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Original Article

Detection of human papillomavirus in squamous cell carcinoma arising from dermoid cysts

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ABSTRACT

Objective: Primary squamous cell carcinoma (SCC) of the ovary in humans is rare. Most cases represent a malignant transformation of ovarian teratoma, Brenner tumor, or endometriosis. The etiology of this cancer remains largely unknown. Human papillomavirus (HPV) infection is a critical factor that induces tumor formation, particularly cervical cancer. Therefore, this study aimed to evaluate the association of HPV with malignant transformation of mature cystic teratoma (MCT) into SCC of the ovary.
Materials and methods: The samples included four formalin-fixed paraffin-embedded SCC-MCT tissues and their adjacent tissues from the cervix to the ovaries, 12 cases of benign teratoma ovarian tissues (dermoid tissues), and 11 cases of benign nonteratoma ovarian tissues (nondermoid tissues). The two squamous carcinoma tissues of the cervix were used as control samples. HPV was detected by immunohistochemistry (IHC) with anti-HPV capsid or E6 (HPV type 16/18) antibodies and *in situ* hybridization (ISH) with three sets of genotyping probes, HPV types 6/11, 16/18, and 31/33.
Results: IHC revealed HPV infection associated with the four cases of SCC-MCT and the two cases of control cervical cancer samples. Importantly, HPV was also detected in adjacent reproductive tissues of the SCC-MCT cases, which suggested that the viral particles might spread in an ascending route through the fallopian tubes, endometrium, endocervix, and cervix to the ovaries. ISH revealed HPV type 16/18 in all SCC-MCT cases and HPV type 31/33 in two, with no HPV type 6/11 in any SCC-MCT cases. However, compared with the SCC-MCT cases, the lower detection rates of HPV in dermoid cysts and nondermoid tissues suggested that HPV might not be associated with normal ovarian tissues or benign ovarian teratomas.
Conclusion: Our data suggest that high-risk HPV infection might be a causal factor that induces malignant transformation of MCT into SCC of the ovary, although further investigation is still required.

Introduction

Ovarian cancer is the fifth leading cause of female cancer death and has the highest mortality rate among all gynecological cancers in the United States [1]. From 2007 to 2011, the annual ovarian cancer incidence and death rates slowly decreased by 0.9% and 2.0%, respectively, in the United States [1]. However, in Taiwan, a study of a 30-year national population-based registry revealed an increasing incidence and a decreasing age at diagnosis of ovarian cancer, which is comparable to that found in other Asian countries [2].

Ovarian cancer represents a heterogeneous group of malignant tumors of ovarian origin that may arise from germ cells, stromal tissue, or epithelial tissue within the ovary. Most ovarian malignancies are epithelial in origin (90%); the remaining 10% are germ-cell tumors, sex-cord stromal tumors, soft-tissue tumors not specific to the ovary, metastatic tumors, and unclassified tumors [3].

A mature cystic teratoma (MCT), or dermoid cyst, is removed with surgery and the condition is then cured. This type of germ-cell tumor is common in women of childbearing age and might consist of mature tissue originating from all three germ-cell layers

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(endoderm, mesoderm, and ectoderm) [4,5]. Although most patients with MCT are asymptomatic, pain and a sensation of abdominal fullness can occur because of the mass; in some cases, somatic malignant transformation of MCT can occur [6]. More than 80% of all malignant transformations of ovarian teratomas are squamous cell carcinomas (SCCs) that arise from the ectoderm; the rest are carcinoid tumors or adenocarcinomas [7–9]. Some cases of SCC arising from the columnar epithelium through squamous metaplasia have been reported [8]. Prolonged exposure to various carcinogens in the female pelvic cavity might cause a malignant change in MCT [10]. High levels of the tumor markers, including SCC antigen, cancer antigen (CA) 125, CA19-9, and CA16-6, were detected in MCT cases; SCC antigen and CA125 may be associated with adverse outcomes [6,11].

Human papillomavirus (HPV) infection is considered a major cause of infection-related cancers of the cervix uteri, vulva, vagina, anus, oropharynx, and penis [12,13]. More than 100 types of HPV have been identified, and at least 20 are associated with cervical cancer [14–16]. HPV types 6 and 11 are low-risk or nononcogenic viruses that cause benign or low-grade cervical cell abnormalities. High-risk oncogenic types (such as types 16, 18, 31, and 33) can cause cancers. Almost all cervical cancers are associated with high-risk HPV infection. Worldwide, approximately one half of all cervical cancers are caused by HPV type 16, and types 16 and 18 together account for approximately 70% of the cases. The oncogenic impact of HPV type 16/18 is critical; however, a recent study in Taiwan found that patients with high-grade squamous intraepithelial lesions were highly infected with high-risk HPV types other than HPV 16/18. This suggests that high-risk HPV-type infections other than type 16/18 should also be managed carefully [17]. Although infection with a high-risk HPV type can cause cervical cancer, the virus infection itself is not sufficient to induce cancer because cancer does not develop in most women with HPV infection [15,18].

