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Human cytomegalovirus in high grade serous ovarian cancer possible implications for patients survival

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Abstract

Patients diagnosed with high grade serous ovarian adenocarcinoma have a poor prognosis. Recently human cytomegalovirus (HCMV) has been detected in several tumors. Here, we evaluated HCMV in ovarian cancer tissue specimens obtained at pre- and postchemotherapy tumor resection.

Available paraffin embedded ovarian cancer tissues from matched pre- and postchemotherapy tumor resection specimens (i.e., diagnostic excisional biopsy prechemotherapy; DEBPC) and neoadjuvant chemotherapy followed by interval debulking surgery (NACT + IDS) from 10 patients with stage IIIC-IV high grade serous ovarian carcinoma (HGS) diagnosed between years 2007 and 2008 at Karolinska University Hospital were examined for HCMV immediate-early protein (HCMV-IE), tegument protein pp65, and nucleic acid (β2.7) by immunohistochemistry and in situ hybridization.

HCMV-IE and pp65 were detected in 8/10 (80%), 4/9 (44%) and in 4/10 (40%), 5/8 in ovarian cancer tissue specimens from DEBPC and NACT + IDS, respectively. HCMV-β2.7 was detected in all available tissue sections obtained from DEBPC and NACT + IDS. Patients with HCMV-IE or pp65 positive cells in their ovarian tumors at IDS after NACT had a median overall survival of 23.4 and 18.2 months, respectively, compared to 29.6 and 54 months, respectively, in those who did not express HCMV proteins in their tumors.

In conclusion, HCMV proteins and nucleic acids are frequently detected at different levels in HGS ovarian carcinoma. Despite the limitation of our study, shorter median overall survival of patients with HCMV-IE and pp65 in their tumor highlights the need to further investigate the role of HCMV in ovarian cancer patients.

Abbreviations: DEBPC = diagnostic excisional biopsy prechemotherapy, HCMV = human cytomegalovirus, HCMV-IE = human cytomegalovirus immediate-early protein, HCMV-pp65 = human cytomegalovirus tegument protein, HGS = high grade serous ovarian carcinoma, IDS = interval debulking surgery, IHC = immunohistochemistry, IL = interleukin, ISH = in situ hybridization, NACT = neoadjuvant chemotherapy.

Keywords: HCMV, serous ovarian cancer

1. Introduction

Ovarian cancer is an aggressive malignancy with a high mortality, despite rapid advances in the treatment of these tumors. Regardless of radical surgery and advanced therapy, the 5-year overall survival rate of women with ovarian cancer is less than 50%.[1,2] It is therefore an urgent need to better understand the biology of this tumor in order to develop new therapies and new methods of prevention and early detection.

Women with ovarian cancer are typically treated with combined primary debulking surgery and platinum-based chemotherapy. Poor survival of these patients is due to a number of factors, including high stage at disease presentation and the development of chemotherapy resistance.[1,2] Inherited genetic factors explain 10% to 15% of ovarian cancers.[3] Immunological and environmental factors including inflammation, infectious agents, and sexual hormones are also suggested to be involved in malignancy development of ovarian cancers.[4] Long-term exposure of Müllerian epithelium to oxidative environment and inflammatory mediators such as interleukin (IL)-6, IL-1beta, tumor necrosis factor-α including prostaglandins, bioactive eicosanoids, plasminogen activators, collagenases may promote tumorigenesis of these cells.[4] The role of pathogens and their ability to drive inflammation is not well studied in the etiology of ovarian cancer. Previous studies have shown evidence of human

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papillomavirus and Epstein–Barr-virus in ovarian cancer.[5–7] Recently, human cytomegalovirus (HCMV) has been detected in several malignancies, including breast, colon, and prostate cancer.[8,9] Shanmughapriya et al[10] found HCMV-glycoprotein DNA by polymerase chain reaction analysis in 50% of tumor tissue specimens from ovarian cancer patients. The goal of this study was to examine the presence of HCMV-protein and DNA in ovarian tissue specimens collected pre- and postchemotherapy in order to determine if HCMV viral proteins and DNA could be detected in ovarian cancer and to investigate whether chemotherapy influenced the expression of virus proteins.

