in this study. For all antibodies except HER2, the level of expression was graded according to the percentage of immunoreactive neoplastic cells of the serous carcinoma component as follows: 0, <10 %; 1+, 10–25 %; 2+, 26–50 %; 3+, >50 %. Tumors with >10 % stained cells were considered positive for expression of that antigen. Immunoreactivity for HER2 was scored semiquantitatively as follows: 0, no immunostaining, or membrane staining in <10 % of cells; 1+, weak or barely perceptible staining in ≥10 % of cells, the cells stained in only part of the membrane; 2+, weak or moderate staining in the whole membrane in ≥10 % of tumor cells; 3+, strong staining in the whole membrane in ≥10 % of tumor cells [5]. We defined cases scoring 2+ and 3+ as HER2–positive. The immunohistochemical evaluation was performed by two observers (S.T. and Y.S.) separately, and the median value was used.

Polymerase Chain Reaction and Sequencing Analysis

DNA samples were extracted from paraffin embedded sections using the QIAamp DNA FFPE tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCR was performed using HPV consensus primers GP5+/6+ as previously described [6]. DNA samples obtained from cervical squamous cell carcinoma with HPV 16 infection and from the HeLa cell line, which is positive for HPV 18 DNA, were used as positive controls. *GAPDH* was amplified to ensure proper DNA extraction using the primer pair 5′-GCAG TGGGGACACGGAAGGC-3′ and 5′-ACTGTGGATG GCCCCTCGG-3′. The PCR products were electrophoresed in a 2 % (w/v) agarose gel and visualized under ultraviolet light with ethidium bromide staining.

The PCR products were purified using QIAquick Spin (Qiagen) and bidirectionally sequenced with the same primers as used for amplification. Sequence data were analyzed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistics

Inter-group comparisons were made by Fisher's exact test. $P<0.05$ was considered statistically significant.

Results

Expression of WT-1, p53, p16, HER2, CEA, and CA125 in Cancer

Table 1 shows a comparison of staining with each antibody in SACC, UPSC, OSA and MEA. WT-1 and p53 staining was nuclear, p16 staining was both cytoplasmic and nuclear, HER2, CEA and CA125 staining was only at the membrane.

Table 1 Immunohistochemical findings

Molecule	Number of cases (%)			
	Immunohistochemistry score			
	0 (0–9 %)	1 (10–25 %)	2 (26–50 %)	3 (50 % <)
SACC (n=12)				
WT-1	12 (100)	0 (0)	0 (0)	0 (0)
p53	6 (50)	2 (17)	0 (0)	4 (33)
p16	0 (0)	0 (0)	2 (17)	10 (83)
HER2	11 (92)	1 (8)	0 (0)	0 (0)
CEA	5 (42)	3 (25)	1 (8)	3 (25)
CA125	0 (0)	1 (8)	0 (0)	11 (92)
UPSC (n=29)				
WT-1	23 (80)	2 (7)	1 (3)	3 (10)
p53	6 (21)	0 (0)	3 (10)	20 (69)
p16	1 (3)	1 (3)	2 (7)	25 (87)
HER2	23 (80)	1 (3)	1 (3)	4 (14)
CEA	20 (69)	4 (14)	2 (7)	3 (10)
CA125	1 (3)	0 (0)	2 (7)	26 (90)
OSA (n=20)				
WT-1	0 (0)	0 (0)	0 (0)	20 (100)
p53	4 (20)	0 (0)	0 (0)	16 (80)
p16	0 (0)	5 (25)	1 (5)	14 (70)
HER2	18 (90)	0 (0)	1 (5)	1 (5)
CEA	18 (90)	2 (10)	0 (0)	0 (0)
CA125	0 (0)	0 (0)	0 (0)	20 (100)
MEA (n=20)				
WT-1	20 (100)	0 (0)	0 (0)	0 (0)
p53	18 (90)	1 (5)	0 (0)	1 (5)
p16	1 (5)	1 (5)	0 (0)	18 (90)
HER2	19 (95)	1 (5)	0 (0)	0 (0)
CEA	1 (5)	4 (20)	1 (5)	14 (70)
CA125	1 (5)	0 (0)	1 (5)	18 (90)

SACC Serous adenocarcinoma of the cervix, *HER2* human epidermal growth factor receptor 2, *CEA* carcinoembryonic antigen, *UPSC* uterine papillary serous carcinoma, *OSA* ovarian serous adenocarcinoma, *MEA* endocervical adenocarcinoma

Serous Adenocarcinoma of the Uterine Cervix (SACC)

Representative immunohistochemical stainings for each antibody are shown in Figs. 1 and 2. WT-1 and HER2 were negative in all SACC (Figs. 1b and 2a). Six SACC cases (50 %) were positive for p53 (Fig. 1c), with 4 of them showing strong (3+) expression. In contrast, p16 had intermediate (2+) or strong expression in all 12 SACC (Fig. 1d), and 11/12 were strongly positive for CA125 (Fig. 2d).

