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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.
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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 intra-epithelial 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

revealed International Federation of Gynecology and Obstetrics (FIGO) Stage Ia SCC-MCT. The patient underwent six courses of chemotherapy and second-look surgery with bilateral pelvic lymph node dissection. No residual disease was detected, and none of the 18 lymph nodes contained malignant cells. Currently, she is alive with no evidence of disease.

The second case was a 54-year-old woman, gravida 4, para 4, who had a 4-week history of a palpable abdominal mass. She received a diagnosis of ovarian malignancy and underwent optimal debulking surgery. She had a 16-cm left ovarian mass with mild ascites and enlarged bilateral pelvic lymph nodes. Pathologic analysis revealed SCC-MCT and metastatic disease in the lymph nodes of the pelvis (8/25) and para-aorta (1/3); however, peritoneal cytology and the remaining staging investigation gave negative results. The patient underwent five courses of adjuvant chemotherapy. She presented 18 months later with liver and lung metastasis and died 6 months later.

The third case was a 39-year-old woman, gravida 3, para 2, who complained of abdominal bloating for 4 weeks. She received a diagnosis of left ovarian dermoid cyst rupture with highly malignant potential on ultrasonography and computed tomography. Exploratory laparotomy revealed an 18-cm left ovarian mass with abdominal carcinomatosis, severe pelvic adhesion, and massive ascites. Suboptimal debulking surgery was performed. Pathology revealed SCC-MCT with pelvic lymph node metastasis (2/27 nodes). The tumor ruptured preoperatively because of abdominal carcinomatosis. The patient underwent adjuvant chemotherapy with taxol, carboplatin, and ifosfamide. The levels of serum SCC antigen were immediately increased to 20 ng/mL (normal range 0–1.5 ng/mL) after three courses of chemotherapy. The patient underwent one-time adjuvant radiation therapy but died 6 months later because of urosepsis.

The fourth case was a 49-year-old woman, gravida 1, para 1, who complained of abdominal pain for 1 day. She received a diagnosis of a right ovarian dermoid cyst with torsion by ultrasonography. She underwent urgent laparoscopic unilateral salpingo-oophorectomy, and a 16-cm right ovarian mass was discovered. The pathology report showed SCC-MCT. The patient received a diagnosis of FIGO Stage Ia. She is still alive and regularly visits our outpatient clinic.

The tumors of the four patients were predominantly cystic, filled with pultaceous material, and hair. Foci of solid areas were identified. All tumors showed keratinized stratified squamous epithelium, hair follicles, sebaceous glands, lobules of mature cartilage, glands lined with respiratory mucosa, and skeletal muscle. The clinical data are presented in Table 1.

IHC of HPV

We subjected four SCC-MCT and two squamous carcinoma samples of the cervix to IHC with antibody for HPV capsid protein. Capsid protein was found in all sections, with strong signaling in Case 2 and control-sample cervical tissue 1 (Figure 1A). HPV 16 was found in epithelial ovarian cancer [31], and therefore, we investigated E6 protein expression of the high-risk HPV type 16/18. The HPV 16/18 E6 protein was detected in all SCC-MCT cases and cervical cancer tissues (Figure 1B). We confirmed the accuracy of IHC findings using HPV 16 E6-positive TC1 cells (HPV-16 E6 and E7 and c-Ha-ras oncogene-transfected primary lung epithelial cells of C57BL/6 mice [30]) and HPV-negative C33A cells (cervical cancer biopsy-derived cells [33]). In IHC analysis, TC-1 and C33A cells were negative for anti-HPV capsid (Figure 1C); however, TC-1 cells were positive for anti-HPV-16/18 E6, but C33A cells were negative (Figure 1D).