2. Material and methods

Paraffin embedded ovarian cancer tissues were available from diagnostic excisional biopsy prechemotherapy (DEBPC) and interval debulking surgery (IDS after neoadjuvant chemotherapy [NACT]) from 10 patients who were diagnosed with high grade serous ovarian carcinoma (HGS) between years 2007 and 2008 at the Gynecological Oncology Department, Karolinska University Hospital (KUH), Stockholm. Median age of these patients was 65 at time of surgery. All patients had an advanced HGS ovarian cancer, stage IIIC-IV and primary debulking surgery was not possible in those patients. Each patient initially had an exploratory laparotomy with confirmation excisional biopsy taken. Thereafter treatment with 4 to 5 cycles of NACT and then an IDS was performed (Table 1). Diagnoses were confirmed by a reference pathologist of gynecological cancer at the Pathology Department, KUH. One representative tumor section was examined for the presence of HCMV proteins and nucleic acid by immunohistochemistry (IHC) and in situ hybridization (ISH), respectively.

This study was approved by Stockholm regional ethical committee and the Regional Ethical committee at Karolinska Institutet Stockholm, Sweden (Dnr: 2008/628-31/2).

2.1. Immunohistochemistry (IHC) and in situ hybridization (ISH)

Previously described IHC staining was performed on paraffin embedded tissue sections with minor modifications.[8] Deparaffination and rehydration of all tissues was performed in xylene (Sigma–Aldrich) and ethanol series (Apoteket Farmaci Stockholm, Sweden), respectively. Antigen retrieval in the tissue sections was performed by treatment with pepsin (BioSite, Täby, Sweden) at 37°C for 15 minutes and overnight incubation in Citra buffer (pH 7.6, BioSite) at 37°C in water bath. Blocking of endogenous unspecific binding was done by treating the tissue sections in 3% H_2O_2 (Histolab, Gotenborg, Sweden), avidin/biotin blocking reagents (Dako, Denmark), FC receptor blocker (Innovex Biosciences, Richmond, CA), and background buster (Innovex Biosciences, CA). Monoclonal antibodies against HCMV immediate-early protein (HCMV-IE) (Chemicon International, MA) and HCMV tegument protein pp65 (BioGenex, CA) were used for the detection of different HCMV proteins and antibodies against keratin 20 (Chemicon) or omitting primary antibodies were used as controls. Three step enzymatic detection system consisting of biotinylated secondary antibodies, HRPO labeled streptavidin, and chromogen diaminobenzidine (all from Innovex Biosciences) were used to detect positive signals. Estimated positive cells within the tissue section were scored as follows: 1, ≤10%; 2, ≥11%–50%; and 3, >50.[8] All stained slides were independently reviewed by a pathologist (JC) and a senior scientist (AR). Furthermore, available tissue sections were examined for HCMV-DNA by ISH using Digoxigenin labeled HCMV-β 2.7 probe for the most abundantly transcribed early *HCMV* gene (ZytoVision). Pretreatment and detection of probes were performed using Vysis paraffin pretreatment IV (CA# 01N31-005, Abbott) and Zytofast Plus chromogenic ISH Implementation Kit AP-NBT-BCIP (CA# T-1061-40, ZytoVision) as recommended by the manufactures. ZytoFast DNA (+) control probe and ZytoFast DNA (−) control probe (ZytoVision) served as positive and negative controls, respectively.

