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Serum levels of interleukins, growth factors and angiogenin in patients with endometrial cancer

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Abstract The purpose of this work was to study changes in serum levels of interleukins, growth factors and angiogenin during different stages of endometrial cancer progression. Serum levels were assayed by enzyme-linked immunosorbant assay in 59 women with stages I–IV of endometrial cancer (study subjects: stage I, $n = 20$; stage II, $n = 8$; stage III, $n = 5$; stage IV, $n = 6$) and compared to the serum levels in 20 women without cancer as control subjects. Patients with endometrial cancer had varied serum levels of interleukins and growth factors. There was a significant increase in serum levels of angiogenin in all stages of tumor progression. Levels of interleukin-8 (IL-8), IL-10 and transforming growth factor β (TGFβ) were significantly elevated in patients with stages I and II carcinoma. The serum levels of tumor necrosis factor α (TNFα), granulocyte/macrophage-colony-stimulating factor, basic fibroblast growth factor (BFGF), IL-7 and IL-2 were significantly elevated in patients with stages II and III carcinoma and the serum level of tumor necrosis factor β (TNFβ) was slightly elevated in patients with stage II carcinoma only. The serum levels of IL-1α, IL-1β and IL-6 were not elevated in endometrial cancer patients in any of the clinical stages. The results showed that progression of endometrial cancer is associated with increased serum levels of cytokines, growth factors and angiogenin, which possibly amplify angiogenesis during different clinical stages.

Key words Angiogenesis · Endometrial · Cytokines · Angiogenin · Growth factors

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Introduction

Endometrial carcinoma is the commonest malignancy occurring in the United States. Although it has a low mortality rate, its incidence exceeds that of cervical cancer (Horn et al. 1993). Significant progress has been made in the identification of various internal and external signals that influence endometrial hyperplasia of glandular epithelial cells of the basal layer; however the stimulus for the transformation to malignancy is unknown (Charnock-Jones et al. 1993). Ovarian steroids, either alone or in conjunction with polypeptide growth factors, are assumed to be the principal mediators of this event (Pearl et al. 1993; Nelson et al. 1991; Irwin et al. 1991). Angiogenic activity occurs in the human endometrium under normal conditions and the precise role of this angiogenic activity is unclear (Folkman 1985a; Folkman and Klagsbrun 1987a). Angiogenic factors that are involved during the normal physiological process of angiogenesis of endometrial tissue are under investigation (Fuchs et al. 1985). There is now increasing evidence that normally occurring cytokines produced by tumors propel the angiogenic process that contributes to the pathophysiology of cancer metastasis (Klagsbrun and D'Amore 1991). Endometrial cancer cell lines produce vascular endothelial growth factor (VEGF) and βFGF-like activity, which has been reported in human endometrium and AN3CA, HEC 1-A, HEC 1-B endometrial carcinoma cell lines (Presta 1988).

Tumor angiogenesis is the growth of new vessels toward and within, a tumor (Folkman and Klagsbrun 1987b; Folkman 1985b; Folkman et al. 1989; Weidner et a. 1993). Such neovascularization may be stimulated by factors released from the tumor cells, tumor-associated inflammatory cells and/or from the extracellular matrix, inducing new blood vessels from the surrounding host tissue. The molecular messengers secreted by tumor cells and immune cells mediate extracellular signals that enhance proliferation and migration of endothelial cells (Leibovich et al. 1987).

The immunomodulatory and angiogenic cytokines and most of the growth factors investigated in the current study

Table 1 Interleukin (*IL*) levels in serum of patients with endometrial cancers during different stages of tumor progression

Interleukins	Level (pg/ml) in endometrial cancer stages:				Level (pg/nl) in controls
	I (n = 20)	II (n = 8)	III (n = 5)	IV (n = 6)	(n = 20)
IL-1α	<10	<10	<10	<10	<10
IL-1β	18.8± 1.6	<10	<10	<10	<10
IL-2	<10	56.8± 6.2*	22.7± 3.2	<10	<10
IL-6	<10	<10	<10	<10	<10
IL-7	<10	42.7± 3.5*	68.4±13.1*	24.7± 4.4	<10
IL-8	282.4±42.5*	334.6±35.2*	164.7±59.4*	122.7±39.8*	28.6±8.9
IL-10	109.5±19.6*	105.5±11.4*	49.4± 2.8*	26.4± 6.0	<10

