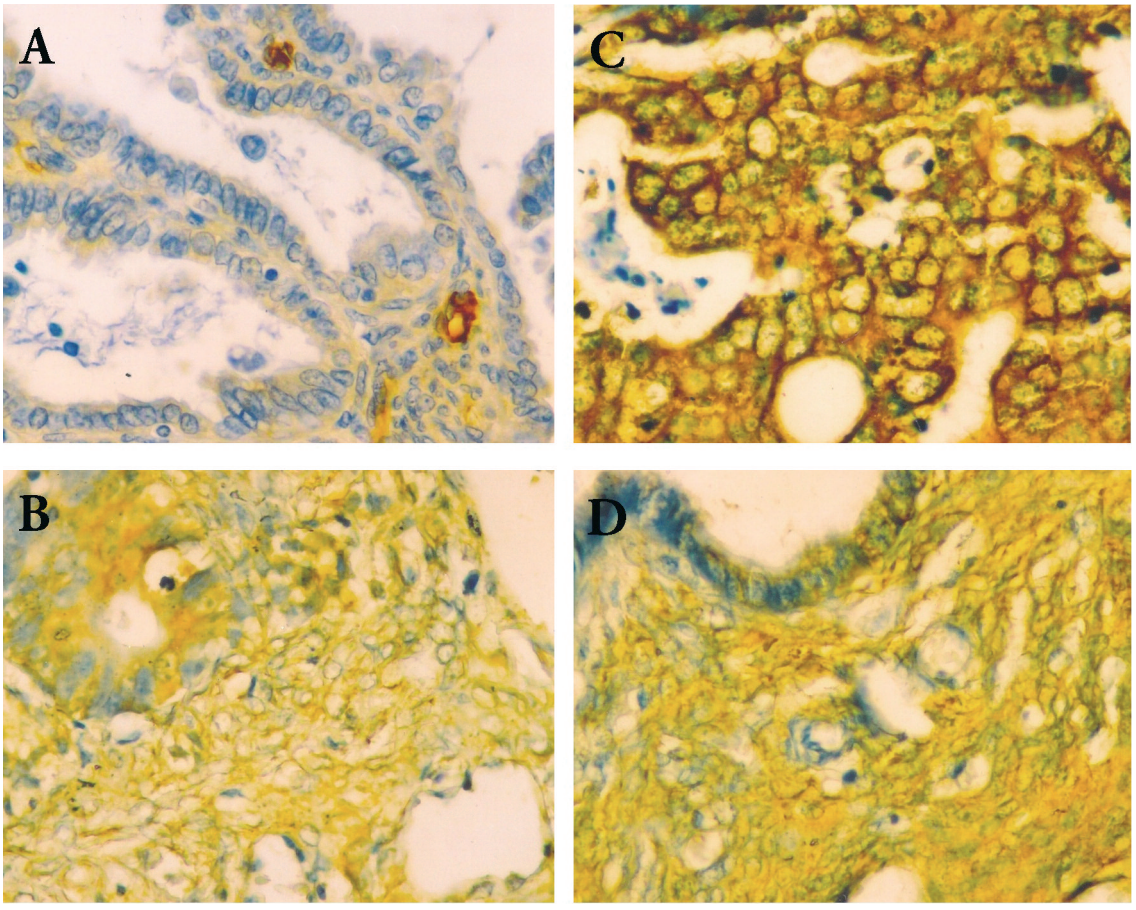


**Fig. 2.** Immunohistochemical stainings of VEGF **(A-D)** in different ovarian tumors x 400 (Original Magnification). Intensities of VEGF were detected as moderate in serous **(A)** and mucinous **(B)** borderline tumors, strong in malignant serous **(C)** and malignant mucinous **(D)** adenocarcinoma.

