Interleukin 16 expression changes in association with ovarian malignant transformation

Aparna Yellapa, MS; Pincas Bitterman, MD; Sameer Sharma, MD; Alfred S. Guirguis, MD; Janice M. Bahr, PhD; Sanjib Basu, PhD; Jacques S. Abramowicz, MD; Animesh Barua, PhD

OBJECTIVE: Long-term unresolved inflammation has been suggested as a risk factor for the development of various malignancies. The goal of this study was to examine whether the expression of interleukin (IL)-16, a proinflammatory cytokine, changes in association with ovarian cancer (OVCA) development.

STUDY DESIGN: In an exploratory study, changes in IL-16 expression in association with OVCA development and progression were determined using ovarian tissues and serum samples from healthy subjects (n = 10) and patients with benign (n = 10) and malignant ovarian tumors at early (n = 8) and late (n = 20) stages. In the prospective study, laying hens, a preclinical model of spontaneous OVCA, were monitored (n = 200) for 45 weeks with serum samples collected at 15-week interval. Changes in serum levels of IL-16 relative to OVCA development were examined.

RESULTS: The frequency of IL-16–expressing cells increased significantly in patients with OVCA ($P < .001$) compared to healthy subjects and patients with benign ovarian tumors. The concentration of serum IL-16 was higher in patients with benign tumors ($P < .05$) than in healthy subjects and increased further in patients with early-stage ($P < .05$) and late-stage ($P < .03$) OVCA. Increase in tissue expression and serum levels of IL-16 in patients with early and late stages of OVCA were positively correlated with the increase in ovarian tumor–associated microvessels. Prospective monitoring showed that serum levels of IL-16 increase significantly ($P < .002$) even before ovarian tumors become grossly detectable in hens.

CONCLUSION: This study showed that tissue expression and serum levels of IL-16 increase in association with malignant ovarian tumor development and progression.

Key words: interleukin 16, laying hen model, malignant transformation, ovarian cancer

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Ovarian cancer (OVCA) is a fatal malignancy in women with the highest incidence-to-death ratio among gynecological cancers.[1] The 5-year survival of OVCA patients is >90% when it is detected at an early stage as compared with those detected at late stages.[2,3] Thus, metastasis of OVCA is the main cause of the high death rate of OVCA patients. Nonspecificity of symptoms at early stage makes early detection of OVCA very difficult.[4] Hence, most cases of OVCA are detected at late stages. Circulating levels of CA-125 alone or in combination with transvaginal ultrasound (TVUS) are used for OVCA detection. Although CA-125 is a better prognostic marker than others, it is not specific for early-stage OVCA. On the other hand, the resolution of traditional TVUS is limited for detecting ovarian tumors at early stage. A combination of serum CA-125 and traditional TVUS scan did not improve early detection of OVCA substantially.[5-7] Thus, an effective early detection test for OVCA remains to be established. Furthermore, the pattern of OVCA dissemination is very different from other solid tumors. The tumor usually spreads in a diffuse intraabdominal fashion in addition to systemic circulation and the tumor microenvironment plays critical roles in early OVCA metastasis.[8] Moreover, emerging information suggests that serous OVCA may originate from malignant transformation of precursor lesions in the fimbria of fallopian tube, which later exfoliate to the surface of the ovary.[9,10] In addition, endometriosis has also been suggested as a risk factor for endometrioid ovarian carcinoma.[11,12]

From the Departments of Pharmacology (Ms Yellapa and Drs Sharma and Barua), Pathology (Drs Bitterman and Barua), Obstetrics and Gynecology (Drs Sharma, Guirguis, and Barua), and Preventive Medicine (Biostatistics) (Dr Basu), Rush University Medical Center, Chicago, and Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana (Dr Bahr), IL, and Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI (Dr Abramowicz).

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Reprints: Animesh Barua, PhD, Department of Pharmacology, Laboratory for Translational Research on Ovarian Cancer, Room 410, Cohn Bldg., Rush University Medical Center, 1735 W. Harrison St., Chicago, IL 60612. Animesh_Barua@rush.edu.

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Therefore, information on early changes associated with OVCA development as well as factors favoring early metastasis of OVCA is critical to establish an early detection test and to prevent recurrence of OVCA as well as to improve the quality of life of OVCA patients.