* P <0.05

were produced by tumor, immune, normal epithelial and/or endothelial cells. Interleukins (IL-1, IL-6, IL-8), colony-stimulating factors [granulocyte-colony-stimulating factor (G-CSF) and granulocyte/macrophage CSF (GM-CSF)], tumor necrosis factor (TNFα and -β), TGFβ and βFGF have been found to have angiogenic and proliferative effects on endothelial cells, many of which are synthesized and secreted by macrophages. Platelet-derived growth factor, prostaglandins, angiotropin, angiotensin, angiogenin and heparin-binding growth factors are additional angiogenic factors that have been studied (Sunderkotter et al. 1991). The angiogenic factors investigated in this study regulated the proliferation, function and interaction of macrophages, T cells, B cells, epithelial, stromal and endothelial cells (Pearl et al. 1993; Leibovich et al. 1987). The roles of these growth factors and cytokines in endometrial cancer angiogenesis and metastasis are not fully understood.

This study was designed (i) to investigate the excess serum levels of various growth factors and cytokines during various stages of endometrial cancer progression, using enzyme-linked immunosorbent assay (ELISA), and (ii) to identify cytokines that could act as regulators of tumor progression and metastasis.

Materials and methods

Patients and controls

We studied 59 women between 30 and 60 years of age, who were nonsmokers and did not use intravenous drugs. Twenty healthy age-matched control subjects without cancer were recruited for comparative study as controls and 39 women with confirmed invasive endometrial cancer served as the study group. Women with autoimmune diseases or chronic immune diseases were not included in this study. Control subjects on medication, drugs or smokers were excluded since they could possibly have abnormal serum levels of cytokines and other growth factors. The control subjects did not have any known concurrent benign uterine conditions such as cervical polyps, cervicitis, endometrial hyperplasia, uterine leiomyomas, etc. The study group was comprised of 19 women with adenocarcinoma, 13 with adenoma and 7 with endometroid carcinoma. This group was clinically, surgically and histopathologically staged: 20 patients had FIGO stage I disease, 8 had stage II disease, 6 had stage III disease and 5 had stage IV disease.

All subjects were recruited and examined by the Division of Gynecological Oncology, Department of Obstetrics and Gynecology, according to the protocol approved by the Institutional Review Board of The University of Texas Medical Branch at Galveston. Venous blood was drawn from each patient and control subject on her first visit prior to treatment or surgery. Serum was separated from clotted blood by centrifugation (1500 *g*), divided into aliquots and stored at −70 °C until analysis. Frozen serum samples were thawed for analysis and assayed in duplicate for angiogenic cytokine and growth factor levels by ELISA, using specific monoclonal antibodies.

ELISA

ELISA (IL-10 kit from Biosource International, Camarillo, Calif.; others from R&D Systems, Minneapolis, Minn.) was used to measure the concentration of specific cytokines in the serum according to the manufacturer's instructions. The assay used the quantitative immunoenzymometric sandwich technique. A group of serially diluted standard samples of the cytokines, diluted in a representative serum sample from control subjects that lacked the cytokine to be tested, was used to generate the standard curves. A human antibody, specific for each cytokine, was coated onto the 96-well polystyrene microtiter plates. Standards, with known amounts of each cytokine and the patient samples (200 µl) were pipetted into each well. Cytokines present in the

Table 2 Angiogenic factor levels in serum of patients with endometrial cancers during different stages of tumor progression. *GM-CSF* granulocyte/macrophage-colony-stimulating factor, βFGF basic fibroblast growth factor, *TNF* tumor necrosis factor, *TGFβ* transforming growth factor β

