Detection of Human Cytomegalovirus in Patients with Epithelial Ovarian Cancer and its Impacts on Survival

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Abstract

Background: The cause of epithelial ovarian cancer(EOC) is not elucidated. It has been proved that infectious agents could contribute to ovarian carcinogenesis. Human cytomegalovirus (HCMV) has been detected in several types of tumors. Objective: To investigate the potential role of HCMV infection in EOC, we evaluated the prevalence of HCMV proteins in EOC tissue sections and its impacts on patients' survival. Methods: Formalin-fixed, paraffin-embedded tissues from 66 patients with EOC and 30 patients with benign ovarian cystadenoma were studied. Specimens were detected for expression of HCMV immediate-early protein (IE) and HCMV tegument protein (pp65) by immunohistochemistry. Results: HCMV-IE protein expression was detected in 82% of EOC and 36% of benign tumors; pp65 was detected in 97% of EOC and 63% of benign tumors. Extensive expression of HCMV-IE protein was associated with higher stage of EOC. Reactivation of latent HCMV within the tumor at interval debulking surery may be induced by neoadjuvant chemotherapy before surgery. Extensive HCMV-IE expression was associated with shorter median overall survival than focal or negative expression (39 versus 41 months, P = 0.03). Conclusions: This study demonstrate a high prevalence of HCMV proteins in tissue sections from patients with EOC. HCMV infections can be potential risks for EOC development. The relationship between HCMV and clinical outcomes highlight the need for further researches on the oncomodulatory role of HCMV in ovarian cancer.

Introduction

Ovarian cancer is a major cause of cancer-related deaths in women. Patients are often diagnosed with advanced-stage due to the lack of effective screening methods and the non-specific symptoms. Ovarian cancer is a highly fatal disease, with a global 5-year survival rate of 30–40% in women at advanced stages of diagnosis[1]. However, our understanding of the exact cause of ovarian cancer is limited. During recent years, serous tubal intraepithelial carcinomas (STIC) have been shown to be precursor lesions of serous EOC [2]. Anatomatically, the female peritoneal cavity and internal genitalia are accessible to outside pathogens through the genital tract. Furthermore the fallopian tubes are easily affected by pelvic inflammatory disease (PID), it is therefore highly hypothesized that microbial infection may contribute to ovarian cancer[3].

HCMV is a member of the β-herpesviruses family, which can establish life-long latency. If the patient's immunological status is impaired, the viral replication cycle will be reactivated[4]. During active infection, HCMV expresses several proteins, some are essential for its replication and a largr amount may interfere with the cellular and immunological functions, enabling the virus to coexist with its host[5]. Recently, several studies provide evidence that HCMV proteins and nucleic acid has been detected in tissue from several malignancies, including cervical[6], breast[7], colorectal[8], as well as glioblastoma[9] and neuroblastoma[10]. Shanmughapriya et al.[11] first found HCMV-glycoprotein DNA by polymerase chain reaction analysis in 50% of tumor tissue specimens from ovarian cancer patients. Carlson et al[12]. reported that HCMV proteins and nucleic acids are frequently detected at different levels in high grade serous ovarian carcinoma, and shorter median overall survival was shown in patients with positive

HCMV-IE and pp65. However, Ingerslev et al. [13]examined the prevalence of Epstein-Barr Virus (EBV) DNA and HCMV DNA in EOC tissue samples, HCMV DNA was detected in only one case sample (0.5%), showing no association between HCMV and EOC.

To elucidate the potential role of HCMV in ovarian cancer and possible impact of HCMV infection on the survival rate, we investigated the prevalence of HCMV protiens in EOC tissue samples and compared findings to those obtained in a benign ovarian cystadenoma group prospectively.

Materials And Methods

Clinical Samples

Between January 1st 2015 and December 31st 2015, 66 patients with EOC and 30 patients with benign ovarian cystadenoma were enrolled in the study. All patients gave informed consent and underwent surgery at the Gynecology Department in The Affiliated Hospital of Qingdao University. Formalin-fixed, paraffin-embedded, surgical specimens were obtained from the pathology tissue procurement archives of the hospital. Thirty-four patients had primary debulking surgery, and 32 had interval debulking surgery after neoadjuvant chemotherapy. All patients received conventional adjuvant chemotherapy after the surgery. Carboplatin AUC5 and paclitaxel 175 mg/m^2 intravenously every third week in total 6 to 8 cycles is recommended according to the guidelines. Clinical follow-up continued to December 31st, 2019.