HPV detection in epithelial ovarian cancer has been inconsistent. Polymerase chain reaction (PCR) findings are frequently negative [19–23]; however, *in situ* hybridization (ISH) and immunohistochemistry (IHC) revealed HPV infection in 52% of all epithelial ovarian malignancy cases in Chinese women [24]. HPV DNA could also be detected by Southern blot hybridization in ovarian and endometrial tissues [25].

There are fewer confirmed cases of high-risk HPV types in ovarian SCC than in epithelial ovarian cancer [26–29]. Thus, understanding a possible association of HPV infection with SCC of mature teratoma in Taiwan is important. In this study, we examined the association of HPV infection in four MCT cases with malignant transformation of MCT into SCC (SCC-MCT).

Materials and methods

Human tissue samples

We conducted a retrospective chart review. Patient information was obtained from radiograph departments, operative reports, pathology reports, and radiation oncology records. We identified only four patients with SCC-MCT of the ovary treated at our institute in Taiwan between 1990 and 2014. Formalin-fixed paraffin-embedded SCC-MCT tissues (tumor part tissues were identified by a pathologist) of these four cases and their adjacent tissues from the cervix to the ovaries were examined. We also examined samples from a random selection of 11 normal (nondermoid) and 12 benign (dermoid) ovary tissues for comparison. The study was approved by the Institutional Review Board of Kaohsiung Veterans General Hospital (Protocol Nos. VGHKS11-CT12-03 and VGHKS15-CT1-07) and conformed to the ethical principles of the Declaration of Helsinki.

Cells

We examined HPV infection in the TC-1 cell line, an HPV-16 E6 and E7 and activated *ras* oncogene transfectant of primary C57BL/6 mouse lung epithelial cells, kindly provided by T.C. Wu (Johns Hopkins University, Baltimore, MD, USA) [30]. The cells were maintained in Roswell Park Memorial Institute medium supplemented with 10% fetal bovine serum (FBS), 2mM glutamine, 1mM sodium pyruvate, 20mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 IU penicillin/mL, and 100 μg streptomycin/mL. HPV-negative C33A cells (Bioresource Collection and Research Center, Taipei, Taiwan) were grown in Dulbecco's Modified Eagle Medium supplemented with 10% FBS.

Immunohistochemistry

The UltraVision Quanto Detection System HRP Dab (Thermo) was used for IHC analysis. In brief, paraffin-embedded tissue-sample sections were prepared from paraffin-embedded tissue blocks. Sections were dried overnight at 60°C, deparaffinized, and dehydrated with ethanol, and then incubated with blocking buffer to avoid the background staining of nonspecific reaction with endogenous peroxidase. Then, sections were incubated with the monoclonal antibody for HPV capsid (SB24) or HPV 16/18 E6 protein (C1P5, sc-460; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. The monoclonal antibody for HPV, clone SB24 (LifeSpan BioSciences, CA), reacts with an epitope of a major capsid protein of HPV, which is broadly expressed among the various HPV subtypes. Sections were then incubated with Primary Antibody Amplifier Quanto for 10 minutes at room temperature, and then with HRP Polymer Quanto for 10 minutes. The horseradish peroxidase (HRP) substrate diaminobenzidine (DAB) was added to generate a brown polymeric oxidation product. Sections were then counterstained with hematoxylin. The HPV capsid protein signal was observed under a microscope.

In situ hybridization

Detection involved paraffin-embedded ovarian or cervix cancer tissue sections as described previously [31,32]. Deparaffinized and hydrated sections were stained with biotin-labeled HPV 6/11, 16/18, or 31/33 DNA probes (PanPath REMBRANDT DISH-HRP detection kit for HPV types, Amsterdam, The Netherlands) according to the manufacturer's instructions and rinsed several times in Tris–saline; hybridization was then visualized using DAB (Thermo) as a substrate of HRP. Sections were counterstained with hematoxylin. After tapping off the excess counterstain and briefly rinsing in distilled or deionized water, sections were mounted on slides using an aqueous mounting medium and viewed under a microscope.

Statistical analysis

Statistical analysis involved the use of GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Chi-square test was used to test differences in HPV infection among dermoid, nondermoid cyst, and SCC-MCT tissues.

Results

Four SCC-MCT cases

The first case was a 32-year-old woman, gravida 0, para 0, who presented a continual “full” feeling. The diagnosis was a right ovarian cystic teratoma. The patient underwent exploratory laparotomy with right salpingo-oophorectomy. Pathologic analysis