Angiogenic factors	Level (pg/ml) in endometrial cancer stage:				Level (pg/nl) in controls
	I (n = 20)	II (n = 8)	III (n = 5)	IV (n = 6)	(n = 20)
GM-CSF	<10	27.5± 4.6	37.9± 4.3	17.8± 4.3	<10
βFGF	<10	33.6± 7.2	38.7± 5.6	29.8± 6.4	<10
TNFα	17.5± 2.3	68.9± 8.8*	99.8± 8.4*	32.6± 9.6	<10
TNFβ	288.3±47.4*	823.7±98.2*	197.3±17.6*	193.5±10.7*	57.3±18.2
TGFβ	114.2±19.2*	135.5±19.8*	118.9±14.5*	95.4±13.8*	22.4± 5.16
Angiogenin	302.3±16.5*	285.6±13.8*	219.2±56.4*	209.2±33.5*	112.6± 8.5

* P <0.001

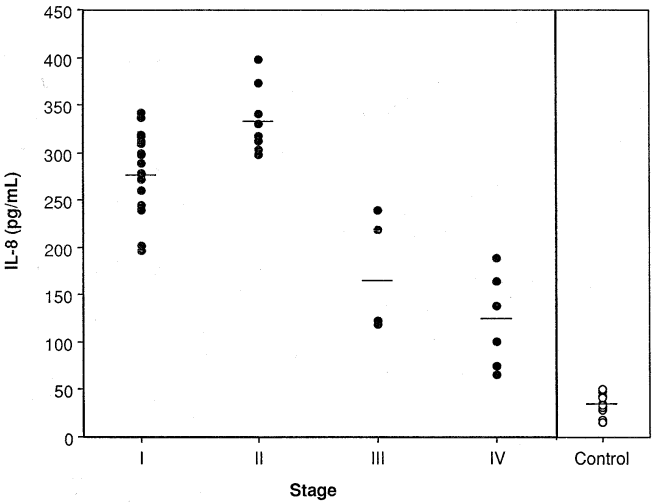

Fig. 1 Serum levels of interleukin-8 (*IL-8*; pg/ml) in patients with endometrial cancer ($n = 49$) during various stages of tumor progression and control subjects ($n = 20$). Each sample was assayed in duplicate

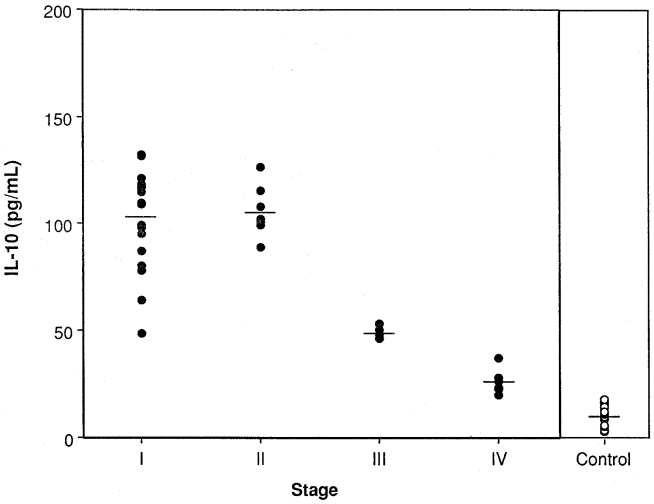

Fig. 2 Serum levels of IL-10 (pg/ml) in patients with endometrial cancer ($n = 49$) during various stages of tumor progression and control subjects ($n = 20$). Each sample was assayed in duplicate

samples were immobilized by the primary antibody during a 2-h incubation at room temperature. The plates were then thoroughly washed (four times) with diluted phosphate buffer to remove unbound proteins. Enzyme-linked monoclonal antibodies, specific for each cytokine and conjugated to horseradish peroxidase, were added to the wells and incubated for 2 h at room temperature. The plates were again washed (four times) to remove the unbound conjugate and the levels of the cytokine were determined by adding a chromogenic substrate consisting of hydrogen peroxide and chromogen tetramethylbenzidine. The amount of color development was proportional to the amount of cytokine bound during the initial step. The reaction was stopped by the addition of 1 M sulfuric acid and the intensity of the color was measured by an ELISA reader at 450 nm with the wavelength correction set at 540 nm. The detection limit of these assays was 10 pg/ml cytokines, based on the standard curve. The ELISA kit to detect angiogenin measured the angiogenin concentration as ng/ml after a 1:10 dilution of serum samples.