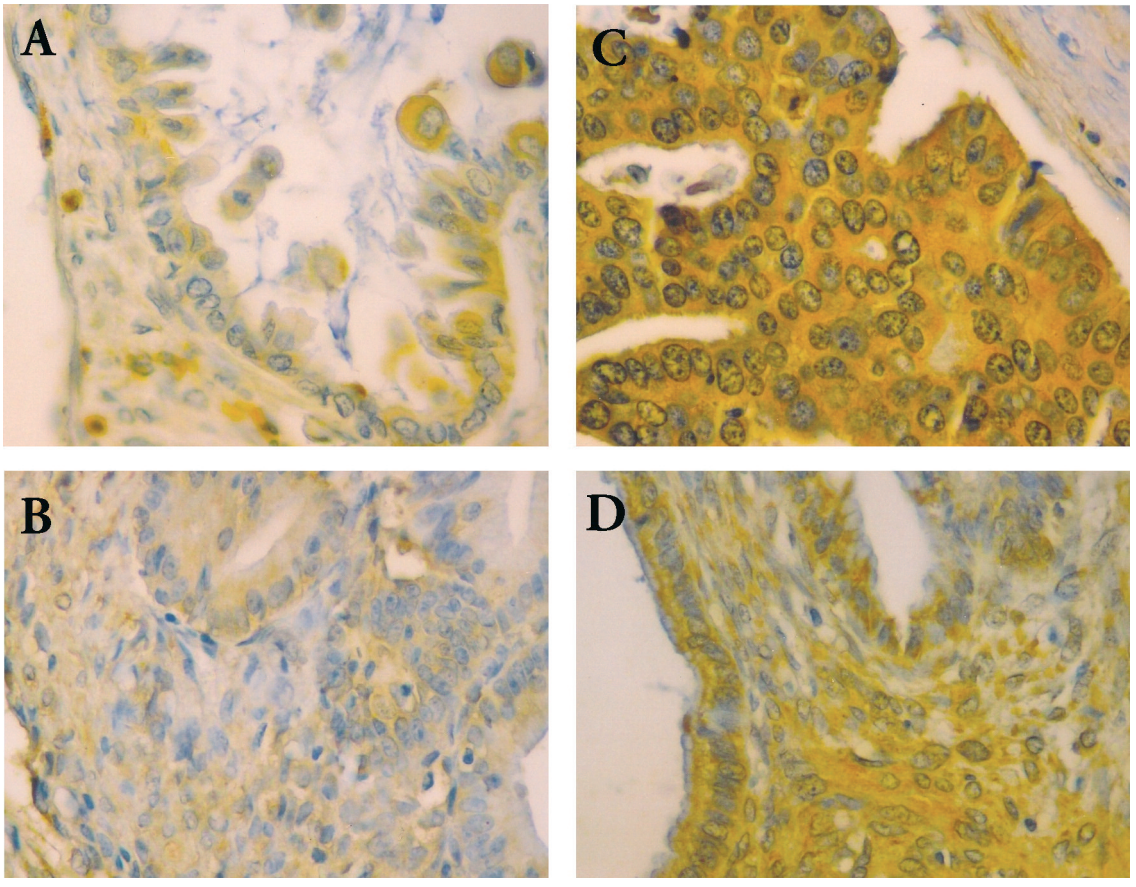


**Fig. 3.** Immunohistochemical stainings of Flt-1 **(A-D)** in different ovarian tumors x 400 (Original Magnification). Immunoreactivity of Flt-1 was detected not only in vascular endothelial cells but also in tumor cells at malignant sites. These immunoreactivities were coexpressed with VEGF immunostaining. Intensities of VEGF were detected as moderate in serous **(A)** and mucinous **(B)** borderline tumors, strong in malignant serous **(C)** and malignant mucinous **(D)** adenocarcinoma.

addition, vascular endothelial cells in the stroma were also stained (Figs. 2-4). Positive immunoreactivity of VEGF was concentrated in a single layer of epithelial cells in the stromal matrix, endothelial cells and clusters of tumor cells in borderline and malign ovarian tumors (Fig. 2A-D). Intensities of VEGF were detected as moderate in serous (Fig. 2A) and mucinous (Fig. 2B) borderline tumors, strong in malignant serous adenocarcinoma (Fig. 2C) and in malignant mucinous adenocarcinoma (Fig. 2D). Immunoreactivities of flt-1 (Fig. 3A-D) and KDR/flk-1 (Fig. 4A-D) were detected not only in vascular endothelial cells but also in tumor cells at malignant sites. These immunoreactivities were coexpressed with VEGF immunostaining. Increased immunoreactivities of VEGF and its receptors were observed in malignant ovarian adenocarcinamas, when compared with the borderline tumors of the ovary. These immunoreactivities were statistically different according to H-SCORE. All of the immunoreactivity intensities and H SCORE of VEGF and its receptors are summarized in Table 2.

