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HPV infection and p16^{INK4A} and TP53 expression in rare cancers of the uterine cervix

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ABSTRACT

Cervix cancer remains among most commonly diagnosed cancer in developing countries. Except squamous cell carcinoma and adenocarcinoma, the etiopathology and oncogenic mechanisms of rare cancers remain largely unknown. The study was performed to investigate the value of HPV infection and the expression of p16^{INK4A} and TP53 in rare primitive cancers of the cervix.

We conducted a retrospective study of rare primitive cancers of the cervix. Main clinicopathological features were reported. HPV infection was detected by *in situ* hybridization. Expression of p16^{INK4A} and TP53 was analyzed by immunohistochemistry.

Overall, seven cases were identified, including basaloid squamous cell carcinoma (BSCC, n = 2), small cell neuroendocrine carcinoma (SCNEC), granulocytic sarcoma without acute myeloid leukemia, leiomyosarcoma, primitive neuroectodermal tumor and botryoid-type embryonic rhabdomyosarcoma. The mean age of patients was 53.7 years. Four cancers were diagnosed at advanced stages. The prognosis was unfavorable and associated with patient death in five cases. HPV types 16/18 were detected in BSCCs and SCNEC. Strong and diffuse p16^{INK4A} overexpression was described in the nucleus and the cytoplasm of all tumor cells of BSCCs and SCNEC. The remaining cancers exhibited only scattered and focal p16^{INK4A} staining. Mutated TP53 protein was detected in BSCC (case 1) and GS.

Rare cancers of the cervix are aggressive and associated with poor prognosis. In contrast to mesenchymal tumors, BSCCs and SCNEC are etiologically related to high-risk HPV infection and could be identified by block positive p16^{INK4A} overexpression as common cancers of the cervix. *TP53* mutations are not a negligible genetic event in rare cervical cancers.

1. Introduction

Cervix cancer is one of the most commonly diagnosed malignancies in women worldwide with estimated 527,624 new cases in 2012 [1]. In developing countries, cervical cancer is the second most common cancer and the third leading cause of cancer death [1]. In Tunisia, cervical cancer ranks third among women with an incidence of 4.8 per 100,000 [1]. Despite this low rate, cervical cancer remains diagnosed at an advanced stage, emphasizing the need to reinforce the early detection of this cancer and its precursor lesions among Tunisian women [2]. Cervix cancer has multiple histological subtypes. Squamous cell carcinoma (SCC) and adenocarcinoma are the most common subtypes, accounting for approximately 75% and 15% of cases, respectively [3,4]. The remaining histological types are rare primitive malignancies accounting for less than 10% of cases. Rare tumors are characterized by a great histological diversity involving diagnostic, prognostic and therapeutic problems and remaining an enigma and a difficult disease to study [4]. Therefore, the etiology and the pathogenic mechanisms of these malignancies have not been well established yet due to their low incidence and unusual localization.

High-risk human papillomavirus (hrHPV) infection plays a preponderant role in the development of the majority of cervix cancers as well as their precursor lesions [5,6]. HrHPVs encode E6 and E7 oncoproteins, multifunctional immortalizing and growth-promoting

Abbreviations: BSCC, basaloid squamous cell carcinoma; BE-RMS, botryoid-type embryonic rhabdomyosarcoma; GS without AML, granulocytic sarcoma without acute myeloid leukemia; LMS, leiomyosarcoma; PNET, primitive neuroectodermal tumor; SCNEC, small cell neuroendocrine carcinoma

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proteins, that bind to the tumor suppressor TP53 protein and the retinoblastoma (RB) protein family, respectively, provoking their inactivation and leading to the overexpression of cyclin-dependent kinase (CDK) inhibitor 16 ($p16^{INK4A}$) [5–8]. $p16^{INK4A}$ protein, encoded by the INK4a/CDKN2A gene, binds to CDK4 and CDK6 and inhibits the RB phosphorylation leading to cell cycle arrest and suppressing cell proliferation [7,8]. During the last years, the interest of $p16^{INK4A}$ overexpression, as an excellent surrogate marker of hrHPV-associated lesions of the cervix, was well established [9–11]. Previously, we confirmed the role of the $p16^{INK4A}$ overexpression as a useful additional marker for the interpretation of problematic precancerous lesions during evaluation of suspicious cervical biopsies [11]. In addition, $p16^{INK4A}$ immunostaining was considered as putative biomarker discriminating cervical adenocarcinoma from benign glandular lesions and from endometrioid adenocarcinoma of the uterine corpus [12,13]. The causal role of hrHPV infection and $p16^{INK4A}$ overexpression in rare primitive cancers of the cervix remains yet unclear due to their scarcity.

The tumor suppressor *TP53* gene plays a key role in controlling genome stability and regulating cell cycle, apoptosis, differentiation and senescence [14]. Mutations of *TP53* gene are the most significant genetic events in various human malignancies and are associated with poor prognosis in several cancers [15,16]. In cervical carcinogenesis, only some studies have analyzed *TP53* mutations with controversial results [16]. Different patterns of *TP*53 alterations were reported in cervical SCC and adenocarcinoma with the highest frequency of the mutated *TP53* gene has been described in adenocarcinoma of Asian (19%) and European (12.2%) women [16]. In rare malignancies of the cervix, TP53 protein alterations remain not well investigated.