Statistical methods

All values were assayed in duplicate. The mean ± SEM was calculated for the total number of samples tested in each group of patients. Statistical differences between means were assessed using Students *t*-test. A p value of less than 0.005 was considered significant. All tests were two-sided and no other adjustment for multiple testing was applied.

Results

An increase in the levels of cytokines and growth factors was observed in the serum of patients with endometrial cancer during different clinical stages as compared to control subjects.

Interleukin levels in the sera of endometrial cancer patients

The serum levels of IL-1α, IL-1β and IL-6 were not detected in the patients with endometrial cancer in stages I–IV and control subjects (Table 1). The values were less than 10 pg/ml and were not in the detectable range of the test. Serum levels of IL-2 were significantly elevated ($P < 0.001$) in patients with stage II carcinoma, but decreased in patients with stage III and IV carcinoma. The increase of IL-2 levels was not associated with tumor progression in the advanced stages (III and IV) of the cancer. The serum levels of IL-7 increased in patients with stage II carcinoma and were highest in patients with stage III carcinoma, but decreased in patients with stage IV carcinoma. The serum levels of IL-8 were consistently high, with dramatic increases in stages I and II, in comparison to stages III and IV (Fig. 1). The serum levels of IL-10 in study subjects were significantly elevated in stages I and II and significantly decreased in stages III and IV (Fig. 2). The cytokine levels in serum of endometrial cancer patients altered in all stages during tumor progression compared to levels in control subjects, hence IL-2, IL-6, IL-7, IL-8 and IL-10 may have the potential to govern the formation of new blood vessels.

Levels of angiogenic factors and angiogenin in the sera of endometrial cancer patients

Serum levels of GM-CSF were elevated in stages II, III and IV of endometrial cancer and remained persistent throughout the tumor progression. Compared to the levels in the control subjects, GM-CSF serum levels were highest in patients with stage III carcinoma (Table 2). The serum levels of βFGF also varied during various clinical stages of tumor development, as compared to serum levels in control subjects: serum levels were highest in patients with stage III carcinoma, were below the detectable range in patients with stage I carcinoma and were also significantly elevated in patients with stage II and III carcinoma (Table 2). The serum levels of TNFα and TNFβ also increased significantly during different stages of endometrial cancer. Levels of TNFα significantly increased and were persistently

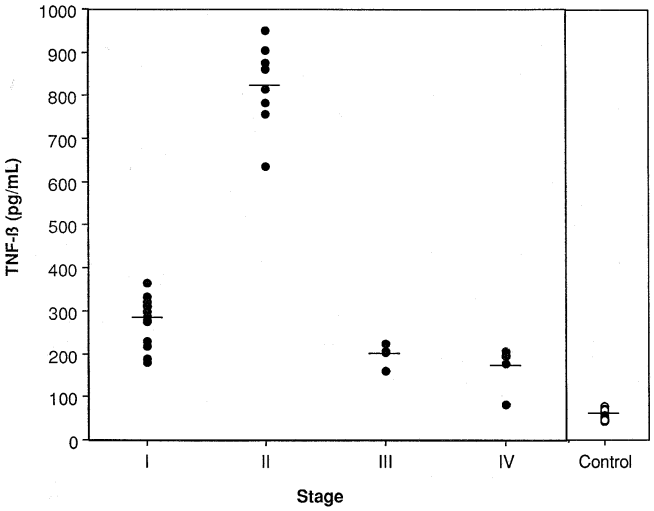

Fig. 3 Serum levels of tumor necrosis factor β (*TNF* β pg/ml) in patients with endometrial cancer (n = 49) during various stages of tumor progression and control subjects (n = 20). Each sample was assayed in duplicate