Immunohistochemical analyses of paraffin sections

4μm paraffin sections were procured from surgery specimens, deparaffinized in xylene, and hydrated them in graded alcohols. For antigen retrieval, tissue sections were performed by treatment with pepsin (BioSite) at 37°C for 15 minutes, and pH 7.60, 37 °C water bath overnight incubation. Endogenous nonspecific binding of antibodies was blocked with 3% H_2O_2 (Bioss,China), avidin/biotin blocking reagents (Bioss,China), FC receptor blocker (Bioss,China). Monoclonal antibodies against HCMV immediate-early protein (HCMV-IE) (Millipore,USA) and HCMV tegument protein pp65 (Bioss,China) were used for the detection of different HCMV proteins. Antibodies against Keratin 20 (Chemicon) were used as controls. The extent of HCMV infection was scored as follows: negative (0% positive cells), focal (<50% positive cells), or extensive (≥50% positive cells), as estimated from the number of cells expressing HCMV proteins, according to the score criteria from Angelique et al.[14]. All staining results were independently reviewed by a pathologist.

Statistical analyses

Immunohistochemical data were determined in EOC and benign ovarian cystadenoma samples. Chi-square test was applied to analyse categorical data. Overall survival data are presented as Kaplan–Meier

survival curves; patients who were alive at the time of the analysis (December 31st, 2019) were censored. P <0.05 was considered significant. Statistical analyses were done with Graph Pad Prism 8.

Results

1.Patients Characteristics

The mean age of the patients with EOC was 57 years old, ranged from 45-71 years. At study closure, 25 (38%) of the patients were alive. Thirty patients with benign ovarian cystadenoma served as controls(Table 1).

Table 1. Patients characteristics

Patients characteristics	
Epithelial ovarian cancer(n=66)	**n(%)**
Age	
<50y	8
≥50y	58
Initial CA125	
<35U/ml	4
≥35U/ml	62
Pathological type	
Serous adenocarcinoma	54
Mucinous adenocarcinoma	2
Endometrioid adenocarcinoma	2
Clear cell carcinoma	8
Stage	
I,II	8
III,IV	58
Neoadjuvant chemotherapy before surgery	32
Benign ovarian cystadenoma (n = 30)	
Iinitial CA125	
<35U/ml	26
≥35U/ml	4

2.Immunohistochemistry for HCMV

HCMV-IE protein was detected in tumor specimens from 82% of EOC patients and 36% of those with benign cystadenomas(Table 2). HCMV-IE protein expression was extensive in 61%, focal in 21% and negative in 18% of EOC tissues(Figure 1A). Respectively, expression was extensive in 23%, focal in 13% and negative in 64% of benign ovarian cystadenoma(Figure 1B). HCMV-pp65 protein was detected in tumor specimens from 97% of EOC patients and 63% of those with benign cystadenomas(Table 2). The expression was extensive in 76%, focal in 21% and negative in 3% in epithelial ovarian cancer tissue(Figure 1C). Respectively, expression was extensive in 33%, focal in 30% and negative in 37% in benign ovarian cystadenoma(Figure 1D).

Table 2. Expression of HCMV-IE and pp65 in Epithelial Ovarian Cancer and Benign Ovarian Cystadenoma

Type of tumor		HCMV IE			HCMV PP65		
		Extensive, n(%)	Focal, n(%)	Negative, n(%)	Extensive, n(%)	Focal, n(%)	Negative, n(%)
Epithelial cancer	ovarian	40/66(61)	14/66(21)	12/66(18)	50/66(76)	14/66(21)	2/66(3)
Benign cystadenoma	ovarian	7/30(23)	4/30(13)	19/30(64)	10/30(33)	9/30(30)	11/30(37)

3.Higher Tumor HCMV Expression is Associated With More Advanced Disease

Next we analyzed the effects of HCMV on theEOC stage. HCMV-IE expression was extensive in 25% of Stage I-II tumors, 66% of Stage III-IV tumors; HCMV-pp65 expression was extensive in 38%, and 64%, respectively. Advanced tumor stage is correlated with extensive expression of HCMV-IE (P =0.0279) (Table 3).

