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Original contribution

TNF-α expression, risk factors, and inflammatory exposures in ovarian cancer: evidence for an inflammatory pathway of ovarian carcinogenesis?☆,☆☆

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Summary Inflammatory cytokines, like tumor necrosis factor–alpha (TNF-α) and interleukin-6 (IL-6), are elevated in ovarian cancer. Differences in cytokine expression by histologic subytpe or ovarian cancer risk factors can provide useful insight into ovarian cancer risk and etiology. We used ribonucleic acid in situ hybridization to assess TNF-α and IL-6 expression on tissue microarray slides from 78 epithelial ovarian carcinomas (51 serous, 12 endometrioid, 7 clear cell, 2 mucinous, 6 other) from a population-based case-control study. Cytokine expression was scored semiquantitatively, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using polytomous logistic regression. TNF-α was expressed in 46% of the tumors, whereas sparse IL-6 expression was seen in only 18% of the tumors. For both markers, expression was most common in high-grade serous carcinomas followed by endometrioid carcinomas. Parity was associated with a reduced risk of TNF-α–positive (OR, 0.3; 95% CI, 0.1-0.7 for 3 or more children versus none) but not TNF-α–negative tumors (*P* heterogeneity = .02). In contrast, current smoking was associated with a nearly 3-fold increase in risk of TNF-α–negative (OR, 2.8; 95% CI, 1.2-6.6) but not TNF-α–positive tumors (*P* heterogeneity = .06). Our data suggest that TNF-α expression in ovarian carcinoma varies by histologic subtype and provides some support for the role of inflammation in ovarian carcinogenesis. The novel associations detected in our study need to be validated in a larger cohort of patients in future studies.

1. Introduction

Epidemiologic and biologic evidence supports the role of inflammation in the pathogenesis of ovarian cancer [1]. Ovulation is an inflammatory process involving localized elevations of prostaglandins and leukotrienes, wound healing, and tissue remodeling [2]. Oral contraceptives (OCs)

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and parity suppress ovulation and are associated with a reduced risk of ovarian cancer. Similarly, tubal ligation, which may block inflammatory mediators from reaching the ovary or fimbria, reduces risk of ovarian cancer by almost 50%, and this protective association is remarkably consistent across studies [3]. In contrast, proinflammatory exposures such as genital powder use, endometriosis, and increased body mass index (BMI) are known to increase ovarian cancer risk [4-6]. Results from several prospective studies suggest that elevated systemic markers of inflammation, specifically, serum levels of C-reactive protein, tumor necrosis factor–alpha (TNF-α), and interleukin-6 (IL-6), are predictive of ovarian cancer development [7–12].

However, ovarian cancer is a heterogeneous disease with several distinct morphological phenotypes of surface epithelial carcinomas. Therefore, it is not surprising that multiple pathways of ovarian carcinogenesis have been proposed, the most notable ones being origin from inclusion cysts of ovarian surface epithelium [13,14], high-grade serous carcinomas with *TP53* mutations originating in the fallopian tubes [15], endometrioid and clear cell carcinomas arising in association with endometriosis [6], and mucinous carcinomas arising in association with teratoma [16] or Brenner tumor [17], with a postulated role for metaplasias at the tuboperitoneal junction [18]. Whether proinflammatory exposures are relevant to risk of ovarian cancer development through all or only some of these pathways remains uncertain.

Prior studies have shown increased TNF-α mRNA levels in ovarian cancer as well as constitutive expression of other cytokines such as IL-1α, IL-6, CCL2, CXCL8, and M-CSF [19,20]. However, most of these mRNA expression array studies used homogenized tumor tissue lysates and could not assess tissue localization of the various cytokines. Cytokine expression by the tumor cell epithelium could not be distinguished from cytokine expression by tumor-infiltrating inflammatory cells. The lack of robust, validated anticytokine antibodies that can work well on paraffin sections has also hampered efforts to assess tissue localization of TNF-α and other cytokines. Recent novel developments in RNA in situ hybridization (ISH) techniques offer a unique opportunity to study inflammatory mediators in formalin-fixed, paraffin-embedded tissue [21]. In this study, we evaluated the association between ovarian cancer risk factors and inflammatory exposures in relation to TNF-α and IL-6 expression in a well-annotated set of ovarian cancers to understand the role of inflammatory mediators in the pathogenesis of ovarian carcinoma.

2. Materials and methods

2.1. Study population

The New England Case Control Study (NECC) is a population-based case-control study conducted in 3 phases between 1992 and 2008 [22]. Cases were recruited in Eastern Massachusetts and New Hampshire through statewide registries and tumor boards, whereas controls were identified through driver's license lists (New Hampshire) and town resident lists (Massachusetts). In the current study, we selected participants from the last phase of the NECC conducted between 2003 and 2008. Participants were excluded if they were younger than 18 years, did not speak English, had moved elsewhere or were not accessible by phone, or had a history of bilateral oophorectomy (controls) or if permission to contact them was denied by their physician (cases). Controls were frequency matched to the cases based on their age and their state of residence. Of 1610 potential cases, 1238 met the eligibility criteria, and 845 (68.3%) were enrolled between 2003 and 2008. We identified 2523 potential controls, where 1673 were eligible to participate, and 857 (51.2%) were enrolled. Information about reproductive and medical history and lifestyle factors was obtained through in-person interview. The study was approved by the institutional review boards at Brigham and Women's Hospital and Dartmouth Medical School.

2.2. Tissue microarray preparation and RNA ISH assays

Pathology reports from the 5-year study period were reviewed by a gynecological pathologist (M. G.) to select cases of invasive surface epithelial carcinomas (low-grade or high-grade serous, mucinous, endometrioid, clear cell, and others) based on the current WHO classification of ovarian cancer [23]. Paraffin-embedded cancer tissue blocks were then requested for participants with invasive tumors who had no history of neoadjuvant chemotherapy and who had surgery at Brigham and Women's Hospital, Boston, MA. Of the initially requested material (n = 207), paraffin blocks were available for 78 tumors, and this formed our final study group. Cases included in the tissue microarray (TMA) had similar characteristics to all the NECC confirmed invasive cases and cases eligible for block collection with respect to ovarian cancer risk factors (age, parity, BMI, OC use, tubal ligation, endometriosis, and family history of breast or ovarian cancer) and tumor characteristics (histology, grade, and stage) (Supplementary Table 1).

For each case, histopathology slides were reviewed by the study pathologist (M. G.) to select representative tumor blocks for inclusion in a tumor TMA. Three separate areas of well-preserved tumor, away from foci of necrosis, were then marked on the tumor tissue slides for TMA construction. TMA blocks were constructed using 1-mm-diameter needles to extract the tissue cores that were then placed into predrilled holes of a recipient paraffin block to create a grid of tissue cores representing approximately 25 cases per TMA with up to 3 cores per case. Normal fallopian tubes and ovaries (n = 18 cores) were also included as controls in the tumor TMA blocks.

Tissue sections, 5 μm in thickness, were cut from each TMA block and baked in a dry oven at 60°C for 1 hour