We tested the tissue distribution of HPV infection in the SCC-MCT samples, cervical cancer tissues, as well as their adjacent tissues from reproductive organs and associated pelvic and para-aortic lymph nodes taken during surgery. HPV capsid and HPV 16/18 E6 proteins were highly detected in the tested samples, which suggested broad HPV infection in the reproductive system of SCC-MCT and cervical cancer patients (Table 2). The selected positive IHC images of SCC-MCT Case 1 are presented in Figure 2 (data of other tested samples are not shown). Our finding suggested that the HPV particles might spread through the reproductive system by an ascending route through the fallopian tubes, endometrium, endocervix, and cervix to the ovaries.

Furthermore, 12 dermoid cyst and 11 nondermoid cyst tissues were subjected to IHC with anticapsid and anti-E6 HPV antibody. Cases 4, 5, and 6 of dermoid cyst were positive for HPV capsid, and no HPV E6-positive case was identified (Figure 3A). Case 7, with a nondermoid cyst, was positive for HPV capsid and E6 (Figure 3B). Compared with the 95% HPV capsid detection rate (all 24 samples from the 4 SCC-MCT cases), the dermoid cyst and nondermoid cyst samples showed significantly lower detection rates of 25% and 9%, respectively (Table 3). Similar results were shown with regard to anti-E6 HPV antibody: 96% of SCC samples were positive for HPV type 16/18 E6, but only 0% of dermoid and 9% of nondermoid cyst samples were positive for this type (Table 3). The lack of findings for anti-E6 positivity in dermoid cysts may due to infection with types of HPV other than type 16/18. Taken together, this evidence suggests that HPV infection in the reproductive system by an ascending route might play a role in the malignant transformation of a benign dermoid cyst into an SCC.

Table 1
Clinical characteristics and outcome of the four cases of squamous cell carcinoma with mature cystic teratoma (SCC-MCT).

Case no.	Age (y)	FIGO Stage	Ultrasonography/computed tomography; tumor diameter (cm)	Tumor markers[a]/cytology	Operation type/ascites	LNs	Outcome
1	32	Ia	Large ovarian cyst, r/o dermoid cyst/postoperative negative findings; 18	CEA: 5, SCC: 0.3 CA125: 68.2, CA199: 69 Cytology: not done	RSO/Nil	Bilateral pelvic: 0/18 (second-look operation)	NED (164 mo)
2	54	IIIc	Large ovarian tumor, r/o malignancy/ovary tumor, r/o malignancy; 16	CEA: 23, CA199: 206 AFP: <3 Cytology: Negative	Optimal debulking/little	Right pelvic: 0/11 Left pelvic: 8/14 Para-aortic: 1/3	DOD (24 mo)
3	39	IIIc	Large ovarian cyst, r/o dermoid cyst with pelvic adhesion/dermoid cyst rupture and malignancy transformation must be considered; 18	CEA: 2, SCC: 9.2 CA125: 57.7, CA199: 87.7 Cytology: Negative	Suboptimal debulking/2400 mL	Right pelvic: 0/10 Left pelvic: 2/17 Para-aortic: 0/1	DOD (6 mo)
4	49	Ia	Large dermoid cyst r/o torsion; 16	SCC: Nil CA125: Nil, CA199: Nil	RSO/Nil	Nil	Alive (37 mo)