Table 3. Expression of HCMV-IE and pp65 in EOC tissues of different stages

HCMV-IE	Extensive, n(%)	Focal/negative, n(%)	Chi-square	P
I,II	2(25)	6(75)	4.834	0.0279
III,IV	38(66)	20(34)		

HCMV-pp65	Extensive, n(%)	Focal/negative, n(%)	Chi-square	P
I,II	3(38)	5(62)	2.036	0.1536
III,IV	37(64)	21(36)		

4.Reactivation of Latent HCMV may be Induced by NACT

HCMV-IE expression was extensive in 75% of cancer tissue with NACT before surgery, 47% of cancer tissue without NACT; HCMV-pp65 expression was extensive in 69%, and 53%, respectively. This observation indicates that reactivation of latent HCMV within the tumor at interval debulking surery (IDS) may be induced with NACT as HCMV-IE viral proteins could be significantly extensive expressed in tumor tissue sections with NACT before surgery(P =0.0279) (Table 4).

Table 4. Expression of HCMV-IE and pp65 in EOC tissues

HCMV-IE	Extensive, n(%)	Focal/negative, n(%)	Chi-square	P
NACT before surgery	24(75)	8(25)	5.390	0.0202
No NACT before surgery	16(47)	18(53)		

HCMV-pp65	Extensive, n(%)	Focal/negative, n(%)	Chi-square	P
NACT before surgery	22(69)	10(31)	1.726	0.1890
No NACT before surgery	18(53)	16(47)		

5.Poor Survival Rate Among EOC Patients With Extensive HCMV-IE Expression

To further confirm the effects of HCMV on EOC clinical outcomes, we analyzed the median overall survival (OS) of EOC patients. At time of study closure, 77% of patients with focal or negative expression of HCMV-IE in their tumors were alive versus 32% of those with extensive expression. EOC patients who had focal or negative HCMV-IE expression in their tumors had significantly longer median OS than those with extensive HCMV-IE expression (41 vs.39 months, P = 0.03)(Figure 2A). Similarly, 26% of patients with focal or negative HCMV-pp65 protein expression were alive versus 61% with extensive expression; however, no significant difference in OS was observed(42 vs. 40 months, P = 0.37) (Figure 2B).

Discussion

In this study, the presence of HCMV IE and pp65 was detected both in EOC and benign ovarian cystadenoma. Advanced tumor stage is correlated with extensive expression of HCMV-IE. The rate of extensive expression of HCMV-IE in cancer tissue with NACT before surgery was higher than those without NACT. Median OS was shorter among ovarian cancer patients who had extensive expression of HCMV-IE in their tumors than in those with focal or negative expression. Thus, HCMV may have an oncomodulatory effect that contributes to disease progression in EOC patients. To our knowledge, this is the first demonstration of HCMV infection in patients with EOC among Chinese population.

HCMV is a widespread opportunistic pathogen which is estimated to be carried by 40–100% of the world's population. The infection rate varies according to geographical location, socioeconomic status and age[15]. HCMV can survive in latent form in an immunocompetent host, while it reactivates during immunosuppression. Several studies have identified high frequency of active HCMV infection in tumor tissues. HCMV is considered to be oncomodulatory, although the mechanisms are not clearly understood[16]. The concept of "oncomodulation" suggests that a virus may modulate cellular pathways such as cell proliferation, tumor progression, vascular disease development, and immune evasion[17]. Therfore, HCMV infection may promote malignant transformation by dysregulating the cell cycle and controlling some key physiological processes. Till now, there have been a largr amount of studies suggesting that HCMV proteins such as IE, pp65 and other encoded proteins enable the virus to play an oncomodulation role[18]. For example, HCMV encodes proteins IE1, IE2, pp71, and pUL97 that can bind or phosphorylate Rb family proteins and inhibit the cell cycle arrest functions of p53. Moreover, CMV induces a mesenchymal-to-epithelial transition[19].