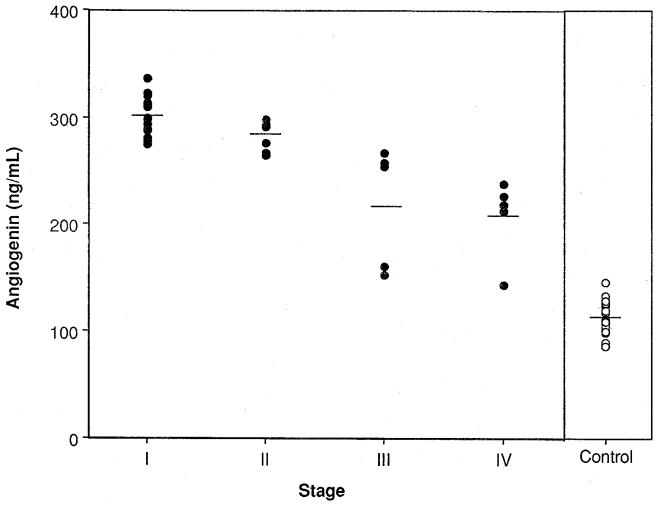

Fig. 5 Serum levels of angiogenin (ng/ml) in patients with endometrial cancer (n = 49) during various stages of tumor progression and control subjects (n = 20). Each sample was assayed in duplicate and angiogenin levels were assayed in 1:10 dilution of the serum sample

elevated and statistically significant ($P < 0.001$) only in stages II and III and again dropped in stage IV (Table 2). Serum levels of TNFβ were elevated significantly in stage II of endometrial cancer progression. They were statistically significant ($P < 0.001$) and reached the highest level in stage II (Fig. 3).

The increase in serum levels of these angiogenic modulators was associated with a significant increase in serum levels of TGFβ and human angiogenin during stages I–IV, above the normal range in the control subjects. Serum levels of TGFβ were significantly elevated in stages I and II ($P < 0.001$) and declined slightly in stages III and IV (Fig. 4). The serum level of angiogenin was statistically significant in stages I and II ($P < 0.001$), but in stages III and IV this level declined (Fig. 5). However, TGFβ and angiogenin levels were elevated throughout cancer progression as compared to those of control subjects. It is evident that endometrial cancer patients have a statistically significant increase in the production of angiogenin, TNFα, TNFβ and TGFβ during different clinical stages of tumor growth.

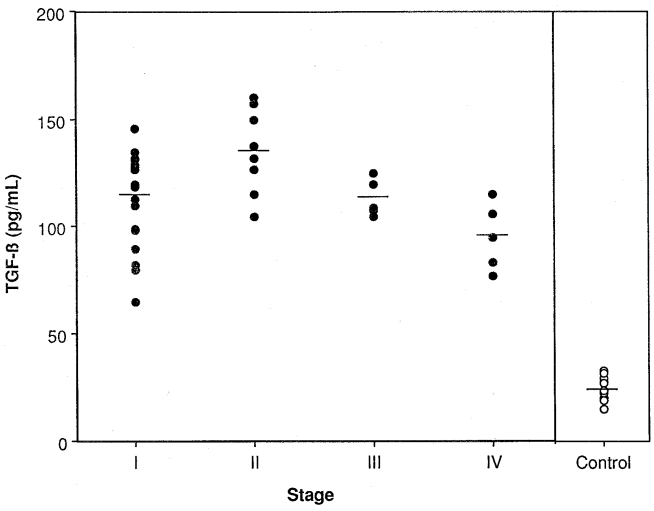

Fig. 4 Serum levels of transforming growth factor β (*TGF*β ; pg/ml) in patients with endometrial cancer (n = 49) during various stages of tumor progression and control subjects (n = 20). Each sample was assayed in duplicate