AFP = alpha-fetoprotein; CA = cancer antigen; CEA = carcinoembryonic antigen; DOD = dead of disease; FIGO = International Federation of Gynecology and Obstetrics; LNs = lymph nodes; MCT = mature cystic teratoma; NED = no evidence of disease; r/o = removal of; RSO = right salpingo-oophorectomy; SCC = squamous cell carcinoma.
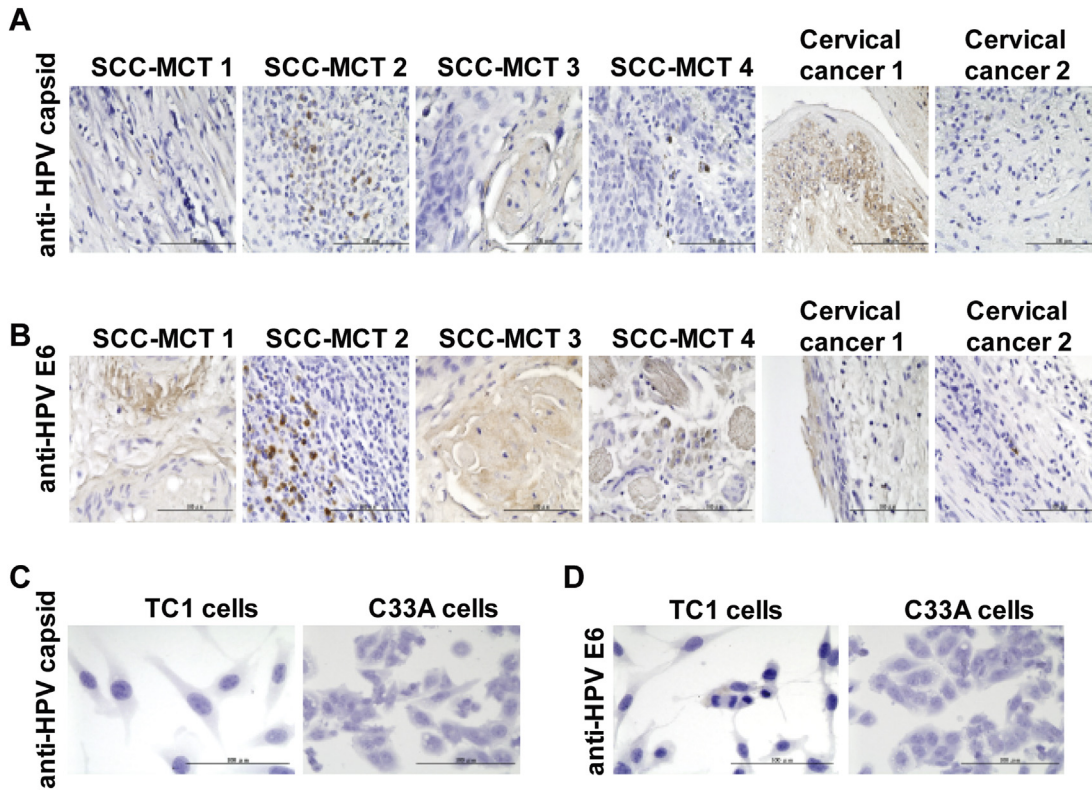[a] Initial diagnosis.

Figure 1. Human papillomavirus (HPV) diagnosis by immunohistochemical (IHC) analysis. (A,B) HPV infection in four cases (Cases 1–4) of squamous cell carcinoma with mature cystic teratoma (SCC-MCT) and two samples of cervical cancer tissue (Cases 1 and 2) detected by IHC with antibodies for HPV capsid protein and HPV 16/18 E6 protein. (C,D) IHC detection of HPV capsid and E6 in TC1 and C33A cells. TC1 cells are capsid-negative and E6-positive cells. C33A cells are HPV-negative cells that do not express HPV capsid and E6 proteins.

Table 2
Human papillomavirus detection by immunohistochemistry in tissue biopsies of cancer patients.