The microscopic characteristics of the nucleus of the cell have long been used in pathology to differentiate malignant cells from normal ones[13] and similar to other cancers, malignant nuclear transformation is an early event in OVCA development. Inflammation has been suggested as a risk factor for malignant transformation.[14] Unresolved inflammation leads to hypoxic conditions accompanied by changes in inflammatory cytokines including interleukin (IL)-16.[14,15] Classic members of the immune system including CD8 T cells and monocytes/macrophages are the primary sources of IL-16.[16-18] IL-16 has been reported to be associated with initiation of proinflammatory processes and chemotaxis of immune cells (eg, CD4 T cells) to the site of inflammation[15,19,20] as well as in several malignancies.[21,22] Ovulation has been reported as an inflammatory process and frequent ovulation has been suggested as a risk factor for OVCA.[23] Thus, ovarian tissues are exposed to sustained inflammatory factors including IL-16, a proinflammatory cytokine and it is possible that IL-16 may be associated with malignant ovarian transformation and progression of OVCA. However, information on the involvement of IL-16 in ovarian tumor development is not known.

Tumors require blood supply for their growth and tumor-associated neoangiogenesis is the formation of new vessels from existing ones.[24] Tumor-associated neoangiogenesis is an early event in tumor development preceded by malignant nuclear transformation.[25] Multiple studies have suggested that tumor-secreted factors in association with other components of the tumor microenvironment stimulate the development of tumor-associated microvessels from existing blood vessels. It has been reported that IL-16 stimulates production of proinflammatory and proangiogenic factors IL-15 and IL-8 by members of immune system including $CD4^+$ macrophages.[26-28] Macrophages as well as different subsets of T cells are present in the tumor microenvironment.[29] It is possible that tumor-secreted factors may induce immune cells including macrophages in the tumor microenvironment to produce IL-16, which may stimulate the development of tumor-associated neoangiogenesis. However, no information is available on ovarian tumor–secreted factors and their involvement in the development of tumor-associated angiogenesis.

The goal of this study was to examine the association of IL-16 with the development and progression of OVCA. We hypothesized that the expression of IL-16 by ovarian tissues and its serum concentrations would be increased in association with malignant ovarian transformation and OVCA progression. This hypothesis was tested by 2 objectives: (1) determine the expression of IL-16 in normal ovaries or ovaries with tumor and serum levels of IL-16 in healthy women and OVCA patients; and (2) determine if the concentration of IL-16 increases in serum before the ovarian tumor forms a solid mass and becomes grossly detectable. Because it is difficult to identify patients at early-stage OVCA, access to patient's specimens remains a significant barrier to study alterations associated with early-stage ovarian tumor development. Thus, human specimens were used for the first objective whereas laying hens, a preclinical model of spontaneous OVCA, were used for the second in a prospective study. Laying hens are the only widely available and easily accessible animal that develop OVCA spontaneously with a high incidence rate.[30] The histopathology and expression of several markers of OVCA in hens are similar to those in human beings.[31-34] We have previously reported that the frequency of IL-16–expressing cells and serum levels were significantly higher in OVCA hens than normal hens.[35] However, the period between the increased levels of IL-16 in serum and the tumor mass becoming detectable by ultrasound imaging is not known.

Materials and Methods

Patient specimen

All tissues and serum samples were collected at Rush University Medical Center, Chicago, IL, and Northwest Oncology, Munster, IN. Benign and malignant ovarian tumor tissues were collected from patients who underwent surgery for a suspected ovarian mass. Corresponding blood samples were collected before surgery. Normal serum and ovarian samples were collected from subjects who had a hysterectomy due to nonovarian cause. All specimens were collected under institutional review board–approved protocol and patient's informed consent. Tumor tissues from 28 OVCA patients [n = 8 early stages (4 stage I and 4 stage II), age range, 53–67 years, and n = 20 late stages (10 stage III and 10 stage IV), age range, 42–79 years], patients with benign ovarian tumors (n = 10, age range, 53–85 years), and normal ovaries from subjects who underwent hysterectomy (n = 10, age range, 40–81 years) were used. Staging of the OVCA for each case was performed comprehensively during the primary surgery and later during histopathological examination of ovarian tumor mass as well as omentum, lymph node, and tubal tissues. Histological tumor types were confirmed by board-certified pathologists.

Animals

Commercial strains of 3- to 4-year-old white Leghorn laying hens (*Gallus domesticus*) were reared at the University of Illinois at Urbana-Champaign experimental Poultry Research Farm under standard poultry husbandry practices. The incidence of OVCA in hens of this age group is approximately 20% and is associated with low or complete cessation of egg laying.[30] Hens with normal or low egg-laying rates were scanned by TVUS[36] and 200 hens without any ovarian abnormality were selected. These hens were monitored by traditional TVUS scanning for 45 weeks at 15-week intervals and serum samples were collected at each interval as reported previously. All experimental