As a result of immunohistochemical staining, in the evaluation of the tumors of the ovary, the distributions of TGF-ß1, TGF-ß2 and TGF-ß3 were observed in the epithelial, stromal and tumor cells (Figs. 5-8). While immunostaining of TGF-ß1 (Figs. 5A, 6A) and TGF-ß2

**Table 2.** Immunostaining intensities and HSCORE of VEGF and its receptors in various ovarian carcinoma.

| | VEGF | | flt-1 | | KDR/flk-1 | |
|---|---|---|---|---|---|---|
| | Intensity | H Score | Intensity | H Score | Intensity | H Score |
| Borderline serous (BLS) tumor | (++) | 214±7.5 | (++) | 220±4.5 | (++) | 222±5.8 |
| Borderline mucinous (BLM) tumor | (++) | 227±8.1 | (++) | 211±6.5 | (++) | 224±9.1 |
| Malignant serous adenocarcinoma (SAC) | (+++) | * 384±9.3 | (+++) | * 373±7.0 | (+++) | * 367±9.9 |
| Malignant mucinous adenocarcinoma MAC) | (+++) | * 378±10.2 | (+++) | * 362±9.6 | (+++) | * 360±8.2 |

(Mean ± SD). * SAC and MAC vs BLS and BLM p< 0.001

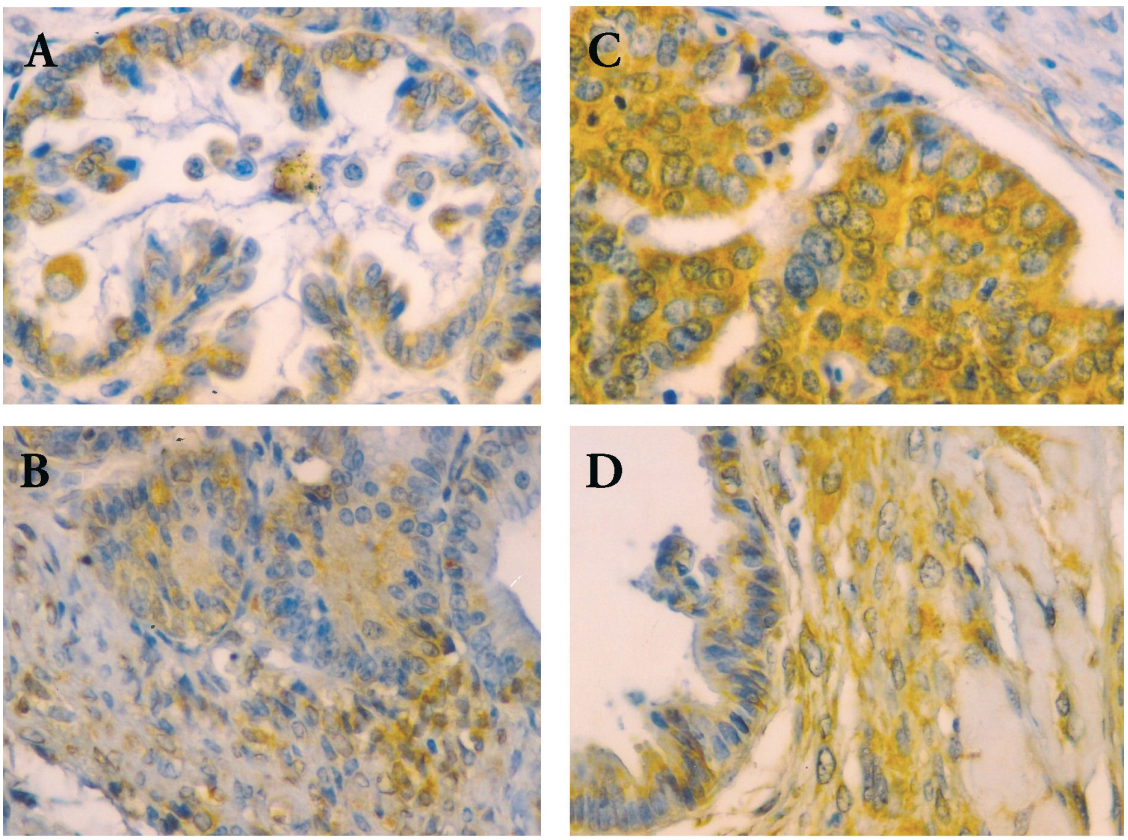


**Fig. 4.** Immunohistochemical stainings of KDR/Flk-1 **(A-D)** in different ovarian tumors x 400 (Original Magnification). Immunoreactivity of KDR/flk-1 was detected not only in vascular endothelial cells but also in tumor cells at malignant sites. These immunoreactivities were coexpressed with VEGF immunostaining. Intensities of VEGF were detected as moderate in serous **(A)** and mucinous **(B)** borderline tumors, strong in malignant serous **(C)** and malignant mucinous **(D)** adenocarcinoma.