Case no.	Total tissue no.	Anticapsid (+) no. (%)	Anti-E6 (+) no. (%)	Positive rate of anticapsid antibody	Positive rate of anti-E6 antibody
SCC-MCT 1	17	15 (88)	16 (94)	L't SCC-MCT (5/7), R't ovary (1/1), uterus (1/1), cervix (1/1), endometrium (1/1), myometrium (1/1), fallopian tube (0/1), R't pelvic LNs (1/1), L't pelvic LNs (1/1), para-aortic LNs (1/1), omentum (1/1)	L't SCC-MCT (6/7), R't ovary (1/1), uterus (1/1), cervix (1/1), endometrium (1/1), myometrium (1/1), fallopian tube (0/1), R't pelvic LNs (1/1), L't pelvic LNs (1/1), para-aortic LNs (1/1), omentum (1/1)
SCC-MCT 2	19	18 (95)	18 (95)	SCC-MCT (4/4), gray-white polypoid tumor (1/1), uterus (1/1), R't bilateral ovaries (1/1), L't bilateral ovaries (1/1), R't bilateral fallopian tubes (1/1), L't bilateral fallopian tubes (1/1), vaginal cuff (1/1), uterine cervix (1/1), bilateral parametrium (1/1), endometrium (1/1), ovary and fallopian tubes (1/1), bilateral pelvic (1/1), para-aortic LNs (2/3)	SCC-MCT (4/4), gray-white polypoid tumor (1/1), uterus (1/1), R't bilateral ovaries (1/1), L't bilateral ovaries (1/1), R't bilateral fallopian tubes (1/1), L't bilateral fallopian tubes (1/1), vaginal cuff (1/1), uterine cervix (1/1), bilateral parametrium (1/1), endometrium (1/1), ovary and fallopian tubes (1/1), bilateral pelvic (1/1), para-aortic LNs (2/3)
SCC-MCT 3	16	15 (94)	15 (94)	SCC-MCT (5/5), cervix (2/2), endometrium (1/1), nontumor ovarian (1/1), omentum (1/1), colon tumor (0/1), peritoneum (1/1), appendix (1/1), R't pelvic LNs (1/1), L't pelvic LNs (1/1), para-aortic LNs (1/1)	SCC-MCT (5/5), cervix (2/2), endometrium (1/1), nontumor ovarian (1/1), omentum (1/1), colon tumor (0/1), peritoneum (1/1), appendix (1/1), R't pelvic LNs (1/1), L't pelvic LNs (1/1), para-aortic LNs (1/1)
SCC-MCT 4	8	8 (100)	8 (100)	SCC-MCT (8/8)	SCC-MCT (8/8)
Cervical cancer 1	16	15 (94)	15 (94)	Cervical tumor (4/4), vaginal cuff (1/1), R't parametrium (1/1), endometrium (1/1), R't ovary and tube (1/1), R't common iliac LNs (1/1), R't external iliac LNs (1/1), R't internal iliac LNs (0/1), R't obturator LNs (1/1), L't external iliac LNs (1/1), L't internal iliac LNs (1/1), L't obturator LNs (1/1), para-aortic LNs	Tumor (4/4), vaginal cuff (1/1), R't parametrium (1/1), endometrium (1/1), R't ovary and tube (1/1), R't common iliac LNs (1/1), R't external iliac LNs (1/1), R't internal iliac LNs (0/1), R't obturator LNs (1/1), L't external iliac LNs (1/1), L't internal iliac LNs (1/1), L't obturator LNs (1/1), para-aortic LNs
Cervical cancer 2	4	4 (100)	4 (100)	Cervix tumor (2/2), R't parametrium (2/2)	Tumor (2/2), R't parametrium (2/2)

L't = left; LNs = lymph nodes; MCT = mature cystic teratoma; R't = right; SCC = squamous cell carcinoma.