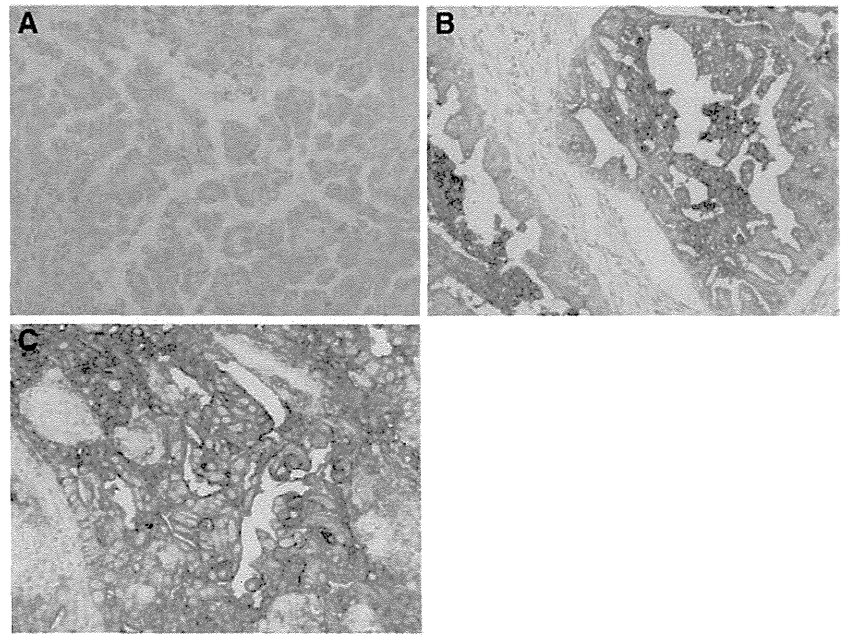

Fig. 2 Histopathological presentation of SACC. (**a**) Immunohistochemical staining showing negative expression of HER2. (x200). Immunohistochemical staining showing expression of (**b**) CEA (x200), (**c**) CA125 (x200)

Uterine Papillary Serous Carcinoma (UPSC)

Representative immunohistochemical stainings for each antibody are shown in Fig. 3. Only 21 % (6/29) of UPSC cases were positive for WT-1 (Fig. 3b) and only one case of UPSC was CK5/6-positive (Fig. 3b). Similarly, 17 % (5/29) of UPSC s were positive for HER2 (Fig. 3d), with 4 cases having strong expression. In contrast, p16 and CA125 showed intermediate or strong positive expression in the majority of cases (93 % and 97 %, respectively). These findings in UPSC are thus similar to SACC. p53 was positive in 23 cases of UPSC (79 %)(Fig. 3c), and strongly positive in most of these (69 %).

Ovarian Serous Adenocarcinoma (OSA)

Representative immunohistochemical stainings for each antibody are shown in Fig. 4. WT-1 (Fig. 4b) and CA125 were positive in all OSA. Thus, the frequency of WT-1 expression was significantly higher in OSA than SACC ($p<0.01$). p53 and p16 were strongly positive in 80 % (16/20) and 70 % (14/20) of OSA cases, respectively (Fig. 4c and d). In contrast,