In our study, we studied IE and pp65 proteins. IE often serve as transcription factors that regulate the expression of both viral and host cellular genes, which are crucial for efficient viral replication. IE can also

activate production of early and late structural viral proteins, including the viral tegument protein pp65[20]. HCMV-pp65 is an immunomodulatory protein. It affects expression of HLA-class II and thereby helps the virus to avoid recognition and killing of infected cells by T cells[21]. Thus, HCMV-pp65 expression might worsen patient outcome by mediating an immunosuppressive state in the tumor microenviroment.

In cancers which are not attributable to infectious agents, chronic inflammation may also play a critical role in the transition from a precancerous condition to invasive malignancy. Ovarian cancer is a highly fatal disease and high-grade serous ovarian carcinoma (HGSOC) is the most aggressive and common subtype of EOC. Recently the fimbriae of the fallopian tube have been suggested as the precancerous site of HGSOC[22]. Pelvic infammatory disease (PID), an infection of the female reproductive organs, also results in the possibility of ovarian oncogenesis. Previous studies have implied a potential role of inflammatory factors in the ovarian malignancy process[3]. Inflammation is a key factor for the reactivation of latent HCMV. Active HCMV infection may aggravate the inflammatory microenvironment by increasing production of inflammatory factors such as viral IL-10、tumor necrosis factor-α, transforming growth factor-β, prostaglandins[23]. Baryawno et al. showed a suppressive role of the cyclooxygenase-2 inhibitor in a xenograft model of medulloblastoma, indicating that antiinflammatory drugs can reduce HCMV replication[24]. Paradowska et al. reported low amounts of viral DNA copies in EOC tissues, suggesting that HCMV exists in ovarian and fallopian tube cells in a latent phase and could be reactivated under the infuence of the infammatory tumor microenvironment. Their study proved the role of HCMV as an oncomodulator rather than involving in direct transformation[25]

There are also a number of reports concerning HCMV reactivation in patients receiving chemotherapy. In our study, we found that the rate of extensive expression of HCMV-IE in cancer tissue with NACT before surgery was higher than those without NACT. Chemotherapy might significantly suppresse cellular immunity and expose patients to a greater risk of HCMV infection. It is possible that latent HCMV could subsequently be reactivated by the chemotherapy before or the dysregulated inflammatory tumor microenvironment[26].

Conclusion

In the present study, HCMV-IE and pp65 were frequently detected in EOC tissue specimens, but only extensive expression of IE was significantly associated with OS. Evidently HCMV affects the clinical outcomes of EOC, this virus may provide a new therapeutic target in ovarian cancer. Our observations suggest that EOC patients who had extensive HCMV-IE expression in their tumors had significantly poor durvival rate. Therefore, antiviral therapy may have place in future cancer treatment.

The main limitations of the present study were the small number of patients. Further studies are merited to validate these findings in a larger cohort of patients. Furthermore antiviral treatment and immunotherapies in addition to standard therapies may improve the prognosis for patients with HCMV-positive tumors.

Abbreviations

AUC: Area under curve

EOC: Epithelial ovarian cancer;

EBV: Epstein-Barr Virus

FIGO: International Federation of Gynecology and Obstetrics;

HCMV: Human cytomegalovirus;

IDS: Interval debulking surery

IL: *Interleukin*

NACT: Neoadjuvant Chemotherapy;

OS：Overall survival

PID: Pelvic inflammatory disease

STIC: Serous tubal intraepithelial carcinomas

Declarations

Ethics approval and consent to participate

The protocol for the research project was approved by the Ethics Committee of our institution.

Consent for publication

Not applicable.

Availability of data and materials

The dataset used during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interests.

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Authors' contributions

MY and APC designed the study. FZ and XCJ performed data collection. MY, FZ and CL performed pathological analyses. CL ,MY and GNW analyzed the data and performed statistical analyses. All authors contributed to the writing of the proposal and manuscript. All authors read and approved the final version of the manuscript.