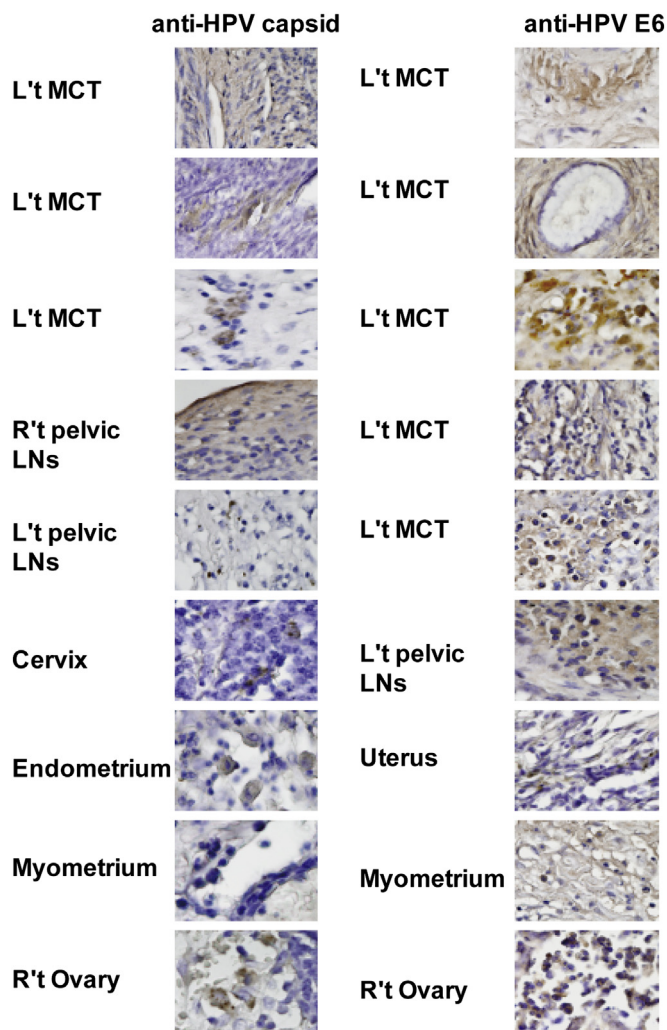## SCC- MCT and adjacent tissues of case1

Figure 2. Positive IHC data of Case 1 SCC-MCT and adjacent tissues. IHC analysis with anti-HPV capsid and E6 protein was performed in tissues including multiple SCC-MCT tissue blocks as indicated. HPV = human papillomavirus; IHC = immunohistochemistry; L't = left; LNs = lymph nodes; MCT = mature cystic teratoma; R't = right; SCC = squamous cell carcinoma.

ISH of HPV

The IHC data showed the HPV capsid and HPV 16/18 E6 detected in the tissue samples of SCC-MCT and adjacent tissues and cervical cancers. It would be important to demonstrate that HPV DNA existed in those specimens; thus, we used the well-established method of ISH analysis for HPV DNA detection [26]. The ISH probes targeting high-risk oncogenic HPV types, types 16/18 and 31/33, and low-risk or nononcogenic HPV types, 6/11, were used to demonstrate that the HPV DNA was incorporated into the cell nucleus. Our data revealed that HPV type 16/18, but not HPV type 6/11, DNA was found in the four SCC-MCT samples, and HPV type 31/33 DNA, high-risk HPV types associated with cervical cancer, was detected in SCC-MCT Cases 2 and 3 (Figure 4). This result revealed co-infection with multiple high-risk types of HPV in ovarian tissue. Two cervical cancer samples were positive for HPV 16/18, and HPV 6/11 DNA was found in one sample (Figure 4). The ISH results were consistent with IHC data (Figure 1).

Discussion

Primary SCC of the ovary is rare. Most cases represent malignant transformation of ovarian teratoma, Brenner tumor, or endometriosis. In this study, we described four cases of ovarian teratoma with malignant transformation of MCT into SCC. We detected HPV infection in SCC-MCT ovarian samples by IHC and ISH, which suggests that HPV infection is associated with SCC-MCT in ovary dermoid cysts. HPV infection was also detected in adjacent reproductive tissues of the SCC-MCT cases, which suggests that the viral particles might spread within the reproductive system. The lower detection rates of HPV in dermoid and nondermoid cysts indicate that HPV might not be associated with normal or benign ovarian teratomas. However, whether HPV causes malignant transformation of benign dermoid cysts into SCC remains to be further explored.

