A Unique Case of Primary Squamous Carcinoma of the Salpinx Associated With Serous Carcinoma of the Omentum: A Pathological and Molecular Study

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Summary: In this article, we report a case of primary squamous cell carcinoma of the salpinx (PSCCS) with immunohistochemical and molecular studies to evaluate the phenotype and define the etiopathogenesis of this neoplasm. A 77-year-old woman, 38 years postmenopausal, was admitted to the Department of Obstetrics and Gynecology for ascites. Her clinical history showed breast carcinoma and left salpingooophorectomy as a result of extrauterine pregnancy. Cytological examination of the free peritoneal fluid showed clusters of malignant cells consistent with ovarian carcinoma. Transvaginal ultrasonography and a pelvic computed tomography scan disclosed a right pelvic mass with solid and cystic areas, measuring 3.2 × 2 × 2.3 cm. The patient underwent exploratory laparotomy. Intraoperative findings showed a mass that had replaced the salpinx and enveloped the ovary and ureter. The surface of the omentum was covered in small white nodules. Pathological examination showed that the right pelvic mass corresponded to PSCCS, whereas the omental white nodules were primary serous carcinoma. On immunohistochemical analysis, the tubal neoplasm showed positivity to Ca-125, keratin 14, and p63 and negativity to WT1 and p16. The hyper-expression of the p53 protein was evident as nuclear positivity. Molecular study by polymerase chain reaction amplification of the tumor DNA did not show any signal for human papilloma virus DNA. In summary, in this case we showed that the PSCCS was not due to human papilloma virus infection, but in all probability due to other pathogenetic mechanisms that cause a mutation of the p53 tumor-suppressor gene. Key Words: Primary squamous carcinoma of salpinx—Polymerase chain reaction—p16INK4a protein expression.

The most frequent type of tubal carcinoma is the serous variety (1) and this is likely not as rare as once thought, because there is a significant (and growing) body of literature to support a tubal origin for many cases of pelvic serous cancer (2–4).

Squamous carcinoma of the fallopian tube, instead, is a very rare malignancy. In this article, we report a case of primary squamous cell carcinoma of the salpinx (PSCCS) associated with primary serous carcinoma of the omentum. Immunohistochemical and molecular studies of PSCCS were performed to evaluate the phenotype and define the etiopathogenesis of this tumor.

MATERIALS AND METHODS

Clinical Findings

A 77-year-old woman, 38 years postmenopausal, was admitted to the Department of Obstetrics and...
Gynecology for ascites. Her clinical history showed breast carcinoma and left salpingo-oophorectomy as a result of extraterine pregnancy. Cytological examination of the free peritoneal fluid showed clusters of malignant cells consistent with ovarian carcinoma.

Transvaginal ultrasonography and a pelvic computed tomography scan disclosed a right pelvic mass measuring $3.2 \times 2 \times 2.3 \text{ cm}$, with solid and cystic areas. Exploratory laparotomy findings showed a mass that had replaced the right salpinx and enveloped the ovary and ureter of the same side. In addition, there were small white nodules on the omental surface. The patient underwent hysterectomy with right salpingooophorectomy and omentectomy.

The surgical specimens were fixed in 10% neutral-buffered formalin for a routine light microscopic examination. Several sections of neoplasms were submitted to histological examination and the samples were embedded in paraffin. Next, the 3-μm sections were cut and stained with hematoxylin-eosin to show mucin secretion. Immunohistochemistry was performed with antibodies anti-Ca-125 (Dako, Carpinteria, CA; dilution 1:125), anti-keratin 14 (Neomarkers, Fremont, CA; dilution 1:50, clone: LL002), anti-p63 (Biocare Medical, Newcastle, UK; dilution 1:100), anti-WT1 (Dako, Carpinteria, CA; dilution 1:100, clone: 6F-H2), anti-p16 (Neomarkers, Fremont, CA; dilution 1:10, clone: 16P04), and anti-p53 (Neomarkers, Fremont, CA; dilution 1:25, clone D0-7).

Polymerase chain reaction (PCR) amplification was performed to evaluate the presence of human papilloma virus (HPV) DNA in the neoplasm. For DNA extraction, 4-μm-thick histological sections stained with hematoxylin-eosin were examined under a stereomicroscope. Neoplastic areas were manually microdissected using sterile scalpels, suspended in a buffer for tissue lysis (Tris-HCl 50 mM, pH 9; 1 mM ethylene diamine tetra-acetate, pH 8.0; 0.5% Tween 20, 5% Chelex 100), and incubated overnight with Proteinase K (0.4 mg/mL) at 55°C. After enzyme inactivation by boiling for 10 minutes, the DNA extracted was directly used in the PCR mix, without further purification.