(Figs. 5B, 6B) were observed as moderate in the borderline tumors, TGF-ß3 (Figs. 5C, 6C) was observed mild or minimal.

Strong immunostaining of TGF-ß1 (Figs. 7A, 8A) was detected in malignant adenocarcinomas of the ovary compared with the borderline tumors. Moderate

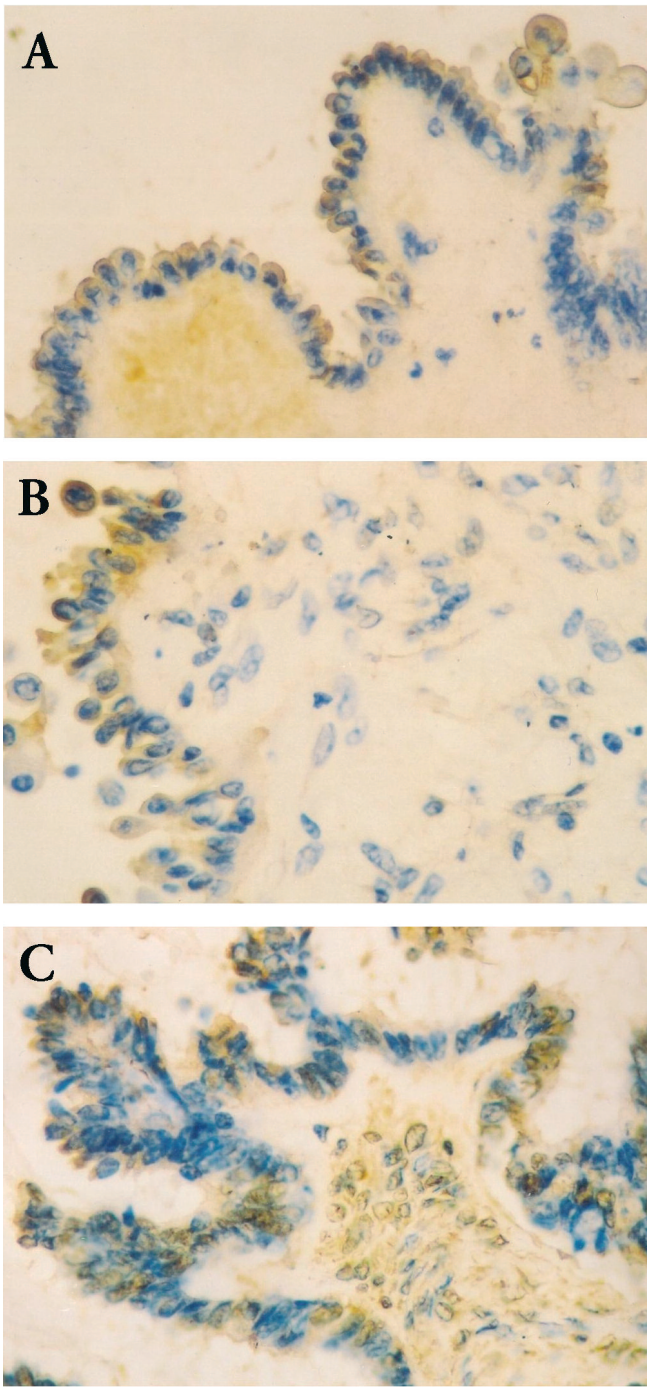


**Fig. 5.** Immunohistochemical stainings of TGF-ß's in borderline serous tumor of ovary. **A.** TGF-ß1, moderate immunoreactivity. **B.** TGF-ß2, moderate immunoreactivity. **C.** TGF-ß3, mild immunoreactivity. x 400 (Original Magnifications).