HPV plays a causal role in cervical cancer. HPV infection is also detected in other cancers of the female lower genital tract, including cancers of the vulva, vagina, and perineum [13,15,34]. However, the role of HPV in the pathogenesis of ovarian cancer is controversial, and the results are conflicting. Two systematic reviews noted the geographical variation in HPV prevalence in epithelial ovarian cancer tissue [35,36]. Overall, HPV prevalence in patients with ovarian cancer was found to be 15.5–17%: 0% in North America, 4.0–18.5% in Europe, and 31.4–45.6% in Asia. Four HPV types, types 6, 16, 18, and 33, were found in ovarian cancer; HPV type 16 was the most common, with a prevalence of 39.7%, followed by type 18 [35,36]. HPV does not play an important role in Western European and North American populations but may have a role in other populations, although HPV-mediated ovarian epithelial tumorigenesis remains inconclusive [37–39].

To the best of our knowledge, only four confirmed cases of high-risk HPV types have been reported in ovarian SCC. The first was case was reported in 1996 in a 40-year-old Canadian woman who had SCC-MCT, recurrent high-grade intraepithelial neoplasia of the vulva, and high-grade cervical intraepithelial neoplasia; HPV type 16/18 DNA was identified by ISH in the invasive carcinoma in the left ovary [26]. A second case was reported in the United States, where a 55-year-old woman who had *in situ* SCC of the cervix with contiguous metastasis and invasion of the endometrium, fallopian tubes, and ovaries; untyped HPV DNA was detected by PCR in SCC tissues [28]. The third case was from the United Kingdom; in 1998, a 52-year-old woman received a diagnosis of synchronous ovarian and cervical SCC; staining was positive for HPV-16 E6 [27]. A fourth case of HPV infection was identified by pan HPV IHC staining in a 48-year-old woman with SCC-MCT in Belgium in 2007 [29]. We herein presented four additional SCC-MCT cases associated with high-risk HPV infection. This report is the first account of HPV associated with ovarian SCC in Taiwan. The previous and present cases suggest that HPV may be involved in the development of ovarian SCC. Nevertheless, there are also four reported SCC-MCT cases that were negative for HPV [22,40–42]. It was thought that the low sensitivity of the techniques might result in a failure to demonstrate the presence of HPV in SCC-MCT [26]. Therefore, a large cohort study with HPV sequence typing should be conducted to validate the oncogenic role of HPV in SCC-MCT.