3. Results

HCMV-IE and human cytomegalovirus tegument protein (HCMV-pp65) were detected in 8/10 (80%), 4/10 (40%) and in 4/9 (44%), 5/8 ovarian cancer tissue specimens from DEBPC and at IDS after NACT, respectively (Fig. 1 and Table 2). HCMV proteins were detected in both the tumor cells and surrounding stromal cells. Estimated number of cells positive for HCMV-IE and pp65 within the tissue sections varied and were scored as previously described.[8,11,12] The expression levels of HCMV IE or pp65 proteins decreased in all patients with a positive score from DEBPC to the score after NACT. However, in those who

Table 1

Patients characteristic.

Patients	BMI	Age at surgery, y	Interval surgery*	Initial CA125 levels	Posttreatment CA125 levels	HGS	Stadium	OS, mo
1†	23	70	Yes	431	4	Yes	IV	4361
2‡	27	57	Yes	429	38	Yes	IIIC	1364
3‡	23	43	Yes	9000	90	Yes	IIIC	1682
4‡	24	84	Yes	96	24	Yes	IIIC	2957
5‡	22	63	Yes	1039	12	Yes	IIIC	2489
6‡	28	79	Yes	1015	11	Yes	IIIC	5392
7‡	25	70	Yes	7142	213	Yes	IV	1817
8‡	20	57	Yes	1700	172	Yes	IIIC	2867
9†,§	20	63	Yes	853	76	Yes	IIIC	12,953
10‡	22	67	Yes	704	32	Yes	IIIC	515

BMI = body mass index, CA = cancer antigen, HGS = high grade serous ovarian carcinoma NN = not none, OS = overall survival.

* Within 4 to 6 mo after 1st surgery.

† Chemotherapy regimens was 5 with Taxol and Paraplatin before interval surgery.

‡ Chemotherapy regimens was 4 with Taxol and Paraplatin before interval surgery.

§ Still alive at study enclosure.