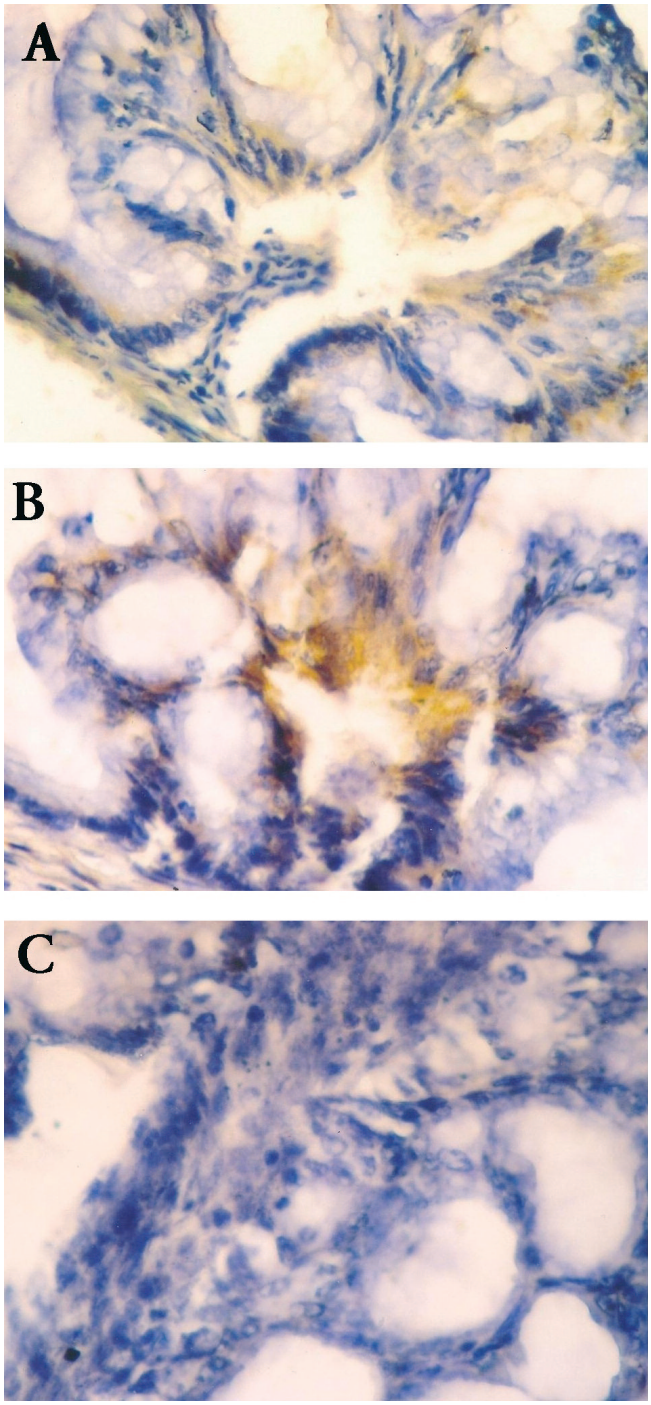


**Fig. 6.** Immunohistochemical stainings of TGF-ß's in borderline mucinous tumor of ovary. **A.** TGF-ß1, moderate immunoreactivity. **B.** TGF-ß2, moderate immunoreactivity. **C.** TGF-ß3, minimal immunoreactivity x 400 (Original Magnifications)

immunoreactivity of TGF-ß2 (Figs. 7B, 8B) and mild or moderate immunoreactivity of TGF-ß3 (Figs. 7C, 8C) were concentrated in only tumor cells. Intencity of TGF-ß1 was significantly more intense in adenocarcinomas of the ovary than the borderline tumors. Although intensity of TGF-ß2 was similar in all ovarian tumours, there was statistically significance in malignant adenocarcinomas according to H-SCORE. Immunoreactivity of TGF-ß3 was increased in only malignant mucinous adenocarcinoma, this intensity was statistically different to the other type of ovarian tumors. All of the intensities of TGF-ß immunoreactivities in the tumors of the ovary are shown in Table 3.