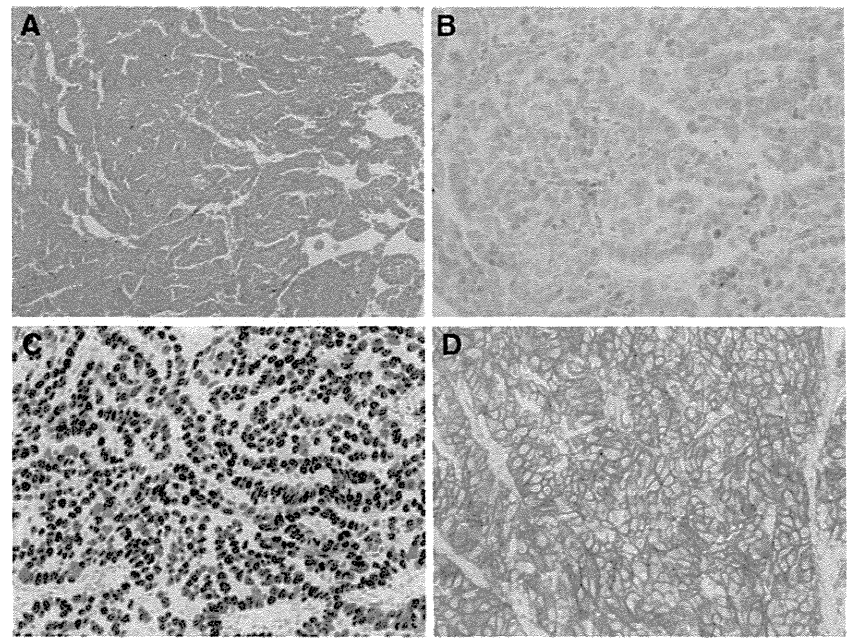

Fig. 3 Histopathological presentation of UPSC. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) WT-1 (x200), (**c**) p53 (x200), (**d**) HER2 (x200)

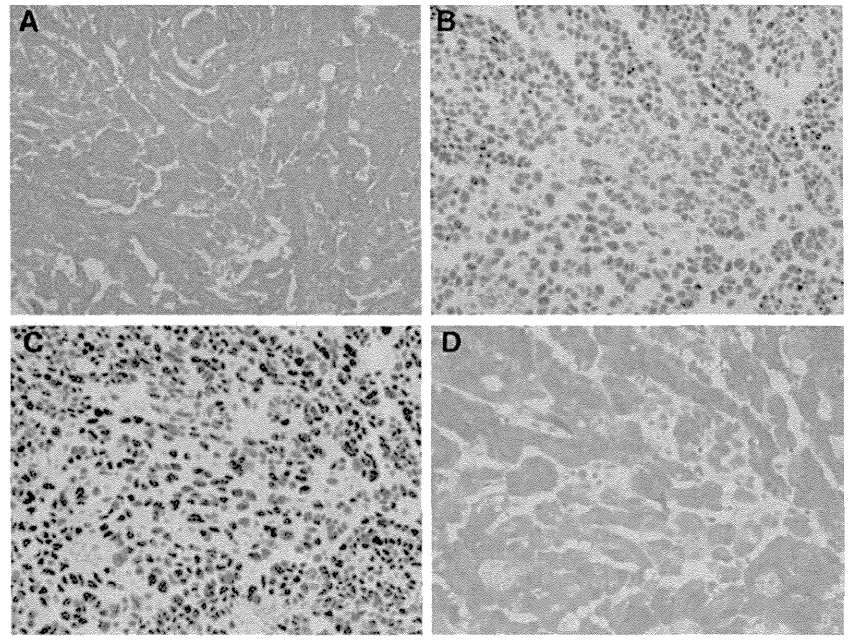

Fig. 4 Histopathological presentation of OSA. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) WT-1 (x200), (**c**) p53 (x200), (**d**) p16 (x200)

HER2 and CEA expression was rare, at only 10 % (2/20) each.

Mucinous Endocervical Adenocarcinoma (MEA)

Representative immunohistochemical stainings for each antibody are shown in Fig. 5. WT-1 and HER2 were negative in all MEA, p53 expression was rare, at 10 % (2/20). In contrast, p16 and CA125 both showed strong positive expression in 90 % (18/20) of cases (Fig. 5b and d).

We suggested binarizing the immunostaining results as positive vs negative, and comparing using the Fisher's exact test. WT-1 and p53 appear to show differences in percent of cases expressing these proteins between SACC, UPSC, OSA and MEA. The difference in WT-1 expression between SACC and UPSC, MEA is not significant, but SACC differ significantly from OSA ($p<0.01$). In the case of p53, overexpression (3+ staining) is seen in 4/12 SACC which differs significantly compared to either endometrial (20/29) or ovarian (16/20) serous carcinomas. There is a tendency between recurrence and p53