PCR amplification was performed with the L1 consensus primers Gp5+ /Gp6+ (5) giving an expected PCR product size of 150 bp: these primers have been developed to allow detection of a broad spectrum of mucosotropic HPV genotypes (6, 11, 13, 16, 18, 30–35, 39, 40, 42, 45, 51–53, 56, 58, 61, 66). Most of these genotypes are correlated with lesions of high oncogenic risk (16, 18, 45, 56, and 58). Five microliters of properly diluted DNA were combined in a 25-μL reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 200 μM of each dNTP (Promega, Madison, WI), 0.4 μM/L of each primer; 2.0 mM MgCl2, 1.25 U Taq polymerase (Promega). Amplification was carried out for 40 cycles in an AB 2700 (Applied Biosystems, Foster City, CA) thermal cycler. Each cycle of amplification consisted of 1-minute denaturation at 94°C, 1-minute annealing at 46°C, and 1-minute elongation at 72°C. The first cycle was preceded by a 7-minute denaturation at 94°C and the last cycle was followed by a 7-minute elongation step at 72°C. PCR for human β-globin gene was performed to establish the presence of amplifiable DNA and to exclude the presence of inhibitory factors of the PCR reaction (6). The presence and correct size of PCR amplimers were checked by electrophoresis on a 2% agarose gel (Qbiogene Inc, Carlsbad, CA).

**Pathological Findings**

On macroscopic examination, the uterus measured $7 \times 3.5 \times 2 \text{ cm}$. The cervix was unremarkable and the endometrium was thin. The right adnexal mass, which measured $5 \times 4 \times 3 \text{ cm}$, corresponded to an enlarged fallopian and a small residual ovary. On sectioning, this lesion showed cystic areas containing serous hemorrhagic fluid.

Histologically, numerous sections of the adnexal mass poorly differentiated squamous carcinoma characterized by nests of neoplastic cells with severe nuclear pleomorphism and frequent mitotic figures. Acantholysis and necrosis of a few neoplastic squamous elements had formed pseudoglandular spaces (Fig. 1A). There was no evidence of glandular formation with mucin secretion.

The neoplasm had entirely replaced the muscular layer of the salpinx and appeared on its serosal surface. There were areas of transition from normal mucosa to malignant areas (Fig. 1B) and areas of severe squamous dysplasia of tubal epithelium (Fig. 1C). The cervix and endometrium, which were entirely sampled and submitted to microscopic examination, showed only chronic inflammation and cystic atrophy, respectively. The small residue of ovarian tissue was recognized microscopically as an area of swirls and a band of small spindle cells with areas of hyalinization.

The multiple white nodules of the omentum corresponded to poorly differentiated serous carcinoma (G3) (Fig. 2A), which on immunohistochemical analysis showed hyperexpression of the p53 protein (Fig. 2B).

On immunohistochemical analysis, the tubal neoplasm showed positivity to Ca-125 (Fig. 3A), keratin...
14 (Fig. 3B), and p63 (Fig. 3C) and negativity was observed to WT1 (Fig. 3D), and p16 (Fig. 3E). The hyperexpression of the p53 protein was evident as nuclear positivity (Fig. 3F).

PCR amplification of the 3 different dilutions of tubal tumor DNA showed no signal for HPV DNA in the presence of an efficiently amplified positive control. The integrity and amplificability of DNA were assessed by PCR for the human β-globin gene. The same 3 DNA dilutions negative for HPV DNA earlier were submitted to β-globin gene amplification and all the dilutions showed the presence of tumoral amplifiable DNA with a band of the customary size (268 bp).

**DISCUSSION**

Primary squamous carcinomas in the female upper tract have been observed in the ovary, endometrium, and salpinx. In all the tracts this neoplasm represents a rare entity.
The occurrence of primary squamous cell carcinoma (SCC) of the ovary is roughly 0.7% to 2% (7). Most cases of primary SCC of the ovary can be observed in a preexisting mature cystic teratoma (dermoid cyst) (8), whereas occasionally they can also occur in a Brenner tumor (9). Pure ovarian SCCs, arising from the metaplasia of the surface epithelium of the ovary and malignant transformation of ovarian endometriosis, have also been reported (10,11).