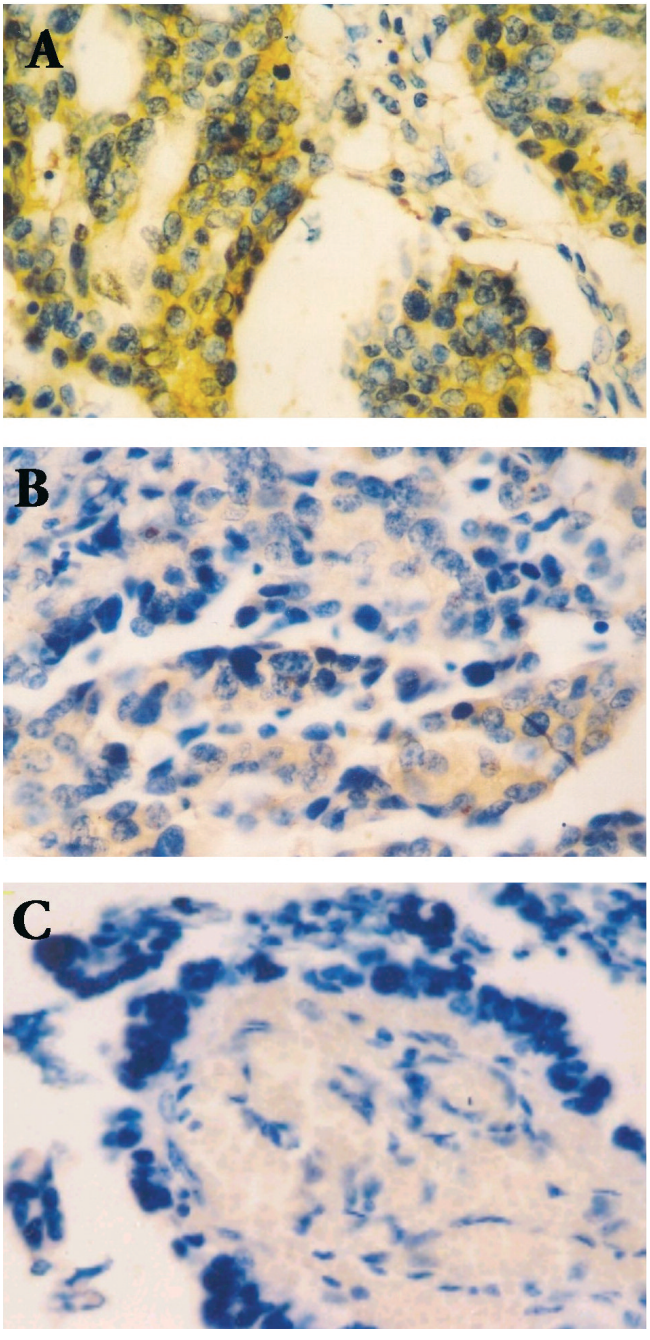


**Fig. 7.** Immunohistochemical stainings of TGF-ß's in malignant serous adenocarcinoma of ovary. **A.** TGF-ß1, strong immunoreactivity. **B.** TGF-ß2, moderate immunoreactivity. **C.** TGF-ß3, mild immunoreactivity x 400 (Original Magnifications)

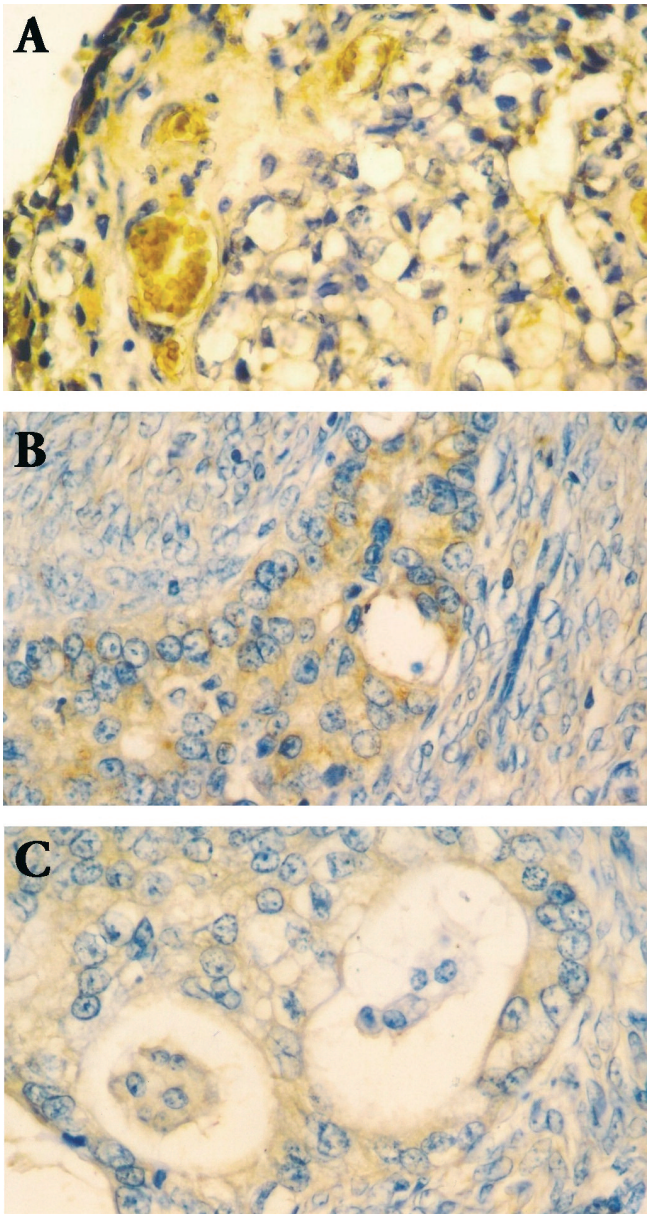


**Fig. 8.** Immunohistochemical stainings of TGF-ß's in malignant mucinous adenocarcinoma of ovary. **A.** TGF-ß1, strong immunoreactivity. **B.** TGF-ß2, moderate immunoreactivity. **C.** TGF-ß3, moderate or mild immunoreactivity. x 400 (Original Magnifications).