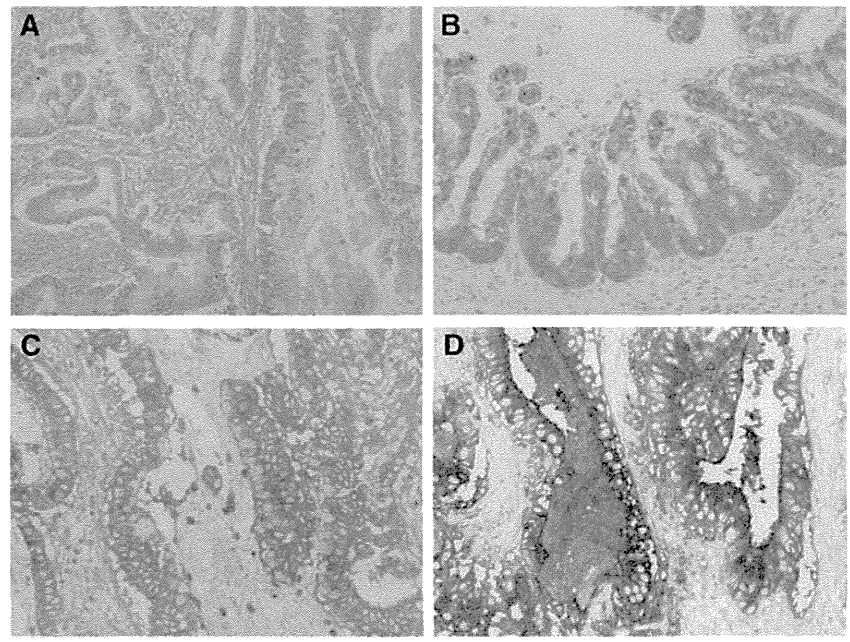

Fig. 5 Histopathological presentation of MEA. (**a**) H&E staining. (x100). Immunohistochemical staining showing expression of (**b**) p16 (x200), (**c**) CEA (x200), (**d**) CA125 (x200)

overexpression (p=0.06). The differences in frequency of HER2 expression are not significant.

HPV Infection

GAPDH was negative in the PCR for 2 of 12 SACC (cases 2 and 6), suggesting poor DNA preservation. These were excluded from further analysis. Among the remaining ten cases, four (cases 1, 3, 7 and 9) were positive when using the HPV consensus primers GP5+/6+ (Fig. 6). Sequencing of the PCR products showed that they had been derived from HPV16 in two samples (cases 1 and 9) and from HPV18 in the other two (cases 3 and 7).

Discussion

The p53 tumor suppressor gene plays a major role in cell cycle control and growth arrest following DNA damage. Mutations of this gene are the most common genetic alterations in human cancers [7]. Overexpression of p53, as detected by immunohistochemistry, has been proposed to indicate a worsened prognosis in some malignancies [8]. In our study, 50 % (6/12) cases of SACC were positive for p53, among them 4 with strong expression. In contrast, 90 % of MEA were negative for p53. Hunt et.al. [8] reported that p53 was not expressed in 86 % (30/35) of their uterine cervical adenocarcinomas, implying a difference in the pathogenetic mechanisms between SACC and MEA in the context of p53 inactivation. However, there is a report that the rates of p53 gene mutation and p53 nuclear immunoreactions in adenocarcinomas of the uterine cervix are relatively high, at 46 % and 32 %, respectively [9]. Therefore, further studies are needed to clarify differences in the pathogenetic mechanisms in SACC and MEA.

Interestingly, 3 of 4 SACC cases which showed strong p53 expression had died, and there is a tendency between recurrence and p53 expression (p=0.06). Zhou et al. [2] reported that nuclear immunoreactivity for p53 was present in 5 of 12 SACC cases, and, of the five patients with p53 positive tumors, 4 developed metastases. Batistatou et al. [10] reported a deceased case of SACC with p53 expression. Recently,

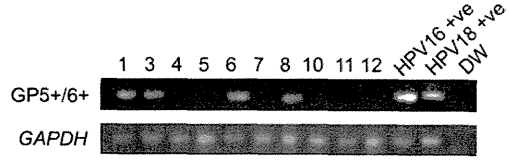

Fig. 6 Detection of HPV DNA by polymerase chain reaction, Four cases (cases 1, 3, 7 and 9) were positive using the HPV consensus primers GP5+/6+. DW, distilled water (no DNA template); +ve, positive control. *GAPDH* served as a positive control

Nofech-Mozes et al. [11] reported p53 immunostaining in 9 of 10 SACC cases, of which 3 had strong expression (>50 % of cells positive). That study [11] included 3 deceased cases of SACC, of whom two had strong expression (>50 % of cells). Thus, the strong p53 expression in SACC seemed to be associated with worse clinical outcome. Establishing p53 status may therefore contribute to prognostic indicators in this disease.