To further explore these malignancies, in the present study, we analyzed the pathogenic role of the HPV infection and the immunoexpression of $p16^{INK4A}$ and TP53 proteins in rare primitive cancers of the cervix.

2. Materials and methods

2.1. Tissue samples

We carried out a retrospective study of rare primitive cancers of the cervix diagnosed in the Pathology Department and treated jointly in Gynecological Obstetrics and Radiotherapy Departments of The Farhet Hached University Hospital, Sousse, Tunisia during 2000–2015.

The inclusion criteria were as follows: (1) all patients whose initial and definitive pathological diagnosis had concluded to a cervix cancer other than SCC or adenocarcinoma; (2) all patients whose initial pathological diagnosis had concluded to SCC or adenocarcinoma and the final diagnosis concluded to another histological type of cervix cancer. The non inclusion criteria included all patients whose final pathological diagnosis had concluded to cervical SCC or adenocarcinoma and all patients with secondary localization in the uterine cervix.

All tissues had been routinely fixed in 4% buffered formalin and paraffin embedded. Hematoxylin and eosin stained sections of selected cases were reviewed by two pathologists (SH and MM). One or two tissues blocks were selected from hysterectomy, cervical cone, and/or cervical biopsy to confirm that diagnostic tissue as originally reported was adequately represented in remaining tissue blocks. This study was approved by the local Human Ethics Committee at the Farhet Hached University Hospital of Sousse (Tunisia) and it conformed to the provisions of the Declaration of Helsinki.

2.2. Clinicopathological data

The collection of clinicopathological data was conducted using patient clinical records from Pathology, Gynecological Obstetrics and Radiotherapy Departments of The Farhet Hached University Hospital, Sousse (Tunisia). Age at diagnosis, cancer discovery circumstances, histological type, TNM classification, International Federation of Gynecology and Obstetrics (FIGO) clinical stage, treatment and survival outcomes were recorded.

2.3. HPV infection

The analysis of HPV infection was carried out by the DNA *in situ* hybridization (ISH) technique as already described [17]. One or two paraffin blocks containing representative portions of the tumors were selected for each case and 3 µm thick serial sections were obtained. A broad-spectrum biotinylated probe that detects common types of HPV has been used according to the protocol suggested by the manufacturer (Dako GenPoint K0620, Dako, Carpinteria, California, USA). The broad spectrum probe (Y1404) targets genomic DNA of HPV 6, 11, 16, 18, 30, 31, 33, 35, 45, 51 and 52. In addition, HPV typing was performed on HPV positive cases using HPV16/18 specific probes (Y1412) and HPV31/33 specific probes (Y1413) according to the manufacturer's protocol. Two cases of uterine cervix SCC were used as positive control cases that were positive in previous reactions.

2.4. Immunohistochemistry

$p16^{INK4A}$ and TP53 protein expression was carried out by immunostaining as previously described [9,11,12,18]. Sections of 4 µ thickness were made. After dewaxing and rehydration, the antigenic unmasking is carried out in a citrate buffer (10 mM, pH 6) at 95 °C for 40 min. The endogenous peroxidase activity was blocked by 3% hydrogen peroxide. $p16^{INK4A}$ immunostaining was performed with the CINtec p16 Histology Ventana System (Ventana Medical Systems, Inc.) according to the manufacturer‘s protocol. For TP53 expression, the slides are incubated 30 min at room temperature with the primary monoclonal antibody (Dako, Do-7, dilution 1/50). The revelation was made by the Envision + Dual Link System HRP kit (Dako, code K4063). Diaminobenzidine was used as an immunogenic chromogen. Finally, sections were counterstained with hematoxylin and mounted. A specific positive control was used for each antibody. Negative controls were obtained by excluding the primary antibodies. Images were captured by the microscopic digital camera Olympus system.

2.5. Evaluation of the immunostaining

The immunostaining was evaluated by two independent pathologists (SH and MM). Both nuclear and cytoplasmic immunolabeling were considered for $p16^{INK4A}$ expression as described [11–13]. In brief, semi quantification of the immunostaining was carried out on both staining intensity (0: no staining; 1: weak; 2: intermediate; 3: strong staining intensity) and percentage of positively stained tumor cells (0: no positive cells; 1: < 5%, 2: 5–20%; 3: 21–50%; 4: 51–99%; 5: 100% positive tumor cells). After multiplication of both values, the immunostaining scores were graded from 0 (no reactivity in tumor cells) to 15 (100% positive tumor cells with strong staining intensity). TP53 immunostaining was evaluated as described [18] and scored as completely absent, wild-type pattern (between 1 and 60% of tumor cell nuclei), or overexpression (> 60%). Complete absence of TP53 indicates TP53 null mutation, whereas TP53 overexpression is indicative of TP53 missense mutation, and any staining pattern in between suggests wild-type TP53 [18].