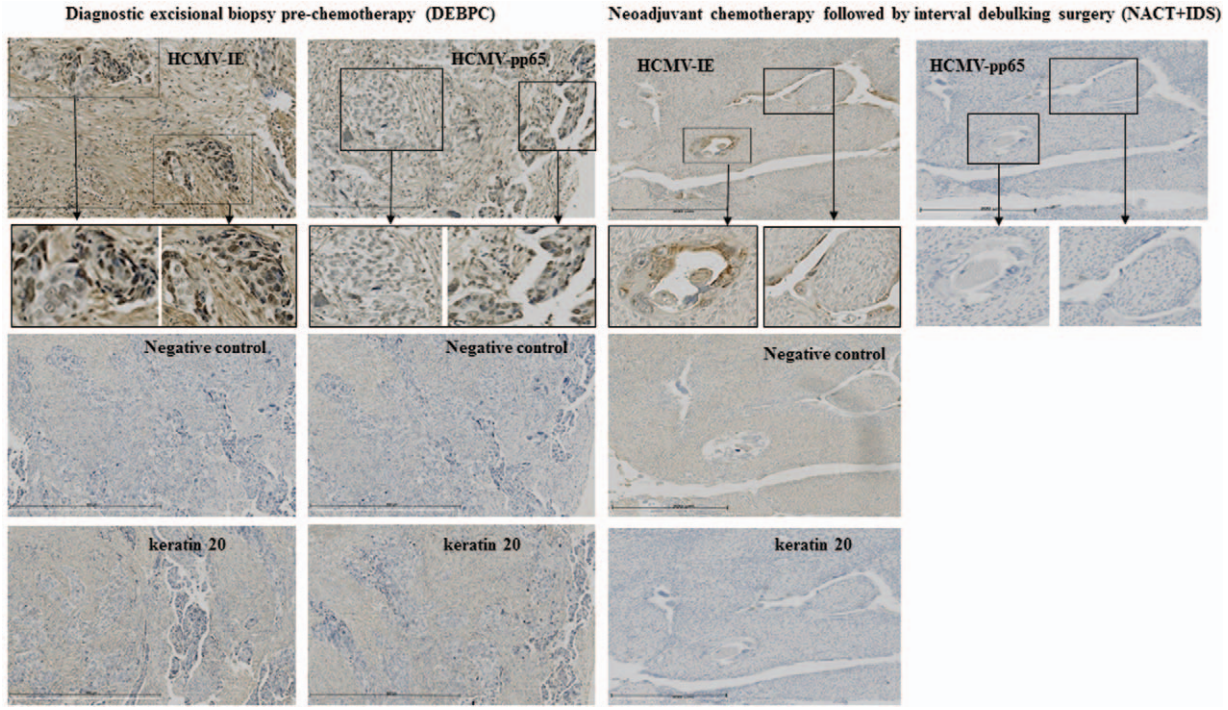

Figure 1. Detection of HCMV-IE and pp65 in HGS ovarian carcinoma tissue sections. HCMV-IE and pp65 were detected frequently at different levels in tumor cells and in the stroma in HGS ovarian cancer tissue sections obtained at DEBPC and IDS after NACT. DEBPC = diagnostic excisional biopsy prechemotherapy, HCMV-IE = human cytomegalovirus immediate-early protein, HCMV-pp65 = human cytomegalovirus tegument protein, HGS = high grade serous ovarian carcinoma, IDS = interval debulking surgery, NACT = neoadjuvant chemotherapy.

were negative, HCMV protein expression was induced after NACT treatment (Table 2). HCMV-IE was detected at score 2 in 3/10 (30%) and at score 3 in 5/10 (50%), and HCMV-pp65 was detected at score 2 in 3/10 (30%) and at score 3 in 1/10 (10%) of patients at DEBPC (Table 2). In tissue sections obtained from patients at IDS after NACT, HCMV-IE was detected at scores 1 and 2 in 2/9 (22%). HCMV-pp65 was detected at score 1 in 5 of 8 available samples and no patients had scores 2 or 3 in their tumor tissue biopsy at IDS after NACT (Table 2). HCMV-β2.7 was detected in all tissue sections obtained from DEBPC (n = 3) and NACT + IDS (n = 5) (Fig. 2 and Table 2). Patients with positive cells for HCMV-IE or pp65 in their ovarian tumors at IDS after NACT had median overall survival of 23.4 (n = 4) and 18.2 (n = 5) months, respectively, compared to 29.6 (n = 5) and 54.0 (n = 3) months in those who did not have detectable HCMV protein expression in their tumor, respectively.

4. Discussion

In this study, tumor samples were collected at DEBPC and at IDS after NACT from 10 patients with HGS ovarian cancer. All patients received NACT (4–5 times Taxol /Paraplatin) before IDS. In the tissue sections from DEBPC, HCMV-IE, HCMV-pp65 proteins, and HCMV-β2.7 DNA were detected in 8/10 (80%), 4/10 (40%), and 3/3 (100%) of the patients. However, at IDS after NACT, which was done at mean time 5 months after DEBPC, HCMV-IE, and pp65 were still detectable in 4/9 (44%), 5/8 of the patients, respectively. HCMV-pp65 was detected only

Table 2

Detection of HCMV proteins and DNA in ovarian tumor tissues by immunohistochemistry and in situ hybridization.

Excisional biopsy prechemotherapy (DEBPC)				Neoadjuvant chemotherapy followed by interval debulking surgery (NACT + IDS)		
Patients	HCMV-IE	HCMV-pp65	HCMV-DNA β2.7	HCMV-IE	HCMV-pp65	HCMV-DNA β2.7
1	2	2	NT	Neg	1	3
2	3	3	3	1	1	3
3	3	Neg	NT	Neg	1	NT
4	2	Neg	3	Neg	NT	NT
5	3	2	NT	Neg	1	3
6	3	2	NT	Neg	Neg	NT
7	3	Neg	NT	1	1	NT
8	Neg	Neg	NT	2	Neg	3
9	Neg	Neg	NT	2	Neg	3
10	2	Neg	2	NT	NT	NT

Estimated number of the cells within the tissue sections expressing HCMV: 1, ≤10%; 2, ≥11%–50%; 3, >50. HCMV = human cytomegalovirus, HCMV-IE = human cytomegalovirus immediate-early protein, HCMV-pp65 = human cytomegalovirus tegument protein, IDS = interval debulking surgery, NACT = neoadjuvant chemotherapy, Neg = negative, NT = no tissue available.