Discussion

In this study, we demonstrated increases in the serum levels of cytokines and growth factors that might act as angiogenic mediators and/or modulators during tumor progression in women with different stages of endometrial cancer. Endothelial cells present both a source of (IL-1, IL-6, IL-8, G-CSF, M-CSF and GM-CSF) and a target for (FGF, G-CSF, GM-CSF, IL-1, TNF and TGF) cytokines and growth factors (Klagsbrun and D'Amore 1991; Folkman and Klagsbrun 1987b; Folkman 1985b; Folkman et al. 1989; Weidner et al. 1993; Presta 1988; Leibovich et al. 1987; Sunderkotter et al. 1991) and their activation dramatically changes their function and surface properties (Koch et al. 1992; Presta et al. 1991). Tumor progression is associated with altered production of most of the immunomodulators. The physiology of the cytokines and growth factors responsible for this dual control of angiogenic activity in the tumor microenvironment or circulating in the host remain to be defined. This study demonstrates that the secretion of angiogenic factors in the sera is altered in advanced stages of endometrial cancer progression.

ELISA studies of the serum samples documented a significant increase in levels of IL-8, TNFα, TNFβ, TGFβ and angiogenin and a moderate increase in the levels of β FGF and GM-CSF in women with endometrial cancer during different clinical stages. Patients with endometroid

carcinoma, adenocarcinoma and adenoma had similar levels of cytokines in circulation during different clinical stages of disease progression as compared to control subjects. Hence the variation of cytokine levels was not related to the type of cancer but to the stage of the disease, as determined by clinical, surgical and histopathological reports.

IL-8 binds basic heparin polypeptides and picomolar concentrations of IL-8 induce a strong angiogenic response in vivo and in vitro; increased levels of IL-8 mRNA were observed in activated endothelial cells (Koch et al. 1992; Presta et al. 1991). Other growth factors (i.e., TNFα, TNFβ, βFGF, TGFβ, GM-CSF and angiogenin) are angiogenic amplifiers and assist in the formation of blood vessels during the advanced stages of cancer (Sunderkotter et al. 1991). TNFα is angiogenic at low concentrations and purified TNFα antibody inhibits the growth of vascular endothelial cells. TNFβ is a major secretory product of T cells and activated macrophages and has been implicated in tumor angiogenesis (Grosen et al. 1992, 1993). βFGF is a very potent heparin-binding angiogenic molecule in vitro and in vivo, even at low concentrations, and is produced by fibroblast, epithelial and endothelial cells (Presta et al. 1991). TGFβ is produced by a wide range of tumor cells and can be instrumental in the transformation of normal cells to tumor cells. It induces the expression of angiogenic activity through activated macrophages by increasing the expression of TNFα, inducing in vivo angiogenesis and inhibiting endothelial cell migration in vitro (Wiseman et al. 1988). GM-CSF is constitutively produced by endothelial and epithelial cells and is associated with macrophage-induced angiogenesis (Baiocchi et al. 1991).

The serum levels of angiogenin, a tumor-specific ribonuclease factor that has a direct effect on vascular endothelium, play a facilitating role for other angiogenic growth factors, which were significantly elevated throughout cancer progression. In vitro, it binds to the extracellular matrix and provides support for and directs the proliferating endothelium. Alternatively, angiogenin is believed to cause the polymerization and release of cell-surface actin, which could be a prerequisite for subsequent cell migration (Hu et al. 1991; Soncin 1992). Angiogenin serum levels were elevated in endometrial cancer patients at earlier stages and could serve as a new prognostic indicator with a clear biological significance. Elevated levels of angiogenin correlate with the disease outcome and could assist in determining which patients are at high risk for carcinoma and require aggressive adjuvant therapy.

