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Impact of Human Cytomegalovirus Infection and its Immune Response on Survival of Patients with Ovarian Cancer[1]

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Abstract

Human cytomegalovirus (HCMV) has been detected in various types of tumors. We studied the prevalence of HCMV in ovarian cancer and its relation to clinical outcome. Paraffin-embedded tissues obtained prospectively from 45 patients with ovarian cancer and 30 patients with benign ovarian cystadenoma were analyzed for expression of HCMV immediate-early protein (IE) and HCMV tegument protein (pp65) by immunohistochemistry. Plasma was analyzed for HCMV serology. HCMV-IgG levels were higher in patients with ovarian cancer or benign cystadenoma than in age-matched controls ($P = .002$, $P < .0001$, respectively). HCMV IgM was detected in 12% of ovarian cancer patients and 3% of patients with benign tumors but was absent in controls. In patients with ovarian cancer, higher IgG levels were associated with better outcomes ($P = .04$). Extensive HCMV-IE protein expression was detected in 75% of ovarian cancers and 26% of benign tumors; pp65 was detected in 67% of ovarian cancers and 14% of benign tumors. A higher grade of HCMV infection was associated with higher stage of disease. Extensive HCMV-pp65 expression was associated with shorter median overall survival than focal expression (39 versus 42.5 months, $P = .03$). At study closure, 58% of ovarian cancer patients with focal pp65 expression were alive versus 27% of patients with extensive pp65 expression ($P = .03$). Thus, HCMV proteins are detected at different levels in ovarian tumors and benign cystadenomas. Ovarian cancer patients with focal HCMV-pp65 expression in their tumors and high IgG levels against HCMV lived longer, highlighting a need for in-depth studies of the oncomodulatory role of HCMV in ovarian cancer.

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Introduction

Ovarian cancer is a major cause of morbidity and mortality in women worldwide [1]. Often diagnosed at an advanced stage, ovarian cancer has a 5-year survival rate of less than 50% [2,3]. Standard care consists of cytoreductive surgery and platinum-based chemotherapy. Despite significant progress in treatments, the 5-year relative survival rate has improved marginally.

The etiology of ovarian cancer is not fully elucidated. Genetic susceptibility is believed to explain about 10–15% of these tumors

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[4]. Hormonal, infectious, and immunological factors have also been implicated in tumor development [5]. Although many key proteins and molecular pathways are potentially important in ovarian carcinogenesis, the early steps leading to malignancy are poorly understood [6]. Factors that may increase the risk of ovarian cancer are inherited gene mutations in breast cancer (BRCA) genes 1 and 2, mutations associated with Lynch syndrome, a family history of ovarian cancer, estrogen hormone replacement therapy, and the age at onset of menstruation and menopause [7]. The tumor microenvironment, including inflammation, may also affect tumor development and should be considered to understand the early steps of oncogenesis.

Human cytomegalovirus (HCMV) proteins and nucleic acid have been detected with optimized protocols in various types of cancers, including glioblastoma multiforme, neuroblastoma, medulloblastoma, and breast, prostate, and colon cancers [8–13]. In a recent study, HCMV-Glycoprotein B (gB) DNA was detected in 50% of ovarian cancers. [14] HCMV is a member of the herpes virus family with a worldwide seroprevalence of 50–100%. The virus infects many cell types and can establish latency in myeloid progenitor cells or specifically in $CD34^+$ cells [15,16]. HCMV can be reactivated in blood monocytes by inflammation and production of cytokines that result in differentiation of monocytes into macrophages or dendritic cells, which can transmit the virus to other cell types. [15] During active infection, HCMV expresses immediate-early proteins (IE), which serve as transcription factors that help regulate the expression of both viral and host cellular genes. These proteins activate production of early and late structural viral proteins, including the viral tegument protein pp65, and several also trans-activate the expression of host and viral genes that are important for efficient viral replication [17]. In the final phase of infection, structural viral proteins are produced and assemble into a new virus particle.