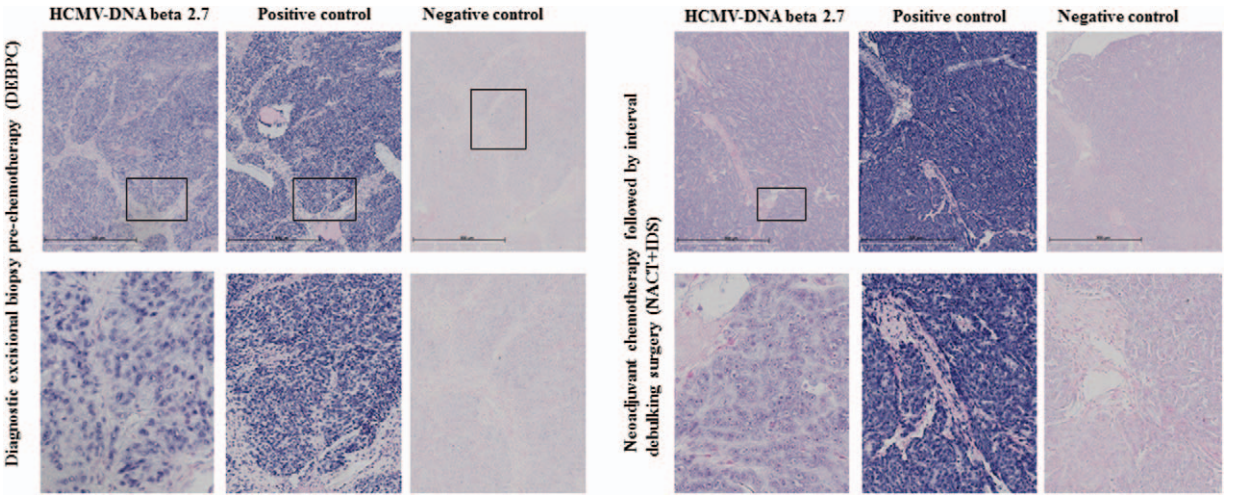

Figure 2. Detection of HCMV-DNA in HGS ovarian carcinoma tissue sections. HCMV-β2 was detected at different intensity in ovarian tissue sections at DEBPC and IDS after NACT. DEBPC = diagnostic excisional biopsy prechemotherapy, HCMV = human cytomegalovirus, HGS = high grade serous ovarian carcinoma, IDS = interval debulking surgery, NACT = neoadjuvant chemotherapy.

at score 1 in the ovarian cancer tissue specimens obtained after NACT. Tumor tissue sections obtained from 2 patients were negative for HCMV proteins at DEPC but the tumor became positive for HCMV-IE protein at IDS after NACT. Both patients were HCMV DNA positive. This observation indicates that reactivation of latent HCMV within the tumor at IDS may be induced with NACT as both viral proteins could be detected in tumor tissue sections obtained from these 2 patients after treatment. Although unlikely, it can however not be excluded that the virus infected the tumor between the 2 test occasions. Furthermore, HCMV-β2.7 was detected at lower intensity in all examined tissue samples (n = 5) obtained at IDS after NACT. The observation that HCMV DNA and low grade HCMV-protein expression were detectable in tissue sections after NACT indicates that HCMV may be present at low activity or in a latent phase in cells in these tissues. It is possible that latent HCMV could subsequently be reactivated under the influence of the inflammatory tumor microenvironment or by the chemotherapy per se. In our study, we could detect HCMV-IE and HCMV-pp65 proteins in the majority of tissue sections by carefully optimized IHC and HCMV-β2.7 DNA detection by ISH. This strengthens the previous report by Shanmughapriya et al,[10] who examined the prevalence of HCMV in ovarian cancer tissues by polymerase chain reaction and found HCMV-glycoprotein DNA in 50% of the patients.