Our findings of positive HPV from ovarian squamous and female genital tract tissues are similar to a previous case of SCC-MCT in the same area [28]. In addition, a PCR-based study found HPV 16/18 DNA in Hong Kong Chinese women with endometrial carcinomas and primary epithelial ovarian cancer [38]. Thus, the upper genital tract may be susceptible to HPV infection. The virus particles likely spread in an ascending route through the fallopian tubes,
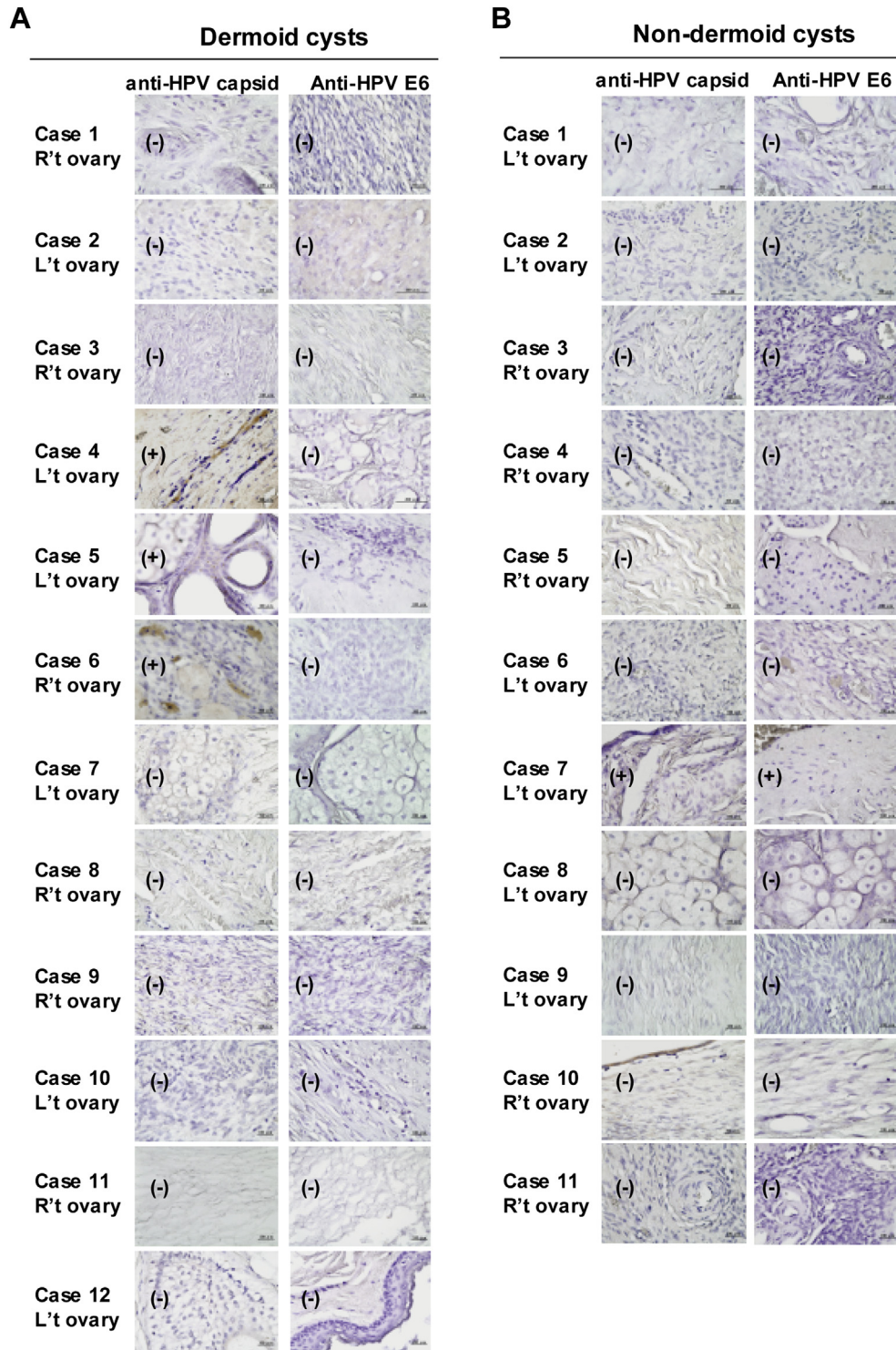
Figure 3. Human papillomavirus (HPV) detection in dermoid and nondermoid ovarian tissues. Immunohistochemical (IHC) analysis of HPV capsid and type 16/18 E6 in (A) 12 dermoid and (B) 11 nondermoid cyst samples from the right or left ovary. R't = right; L't = left; (+) = positive IHC staining; (−) = negative IHC staining.

endometrium, endocervix, and cervix to the ovaries. This suggestion is further supported by our finding of HPV positivity in tissues from the ovarian dermoid cyst, suggesting retrograde passage along the genital tract of tissues and the involvement of viral particles that facilitated the development of squamous lesions in the ovary.

Comparison of the low positive rate of HPV infection in dermoid and nondermoid cysts with a high positive rate of HPV infection in SCC-MCT tissues and their adjacent tissues revealed an association

between HPV infection and malignant transformation of teratoma. It would be important to understand whether HPV infection is correlated with poor prognosis of ovarian teratomas.

Because this is a retrospective study, the specimens were prepared from paraffin-embedded tissues for ISH and IHC analysis. The DNA extraction, PCR, and sequencing were poorly implemented because of the limited tissue blocks. However, the applied methods (IHC and ISH) are well established [26,27]. In addition, the

Table 3
HPV detection by immunohistochemical analysis in dermoid and nondermoid cyst tissues.