In addition, overexpression of p16 induced by HPV has been found to be associated with cervical squamous carcinoma [12–15]. It was also reported to be expressed in cervical adenocarcinoma [16–18]. In our study, 90 % (18/20) of MEA and 83 % (10/12) of SACC were strongly positive for p16, with no significant difference between SACC and MEA. Chiesa-Vottero et al. [19] reported that p16 overexpression was present in uterine and ovarian high grade serous adenocarcinomas. In our study, both OSA and UPSC showed p16 expression, with no significant differences among serous adenocarcinomas arising from different female genital tract organs and MEA.

Some studies have shown that persistent infection with high risk HPV is an important etiological factor for the occurrence of cervical adenocarcinoma [20, 21]. In the present study, 2 cases had HPV 16 DNA and 2 had HPV 18 DNA (both high risk HPV types). Nofech-Mozes et al. [22] reported that high-risk HPV DNA was found in 3 of 4 SACCs. Another study reported that HPV was detected in 1 of 3 SACCs [23]. We suggest that high risk HPV infection affects the occurrence of SACC.

Despite different origins, serous adenocarcinomas of the female genital tract show similar morphologic features, characterized by the presence of a prominent papillary structure and/or slit-like glandular spaces, and usually moderate to marked cytologic atypia. It has been shown that among the various histological types of ovaian carcinoma, the incidence of WT-1 positive tumors is highest in ovarian serous adenocarcinoma [24–27]. Nofech-Mozes et al. [28] concluded that strong WT-1 expression was associated with OSA rather than UPSC. In our study, WT-1 was positive in all OSA, whereas only 3 of 29 (10 %) UPSC showed strong expression of this antigen. In addition, WT-1 was negative in all SACC in this study. Nofech-Mozes et al. [11] also reported that only two cases of 10 SACC showed immunoreactivity to WT-1 where staining was seen in <50 % of all neoplastic cells. This is similar to the findings in our study. We suggest that SACC has biological features similar to UPSC. These may be associated with its embryologic developmental origin, in that both the uterine cervix and the uterine corpus are derived from the mullerian duct. On the other hand, the ovary is derived from indifferent gonad.

Generally, a majority of endocervical adenocarcinomas is CEA positive [29]. Alkushi et al. [30] reported that all endocervical type cervical adenocarcinomas expressed CEA

as detected by the polyclonal antibody. In our study, 95 % (19/20) of MEA were positive (>10 % of cells) and 58 % (7/12) of SACC were also positive (>10 % of cells). Zhou et al. [2] reported that 50 % (6/12) of SACC were positive for CEA, but Nofech-Mozes et al. [11] reported that only 30 % (3/10) were. Another two SACC cases were reported to be negative for CEA. The frequency of CEA expression in SACC tended to be low compared with MEA. Therefore, it was thought to be useful to distinguish SACC from MEA with respect to CEA immunostaining.

Although CA125 is a valuable serum marker for gynecologic cancer, its utility as an immunohistochemical marker is limited. Zhou et al. [2] reported that 75 % (9/12) of SACC were positive for CA125, and, in our study, 92 % (11/12) of SACC were strongly positive. Similarly, all OSA, 90 % (26/29) of UPSC, and 90 % (18/20) of MEA showed strong expression of CA125. There was no significant difference in CA125 expression among serous adenocarcinomas arising in different female genital tract organs and MEA.

HER2 immunostaining is associated with poor patient outcome in UPSC [31–33]. However, 92 % (11/12) of SACC were negative for HER2, and the differences in frequency of HER2 expression are not significant. HER2 expression appears not to be associated with poor prognosis in SACC, but this may due to the small number of SACC patients studied.

In summary, we have found that p53, p16 and CA125 expression is common in SACC. p53 expression seems to be associated with worse clinical outcome, and HPV infection is related to its pathogenesis. The immunohistochemical expression pattern in SACC samples was similar to UPSC. Although SACC is a very rare tumor, it is hoped that appropriate immunoprofiling will contribute to the introduction of improved management of patients with this tumor.

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Conflict of Interests The authors declare no conflicts of interest.

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