Inflammation is a key factor for the reactivation of latent HCMV. Previous studies have implied a potential role of inflammatory factors in the ovarian malignancy process.[13] Active HCMV infection may aggravate the inflammatory microenvironment by increasing production of inflammatory factors such as IL-1β, IL-6, IL-8, tumor necrosis factor-α, transforming growth factor-β, viral IL-10, prostaglandins, and leukotrienes.[14,15] Antiinflammatory drugs reduce HCMV replication,[10] and we earlier showed a suppressive role of the cyclooxygenase-2 inhibitor Celecoxib in a xenograft model of medulloblastoma.[16]

Oncomodulation is the process by which HCMV and its expressed viral proteins can affect tumor-related processes. The HCMV-IE proteins interfere with key cellular factors including retinoblastomprotein family (Rb), cyclins, p53, Wnt, PI3K/Akt, and NF-κB and this modulation affects cellular apoptosis, proliferation, differentiation, migration, and elimination by the immune system.[15,17,18] Further, a number of proteins including both HCMV-pp65 and HCMV-β2.7 are important in providing immune evasion and antiapoptotic strategies. HCMV-pp65 is a major abundant viral tegument protein with enzymatic kinase activity that is involved in oncomodulation via immune evasion by downregulation of histocompatibility complex class (HLA)-I and II and preventing antigen presentation and immune system activity and preventing crosstalk between natural killer (NK) and dendritic (DC) cells by interfering with NKp30 activating receptor.[19,20] Moreover, HCMV-pp65 contributes to immunosuppression by downregulation of the interferon response.[21] By direct interaction of HCMV-β2.7 with complex I of the respiratory chain in mitochondria, HCMV infection inhibits apoptosis and preserves metabolic activity in the target cells by maintaining ATP production.[22] The virus ability to delay and prevent apoptosis may as a consequence prevent the therapeutic action of chemotherapy in HCMV-infected tumor cells. Thus, the activity of HCMV in a tumor may promote disease progression and prevent desired effects of chemotherapy in some cancer patients. Indeed, we found the activity of HCMV is linked to patient outcome; patients with lower activity of HCMV in their tumors have a better prognosis.[11,12] Interestingly, in our study, the 5 patients with no detectable HCMV-IE and 3 patients with no detectable HCMV-pp65 in their tumor at IDS after NACT lived 6.2 (29.5 vs 23.4 months) and 35.3 (54 vs 18.2 months) months longer than those who had HCMV pp65 detected in their tumors. These observations suggest that lower activity of HCMV is associated with better patient outcome. Therefore, antiviral therapy may have place in future cancer treatment. In support of this statement, anti-HCMV treatment reduces growth of Medulloblastomas and Neuroblastoma xenografts in animal models[16,23] and anti-HCMV therapy as add on to standard therapy indicate highly improved survival in optimally treated glioblastoma patients.[24]

Ovarian cancer patients have poor survival despite advances in molecular biology, diagnostic imaging, multidisciplinary surgical intervention, and oncological treatment. In this study, patients with HCMV-IE or pp65 expression in their tumor samples had

shorter survival compared to those without HCMV in their tumor (29.6, 23.4 vs 54.0, 18.2 months). Despite a limited number of specimens in this study, our findings may indicate a role for HCMV in ovarian cancer. Further studies should focus on validating these findings in a larger cohort of patients. Elucidating the potential role of HCMV in epithelial ovarian cancer is of great interest given the availability of antiviral therapies that may be active in this disease.

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