Our studies also indicate that the serum levels of IL-1α, IL-1β and IL-6, which are considered as acute-phase cytokines and mediators of inflammation and immune responses, were not altered. This could be attributed to the binding of the cytokine to cells expressing specific receptors, reducing the measurable levels of IL-1α. We were interested in measuring the serum levels of these cytokines because they stimulate the proliferation of vascular endothelial cells, induce angiogenesis in several in vivo models and act positively with IL-8 to enhance angiogenesis in vitro (Dejana et al. 1991; Kristensen et al. 1991). IL-6 enhances the motility and adherence of various tumor cell lines. It is angiogenic in normal physiological conditions, promoting tumor metastasis (Cozzolino et al. 1993). IL-2, a predominant growth and differentiating factor, is strongly mitogenic, along with interferon α, for endothelial cells and enhances the synthesis and release of βFGF from activated endothelial cells (Detmar et al. 1992). IL-7, like other cytokines, is a stroma-derived cytokine and induces production and secretion of IL-6 and the secretion of immunoreactive IL-1α, IL-1β and TNFα by peripheral blood monocytes (Alderson et al. 1991). The slight increase in the levels of IL-7 reflects its role in immune functions. Conceivably, IL-2 and IL-7 could induce other cytokines and angiogenic modulators indirectly, mediated by cytokine cascade, to induce neovascularization. IL-10 is a potent inhibitor of T helper cell function, blocking synthesis and production of pro-inflammatory cytokine molecules (IL-α, IL-6, IL-8 and TNFα) and exhibiting cytokine-synthesis-inhibitor factor activity in vitro (Moore et al. 1993). The rise in serum levels of IL-10 in patients with endometrial cancer is associated with tumor development in early stages (I and II), but is down-regulated in the advanced stages III and IV, reflecting the immunosuppressive role of IL-10. Further, the decrease in the serum levels of IL-10 up-regulates the angiogenic response of other cytokines associated with tumor progression in later stages. Thus IL-10 alters and regulates the production of angiogenic cytokines during tumor progression.

These results indicate an increase in the serum levels of angiogenin, TNFβ, TGFβ and IL-8 in the early stages of endometrial cancers. However, in the later stages the serum levels of immunoregulatory cytokines with angiogenic potential decrease. These results strongly suggest that cytokines and growth factors play important roles, not only in the tumor progression, metastasis and immunosurveillance but also in tumor angiogenesis

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References

1. Alderson MR, Tough TW, Ziegler SF, Grabstein H (1991) Interleukin-7 induces cytokine secretion and tumoricidal activity by human peripheral blood monocytes. J Exp Med 173:923–930
2. Baiocchi G, Kavanagh JJ, Talpaz M, Wharton JT, Gutterman JU, Kurzrock R (1991) Expression of the macrophage colony-stimulating factor and its receptor in gynecologic malignancies. Cancer 67:990–996
3. Charnock-Jones DS, Sharkey AM, Rajput-Williams J, Burch D, Schofield JP, Fountain SA (1993) Identification and localization of alternately spliced mRNA for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines. Biol Reprod 48:1120–1128