Primary squamous cell carcinoma of the endometrium is a very rare neoplasm, with fewer than 100 cases reported in the English literature (12).

**FIG. 3.** On immunohistochemical analysis, the tubal neoplasm showed positivity to Ca-125 [(A) 100], Keratin 14 [(B) × 200], p63 [(C) × 100], negativity to WT1 [(D) × 200], and p16 [(E) × 100]. Hyperexpression of the p53 protein was evident as nuclear positivity [(F) × 200].
Examples of squamous carcinoma involving different female genital tracts at the same time have been reported in the literature (13–19) and in some instances, these have been related to HPV infection (15,18).

PSCCS is 1 of the rarest neoplasms of the female genital tract, with only 4 cases reported earlier in the literature (20–23). In all these examples, the patients were postmenopausal. Clinical manifestations, such as intermittent, colicky pain, sudden discharge per vaginam of a watery fluid rich in cholesterol that sometimes accompanies neoplasms of the salpinx, were absent in all the 4 cases reported earlier (20–23).

Three of these cases had ovarian involvement and were in an advanced stage of development of the disease. Clinical manifestations include the effect of the mass, characterized by painful bowel movement or leg swelling (17,18,20). In all these cases, the patients died despite aggressive surgery and chemotherapy (20,21,23).

The remaining case reported by Cormio et al. (22) presented with massive groin node metastasis, initially misdiagnosed as inguinal hernia. Despite lymphatic involvement, this patient showed no evidence of any recurrence 6 years after diagnosis (22). Our case of PSCCS is very peculiar because it is associated with ascites, which was not related to intraperitoneal diffusion of tubal carcinoma, but to the presence of simultaneous primary serous carcinoma of the omentum. Unlike the other numerous cases reported in literature, our example did not show a simultaneous cervical or endometrial squamous carcinoma (13–19), but ovarian involvement, as in the other 3 cases described earlier (20,21,23). An ovarian origin for the neoplasm has been excluded given that severe squamous dysplasia in the tubal luminal epithelium and areas of transition from benign to malignant tubal epithelium were observed, in line with the criteria for the diagnosis of PSCCS established by Hu et al. (24).

In addition, immunohistochemical analysis showed negativity to WT1, a marker positive in tubo-ovarian serous cancers and positivity to keratin 14, p63, and C2-125, which, are squamous and müllerian markers, respectively (18).

The negativity to p16 and the absence of HPV DNA on PCR study in the neoplastic elements showed that our case was not related to HPV infection. The hyper-expression of the p53 protein suggested that in all probability some other pathogenetic mechanisms that cause a mutation of the p53 tumor-suppressor gene may have been responsible for this rare malignancy.

The presence of a simultaneous primary serous carcinoma of the omentum could be because of the simultaneous malignant transformation of the embryonic germ cell, which might have remained along the gonadal embryonic pathway (25) or perhaps a malignant transformation of the coelomic epithelium lining the abdominal cavity (26).

Consequently, in our opinion, the association of PSCCS with primary serous carcinoma of the omentum in our case of PSCCS may be nothing more than a rare concurrence.

Other cases of PSCCS with primary serous carcinoma of the peritoneum should be reported to hypothesize that both the neoplasms could be the result of a common malignant transformation of the müllerian and coelomic epithelium, caused by the mutation of the p53 tumor-suppressor gene.

Although both the squamous adnexal tumor and the peritoneal serous tumor, which were well-sectioned and entirely examined on several histological sections and did not show any area of simultaneous squamous and serous differentiation, it is possible that the tumors share an origin. Both the squamous adnexal tumor and the peritoneal serous tumor, in fact, on immunohistochemical analysis were characterized by the over-expression of the p53 protein and probably also by the mutation of the p53 tumor-suppressor gene.

Some investigations, in fact, have shown that mutant p53 proteins generally have a longer half-life than wild-type p53 proteins and lead to nuclear accumulation (27,28), which can be detected by immunohistochemistry as nuclear positivity (29,30).

Further molecular studies could be useful to show the presence of p53 gene mutations, in other cases of tubal carcinoma with a squamous phenotype, concurrent with a serous carcinoma involving the peritoneum. In addition, it could be interesting to find the same alterations in both the tumors.

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REFERENCES