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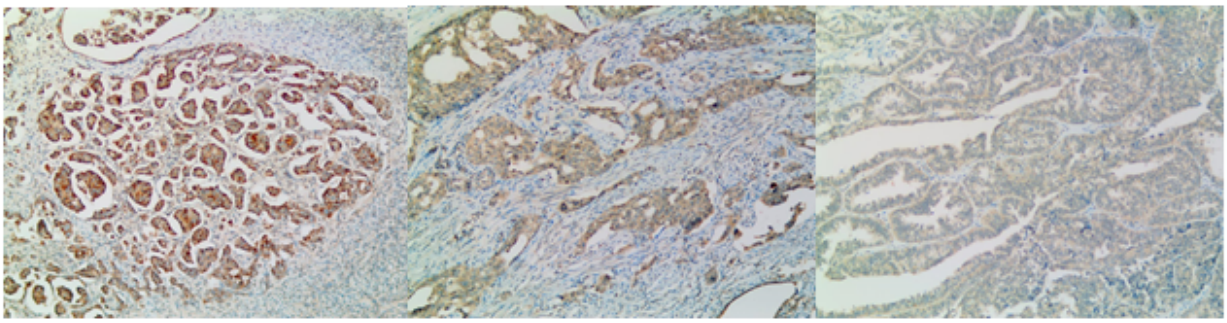
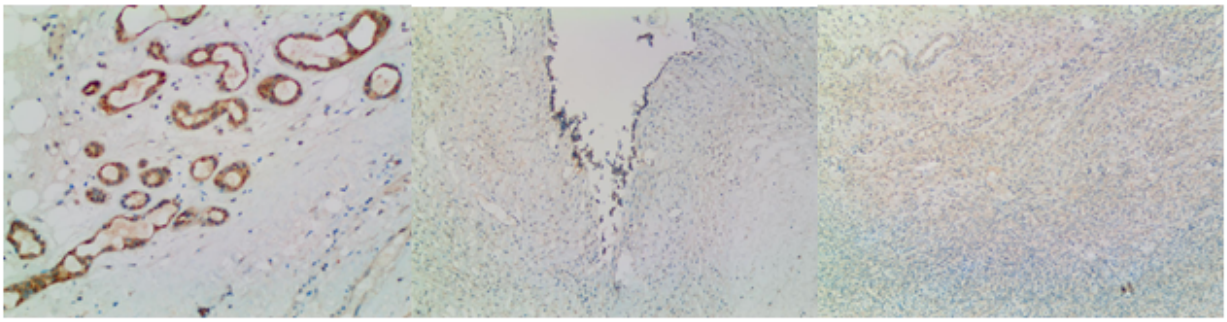
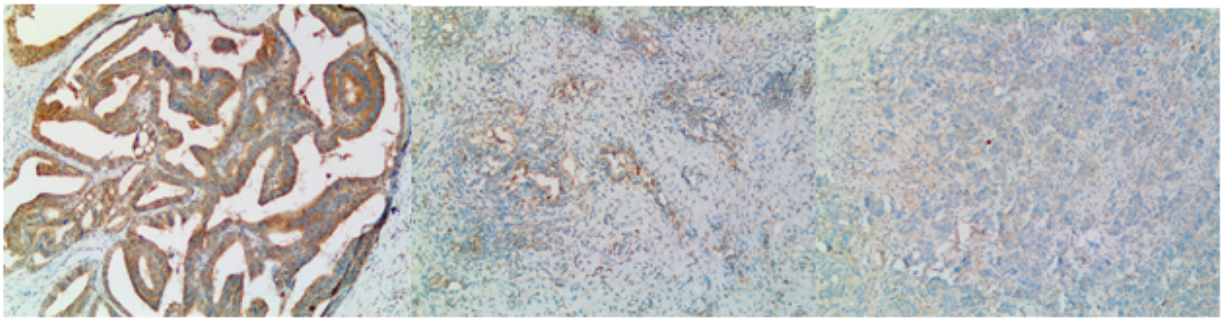
Figures