**Table 3.** Immunostaining intensities and HSCORE of TGF-ß1, TGF-ß2 and TGF-ß3 in various ovarian carcinoma.

| | TGF-ß1 | | TGF-ß2 | | TGF-ß3 | |
|---|---|---|---|---|---|---|
| | Intensity | H Score | Intensity | H Score | Intensity | H Score |
| Borderline serous (BLS) tumor | (++) | 110±16.4 | (++) | 88±3.0 | (+) | 26±4.5 |
| Borderline mucinous (BLM) tumor | (++) | 100±19.2 | (++) | 102±18.0 | (+/-) | 18±2.7 |
| Malignant serous adenocarcinoma (SAC) | (+++) | * 286±15.0 | (++) | * 162±23.2 | (+) | 24±4.0 |
| Malignant mucinous adenocarcinoma (MAC) | (+++) | * 318±26.6 | (++) | * 186±21.0 | (+/++) | ** 52±7.3 |

* SAC, MAC TGF ß1 and ß2 vs BLS and BLM p<0.001. ** MAC TGF ß3 vs SAC, BLS, MBL p<0.001

## Discussion

In this study, angiogenesis was studied in patients with epithelial ovarian neoplasia, such as borderline serous and mucinous tumors, malignant serous and mucinous adenocarcinomas of the ovary. The relationship between immunohistochemical staining for VEGF, its receptors, and TGF-ß's were graded on a semi-quantitative scalei. Analysis of immunohistochemical staining demonstrated that increased immunoreactivities of VEGF, flt-1 and KDR/flk-1 were detected in all malignant ovarian carcinomas. TGF-ß's were present in all of the tissue specimens examined, including borderline and malignant tumor specimens but immunostaining intensities of sub groups of TGF-ß's (TGF-ß1, TGF-ß2 and TGF-ß3) were distributed differently. While immunoreactivities of TGF-ß1 and TGF-ß2 were significantly more intense in adenocarcinomas of the ovary than the borderline tumors, immunoreactivity of TGF-ß3 was increased only in malignant mucinous ovarian tumors.

Angiogenesis, the formation of new vessels from pre-existing vasculature, is critical for tumor development and metastasis; and many growth factors important to ovarian cancer invasion are also prominent in its associated angiogenesis (Folkman, 1986; Abulafia and Sherer, 2000; Patan, 2004;). Deregulation of normal angiogenic processes occurs with the cancer's acquisition of the ability to secrete pro-angiogenic factors (Gadducci et al., 2003; Sonoda et al., 2003; Tanir et al., 2003). The local imbalance of endogenous angio-stimulators and angio-inhibitors promotes angiogenesis. Assessment of these pro-angiogenic growth factors and enumeration of tumor-associated microvessels have been shown to be prognosticators of ovarian cancer outcome, and may also be surrogates of ovarian cancer formation (Hazelton and Hamilton, 1999). VEGF is the angiogenic growth factor most strongly implicated in tumor angiogenesis. Its special role in the pathophysiology of ovarian cancer emerges from its dual functional capability as an endothelial cell mitogen and a potent stimulator of vascular permeability (Sowter et al., 1997). Thus, when a tumor increases in volume, new blood vessels must form and invade the expanding tumor.

The molecular regulation of these distinct mechanisms is discussed with respect to the most important positive regulators, VEGF and its receptors flt-1 and KDR/flk-1. VEGF and its receptor KDR are highly expressed in a majority of ovarian epithelial tumors, and VEGF expression is a negative prognostic factor for this disease (Boocock et al., 1995; Abu-Jawdeh et al., 1996; Neufeld et al., 1999). Boocock et al. demonstrated that elevated expression of VEGF mRNA was found in all primary ovarian tumors and metastases. Receptors flt-1 and KDR were expressed by some tumor blood vessels, whereas KDR was also expressed by some tumor cells that co-expressed with VEGF (Boocock et al., 1995). Abu-Jawdeh et al., defined the expression of VEGF and flt-1, KDR receptors in normal ovarian cortex and in benign, borderline and malignant ovarian tumors. No strong expression of VEGF mRNA was found in normal ovarian cortex and benign tumors, whereas borderline tumors had variable VEGF mRNA expression (Abu-Jawdeh et al., 1996).