HCMV is estimated to produce about 200 proteins, of which 50 are essential for viral replication. New data from ribosome profiling analysis suggest that the virus encodes over 750 unique RNAs that may encode viral proteins. Many of these proteins will affect cellular and immunological functions that are highly relevant to tumor development. Indeed, emerging research suggests that HCMV's oncomodulatory properties are important in carcinogenesis; HCMV proteins interfere with the retinoblastoma protein family (Rb) [18], cyclins, p53, Wnt, PI3K/Akt, NF-κB [18–23], and STAT3 and modulate cellular functions though the effects on cellular differentiation, proliferation, and migration [24]. HCMV can block apoptosis and avoid immune surveillance, giving infected cells a survival advantage. Furthermore, HCMV infection alters expression of matrix metalloproteinases [25] and MMP2 and 9 have been shown to be strongly expressed in both stromal and epithelial tumor cells of serous invasive carcinomas [26].

Since HCMV is highly prevalent in breast cancers, which are morphologically similar to ovarian cancer, and mutations in *BRCA* are found in both types of tumors, we set out to study the prevalence and possible impact of HCMV infection on the survival rate of ovarian cancer patients in a prospective study.

Materials and Methods

Patient Characteristics and Treatment

Between February 2010 and July 2012, 45 consecutive patients with presumed epithelial ovarian cancer were enrolled in the study. All patients gave informed consent and underwent surgery at the Department of Obstetrics and Gynecology, Surgery at Karolinska University Hospital, the only referral center for gynecological malignancy in the Stockholm / Gotland region in Sweden. Clinical follow-up continued to June 1, 2015. Thereafter, clinical data were retrospectively collected into a database by a gynecology surgeon (AFR) (Table 1).

Table 1. Patient Characteristics

Patients Characteristics	
Ovarian cancer (n = 45)	
Median age, years (range)	66 (25–90)
Stage	
IA, IB, IC, IIA, IIB, IIC, IIIC, IV	n = 12
IIIC-IV	n = 33
Median BMI (range)	25.6 (19–37.8)
Median CA125 level	
Initial	436 U/ml
After treatment	18 U/ml
Surgery	n = 45
R0	n = 25 (57%)
R1	n = 12 (27%)
R2	n = 8 (18%)
Neoadjuvant chemotherapy before surgery	
Yes	n = 15 (33%)
No	n = 30 (65%)
Adjuvant chemotherapy after surgery	
Yes	n = 41 (91%)
No	n = 4 (9%)
Dead at study closure	n = 20 (44%)
Alive at study closure	n = 25 (56%)
Benign tumors (n = 30)	
Median age, years (range)	57 (31–82)
BMI (range)	19–36
Median initial CA125 (n = 28)	34 U/ml

BMI, body mass index.

The median age of the patients with ovarian carcinoma was 66 years (range 25–90 years). All underwent surgery during 2010–2012 by a gynecology surgeon; 30 patients had primary debulking surgery, and 15 had interval debulking surgery after neoadjuvant chemotherapy. Fifty-seven percent of the patients had a complete cytoreduction (R0, no visible tumor at the end of surgery), 27% had a R1 resection (1–2 cm^2 of tumor left at the end of surgery) and 18% had a R2 resection (>2 cm^2 of tumor left at the end of surgery) (Table 1). All patients received conventional adjuvant chemotherapy (carboplatin AUC5 and paclitaxel 175 mg/m^2 intravenously every third week in total 6 cycles) according to the Swedish guidelines for standard of care of these patients (Table 1). At study closure, 22 (49%) of the patients were alive. Thirty-one women who had a benign ovarian cystadenoma served as controls; their median age was 57 years (range 31–82 years). In addition to tissue specimens, blood samples were also collected before surgery from 34 patients with ovarian cancer and 30 patients with benign tumors. All diagnoses were confirmed by a reference pathologist of gynecological cancer (JC) at the Department of Pathology, Karolinska University Hospital. Blood plasma samples from 31 healthy aged-matched women in a biobank at our hospital served as controls for HCMV serology. This study was approved by the Stockholm regional ethical committee and the regional ethical committee at the Karolinska Institutet (Dnr: 2008/628–31/2, Dnr: 01–420, Dnr: 2009/1412–31).

Immunohistochemistry

Paraffin-embedded tissue sections were analyzed by immunohistochemistry as described but with minor modifications as follows [10,12]. The sections (4 μm) were deparaffinized in xylene (Sigma-