Tumor type	Total sample no.[a]	Anticapsid (+) (%)	Anti-E6 (+) (%)
Dermoid cyst (12 patients)	12	3 (25)	0 (0)
Nondermoid cyst (11 patients)	11	1 (9)	1 (9)
SCC-MCT (4 patients)[a]	24	22 (92)	23 (96)

Anticapsid, $p < 0.001$, χ^2 test; anti-E6, $p < 0.001$, χ^2 test.
HPV = human papillomavirus; MCT = mature cystic teratoma; SCC = squamous cell carcinoma.

[a] One sample from each case of dermoid and nondermoid tissues was analyzed. The number of tested SCC-MCT tissue blocks (tumor part) from the four SCC-MCT cases is as follows: Case 1, seven blocks; Case 2, four blocks; Case 3, five blocks; Case 4, eight blocks.

individual HPV type in SCC-MCT tissues could be identified by sequencing. In this study, all cases of SCC-MCT (4 patients) diagnosed in our hospital were included; to further validate the role of HPV in SCC-MCT development, a nationwide large-scale cohort trial should be conducted in the future. An investigation with fresh
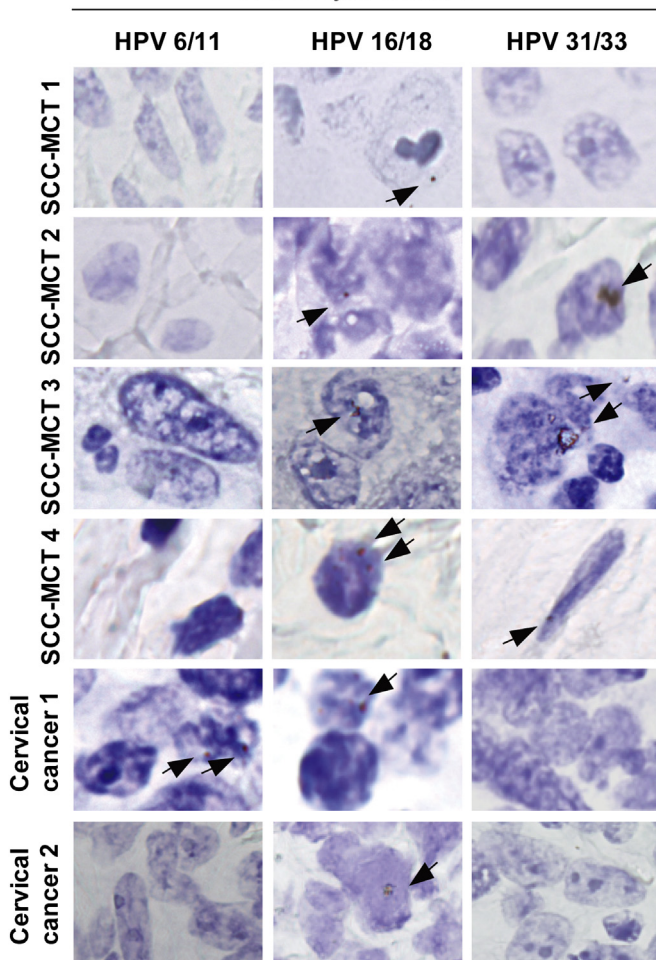

Figure 4. Human papillomavirus (HPV) detection by *in situ* hybridization (ISH). HPV infection in four cases of SCC-MCT and two samples of cervical cancer tissue detected by ISH with DNA probes HPV 6/11, 16/18, and 31/33. The arrows indicate the positive ISH signals of HPV DNA. MCT = mature cystic teratoma; SCC = squamous cell carcinoma.

surgical specimens will also allow the sequencing analysis of HPV typing.

Validating the role of HPV in SCC or other cell types of ovarian cancer may support HPV vaccination to reduce the incidence of ovarian cancer. However, further studies are required to determine the exact role of HPV in the etiology of this condition.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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