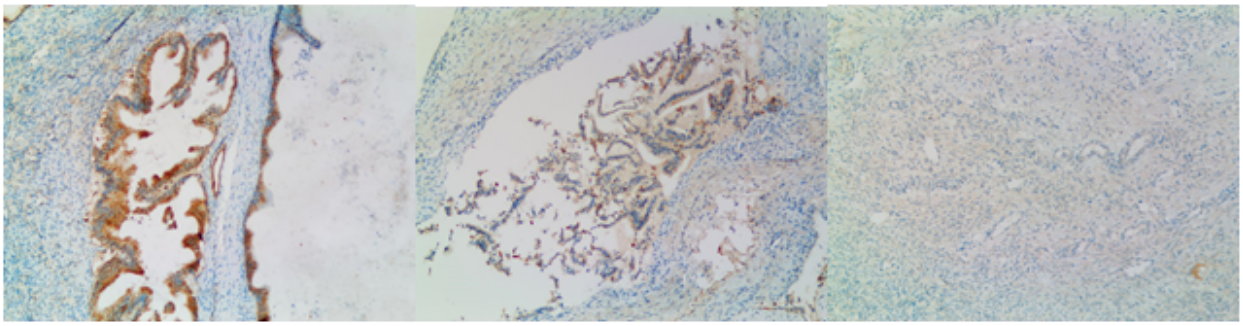
A. HCMV IE extensive, focal and negative expression in EOC sections(10×10).



B. HCMV IE extensive, focal and negative expression in benign ovarian cystadenoma sections(10×10).



C. HCMV pp65 extensive, focal and negative expression in EOC sections(10×10).



D. HCMV pp65 extensive, focal and negative expression in benign ovarian cystadenoma sections(10×10).

Figure 1

Detection of HCMV-IE and pp65 in EOC tissue sections and benign ovarian cytstadenomas.

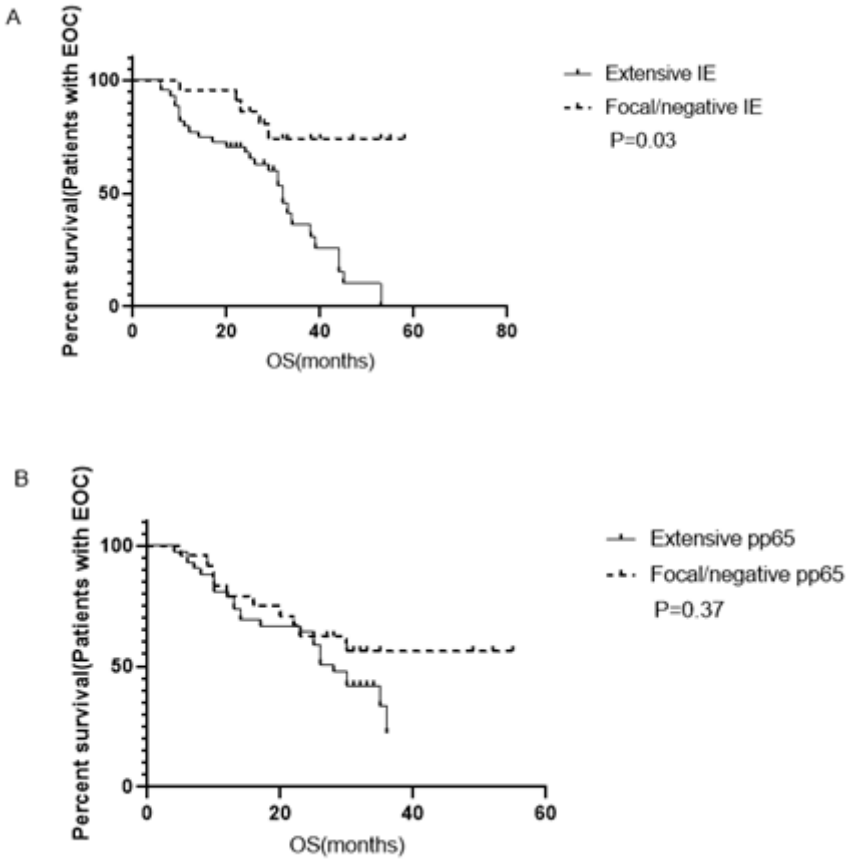


Figure 2

Survival curves of EOC patients with HCMV protein expression. (A) EOC patients who had focal or negative HCMV-IE expression in their tumors had significantly longer median OS than those with extensive HCMV-IE expression (41 vs.39 months, P = 0.03) (B) No significant difference in OS was observed in EOC patients with focal/negative or extensive HCMV-pp65 expression in their tumors (42 vs. 40months, P = 0.37).