In our study we observed that expression of VEGF and its receptors increased in malignant ovarian tumors when compared with borderline tumors. Our results suggest that the expression of VEGF and its receptors were not statistically different in both serous and mucinous adenocarcinomas. Therefore, VEGF and its receptors might play a role in tumor development by malignant transformation of the cells via angiogenesis. Inhibition of VEGF signaling abrogates the development of a wide variety of tumor mechanisms that control VEGF production and VEGF signal transduction. Recent studies have shed light on the mechanisms by which VEGF regulates angiogenesis (Brown et al., 2000; Bamberger and Perrett, 2002; Wong et al., 2003).

On the other hand, the role of TGF-ß in ovarian cancer remains poorly understood. TGF-ß is a bifunctional regulator of cell growth; it stimulates proliferation of mesenchymal cells and inhibits growth of other cell types, primarily epithelial cells (Pepper, 1997; Ingman and Robertson, 2002). It is possible that dedifferentiation may alter the effects of TGF-ß on tumor cell growth, even leading to stimulation of growth in some cases (Henriksen et al., 1995). Additionally, TGF-ß may be an angiogenic factor, because TGF-ß promotes basement membrane deposition, capillary sprout formation, and differentiation of smooth muscle cells (Bartlett et al., 1997; Rodriguez et al., 2001) .

The roles of TGF-ß1 and TGF-ß2 mRNA have been

documented in ovarian cancer cell lines, and these isotypes, in addition to TGF-ß3 have been detected in human ovarian carcinoma tissues (Bartlett et al., 1997; Rodriguez et al., 2001). It was demonstrated that expression patterns were similar between malignant, borderline and benign tumors, although TGF-ß1 incidence was reduced in benign tumours (Bartlett et al., 1997). Results of our immunohistochemical study demonstrated that immunoreactivities of TGF-ß1 and TGF-ß2 were overexpressed in malignant ovarian adenocarcinomas when compared with borderline tumors. Immunoreactivity of TGF-ß3 was increased only in malignant mucinous adenocarcinoma, this intensity was significantly different from the other types of ovarian tumors. Although TGF-ß1 was a more intense transforming growth factor in malignant ovarian tumors; TGF-ß3 is likely to be involved in the progression of some malignant tumor cells. TGF-ß's might be controlling epithelial proliferation on borderline tumors, however, they might not be able to promote malignant neoplastic behaviours, due to its bifunctional effects. In addition, Nilsson et al. argued that TGF-ß acts to inhibit proliferation of normal ovarian surface epithelium and progress into early stage ovarian carcinomas (Nilsson et al., 2001).

Angiogenesis appears to be an early event in epithelial ovarian cancer and may be induced differently in tumors of different histological types. The expressions of VEGF and TGF-ß are associated with the promotion of angiogenesis and the expression of TGF-ß is a prognostic indicator in epithelial ovarian cancers. Nakanishi et al. demonstrated that the microvessel density of VEGF-rich and TGF-ß positive tumors was significantly higher than that of VEGF-poor and TGF-ß negative tumors (Nakanishi et al., 1997). Gordinier et al. showed that TGF-ß1 and TGF-ß2 were overexpressed in both primary and metastatic tumor specimens in comparison to normal ovarian tissue (Gordinier et al., 1999). Our results suggest that both growth factors may render normal cells to acquire malignant potential with an increase in the expression of angiogenic factors in epithelial ovarian neoplasia.

In summary, our findings showed that VEGF, its receptors flt-1, KDR, and TGF-ß1 and TGF-ß2 were overexpressed in malignant ovarian tumors. Co-expression of VEGF, its receptors and TGF ß's by tumor cells in ovarian carcinoma may provide paracrine stimulus needed for new blood vessel formation. Overexpression of VEGF and TGF-ß marks these cytokines as important in ovarian cancer biology, with potential effects on tumor growth, differentiation and angiogenesis. Many growth factors important to ovarian cancer invasion are also prominent in its associated angiogenesis. Deregulation of normal angiogenic processes occurs with the cancer's acquisition of the ability to secrete pro-angiogenic factors. Our results suggest that new therapeutic strategies using VEGF and TGF-ß antagonists could secure an additional approach for the treatment of epithelial ovarian carcinoma by inhibiting angiogenesis. Thus, further understanding of the molecular and cell biological foundations of angiogenesis in the epithelial ovarian carcinoma offers important directions for predicting the patient outcome and treatment.

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