4. Cozzolino F, Torcia M, Lucibello M, Morbidelli L, Ziche M, Platt J, Fabiani S, Brett J, Stern D (1993) Interferon-α and interleukin-2 synergistically enhance basic fibroblast growth factor synthesis and induce release, promoting endothelial cell growth. J Clin Invest 91:2504–2512
5. Dejana E, Bussolino F, Wang JM, Mantovani A (1991) Cytokine regulation of endothelial cells. In: F. R. Balkwell (ed) Cytokines, a practical approach. IRL London, pp 215–227
6. Detmar M, Tenorio S, Hettmannsperger U, Ruszczak Z, Orfanos CE (1992) Cytokine regulation of proliferation and ICAM-1 expression of human dermal microvascular endothelial cells in vitro. J Invest Dermatol 98:147–153
7. Folkman J (1985a) Tumor angiogenesis. Adv Cancer Res 43:175–203
8. Folkman J (1985b) Toward an understanding of angiogenesis: search and discovery. Perspect Biol Med 29:10–36
9. Folkman J, Klagsbrun M (1987a) Vascular physiology. A family of angiogenic peptides. Nature 329:671–672
10. Folkman J, Klagsburn M (1987b) Angiogenic factors. Science 235:442–447
11. Folkman J, Watson K, Ingber D, Hanahan D (1989) Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339:58–61
12. Fuchs A, Lindenbaum E, Maecoudas NG (1985) Endometrial angiogenesis. Acta Anat (Basel) 124:241–244
13. Grosen EA, Yamamoto RS, Ioli G, Ininns WK, Gatanaga M, Gatanaga T, DiSaia PJ, Berman M, Manetta A, Granger GA (1992) Blocking factors (soluble membrane receptors) for tumor necrosis factor and lymphotoxin detected in ascites and released in short-term cultures obtained from ascites and solid tumors in women with gynecologic malignancy. Lymphokine Cytokine 11:347–353
14. Grosen EA, Granger GA, Gatanaga M, Innins EK, Hwang C, DiSaia P, Berman M, Manetta A, Emma D, Gatanaga T (1993) Measurement of the soluble membrane receptors for tumor necrosis factor and lymphotoxin in the sera of patients with gynecologic malignancy. Gynecol Oncol 50:68–77
15. Horn DW, Vollmer G, Deerberg F, Schneider MR (1993) The EnDA endometrial adenocarcinoma: an estrogen-sensitive, metastasizing, in vivo tumor model of the rat. J Cancer Res Clin Oncol 119:450–456
16. Hu GF, Chang SI, Riordan JF, Vallee BL (1991) An angiogenin-binding protein from endothelial cells. Proc Natl Acad Sci USA 88:2227–2231
17. Irwin JC, Utian WH, Eckert RL (1991) Sex steroids and growth factors differentially regulate the growth and differentiation of cultured human endometrial stromal cells. Endocrinology 129:2385–2392
18. Klagsbrun M, DIAmore PA (1991) Regulators of angiogenesis. Annu Rev Physiol 53:217–239
19. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, Elner SG, Strieter RM (1992) Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 258:1798–1801
20. Kristensen MS, Paludan K, Larsen CG, Zachariae CO, Deleuran BW, Jensen PK, Jorgensen P, Thestrup-Pedersen K (1991) Quantitative determination of IL-1α-induced IL-8 mRNA levels in cultured human keratinocytes, dermal fibroblasts, endothelial cells and monocytes. J Invest Dermatol 97:506–510
21. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N (1987) Macrophage-induced angiogenesis is mediated by tumor necrosis factor-α. Nature 329:630–632
22. Moore KW, O'Garra A, de Waal Malefyt RW, Vieira P, Mosmann TR (1993) Interleukin-10. Annu Rev Immunol 11:165–190
23. Nelson KG, Takahashi T, Bossert NL, Walmer DK, McLachlan JA (1991) Epidermal growth factor replaces estogen in the stimulation of female genital-tract growth and differentiation. Proc Natl Acad Sci USA 88:21–25
24. Pearl ML, Talavera E, Gretz HF III, Roberts JA, Menon KM (1993) Mitogenic activity of growth factors in the human endometrial adenocarcinoma cell lines HEC-1-A and KLE. Gynecol Oncol 49:325–333
25. Presta M (1988) Sex hormones modulate the synthesis of basic fibroblast growth factor in human endometrial adenocarcinoma cells: implications for the neovascularization of normal and neoplastic endometrium. J Cell Physiol 137:593–597
26. Presta M, Rusnati M, Maier JAM, et al (1991) Basic fibroblast growth factor and endothelial cells: receptor interaction, signal transduction, cellular response-dissociation of the mitogenic activity of β-FGF from its plasminogen activator inducing activator inducing capacity. In: Maragoudakis ME, Gullino P, Lelkes PI (eds) Angiogenesis in health and disease 227:63–78
27. Soncin F (1992) Angiogenin supports endothelial and fibroblast cell adhesion. Proc Natl Acad Sci USA 89:2232–2236
28. Sunderkotter C, Goebeler M, Schulze-Osthoff K, Bhardwaj R, Sorg C (1991) Macrophage-derived angiogenesis factors. Pharmacol Ther 51:195–216
29. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J (1993) Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 143:401–409
30. Wiseman DM, Polverini PJ, Kamp DW, Leibovich SJ (1988) Transforming growth factor-beta (TGF-β) is chemotactic for human monocytes and induces their expression of angiogenic activity. Biochem Biophys Res Commun 157:793–800