3. Results

3.1. Clinicopathological findings

A total of 7 cases were identified during the study period. The patient age ranged from 34 to 82 years with a mean age of 53.6. Selected cases were diagnosed as: two cases of basaloid squamous cell carcinomas (BSCC), one small cell neuroendocrine carcinoma (SCNEC), one granulocytic sarcoma without acute myeloid leukemia (GS), one

Table 1
Clinicopathological features of rare primitive cancers of the uterine cervix.

No.	Age	Clinical Symptoms	Histology	TNM Stage	FIGO stage	Treatment	Clinical outcome
1	52	Postmenopausal metrorrhagia	BSCC	T2aN0M0	IIA	TAH with BA and LND followed by brachytherapy	Alive 10 years after first diagnosis
2	82	Postmenopausal metrorrhagia	BSCC	T2bN1M1	IVB	Concomitant CT and RT	Died during treatment 4 months after first diagnosis
3	55	Postmenopausal metrorrhagia	SCNEC	T3bN1M1	IVB	Palliative chemotherapy (vincristin, etoposide)	Died during treatment 2 months after first diagnosis
4	34	Pelvic pain, leukorrhea, metrorrhagia, transit disorder and dysuria	GS without AML	T2aN1M0	IIIB	TAH with BA and LND Adjuvant CT and RT (adriamycin, rubicin)	Died during adjuvant treatment
5	51	Metrorrhagia	LMS	T1bN0M0	IB	TAH with BA Adjuvant CT (adriamycin) Palliative CT (holoxan, epirubicin, mesna) for recurrent tumor	Died by tumor recurrence 8 months after the end of the adjuvant CT
6	51	Sensation of ball in the vagina	BE-RMS	T2aN0M0	IIA	TAH with BA Adjuvant CT (vincristin, adriamycin, cyclophosphamide)	Alive without recurrence 18 months after the adjuvant CT end
7	50	Postmenopausal bleeding of low abundance	PNET	T2aN0M1	IVB	Neoadjuvant CT (adriamycin, endoxan, cisplatin) TAH with BA and LND Adjuvant CT (adriamycin, endoxan, cisplatin) Palliative CT (adriamycin, endoxan)	Died after local recurrence with bone metastases 14 months after the end of adjuvant CT

Note: BA, bilateral adnexectomy; BSCC, basaloid squamous cell carcinoma; BE-RMS, botryoid-type embryonic rhabdomyosarcoma; CT, chemotherapy; GS without AML, granulocytic sarcoma without acute myeloid leukemia; LMS, leiomyosarcoma; LND, lymph node dissection; PNET, primitive neuroectodermal tumor; SCNEC, small cell neuroendocrine carcinoma; TAH, total abdominal hysterectomy; RT, radiation therapy.

leiomyosarcoma (LMS), one primitive neuroectodermal tumor (PNET), and one botryoid-type embryonic rhabdomyosarcoma (BE-RMS). The clinicopathological features were summarized in Table 1.

Case 1 and case 2 were identified as BSCC (Fig. 1A–F). The first case showed undifferentiated epithelial tumor proliferation of basaloid type and largely ulcerated. The cells were arranged in clusters and in diffuse ranges within a moderately fibrous and inflammatory stroma (Fig. 1A–C). The tumor was classified as clinical stage IIA. The prognosis was good with the absence of local tumor recurrences and metastases, 10 years after first diagnosis. In the second BSCC, the biopsy showed undifferentiated tumor proliferation of basaloid type made of casings and masses of polygonal cells with numerous cytonuclear atypias and frequent mitoses (Fig. 1D–F). The tumor was classified as clinical IVB stage. The patient died four months after diagnosis during concomitant chemo-radiotherapy.

In SCNEC, the tumor showed a monomorphic proliferation of small and atypical cells with largely necrotic tumor proliferation (Fig. 1G–I). Tumor cells exhibited a diffuse immunostaining with anti-CD56 antibody (Fig. 1H) and a focal immunolabeling with anti-CK and anti-chromogranin A. No synaptophysin and LCA immunostaining was observed. SCNEC was diagnosed at a metastatic stage and the patient died only two months after diagnosis during palliative chemotherapy.

The 4th case was diagnosed as GS of the cervix without AML (Fig. 2A–D). The tumor contained proliferation of non-cohesive and medium-sized monomorphic cells with plasmocytic appearance. The chromatin was finely nucleated. There were many figures of mitosis. The interstitial tissue was fibrous and slightly inflammatory. Tumor cells showed a diffuse immunostaining with anti-myeloperoxidase (Fig. 2B). No immunostaining was observed with LCA, CK, chromogenin, desmin, C-KIT, mic2 and CD34. The clinical tumor stage was IIIB. The patient died shortly after radical surgery followed by adjuvant chemotherapy.

The 5th case was identified as moderately-differentiated LMS of the cervix and classified as clinical IB2 stage. The case revealed mesenchymal proliferation with muscle differentiation. There were some atypias with large nuclei and important mitotic index. There were also some foci of tumor necrosis with edematous and hemorrhagic zones (Fig. 2E–G). The patient died of tumor recurrence eight months after first diagnosis.

The 6th case was identified as BE-RMS of the cervix. The biopsy showed a malignant tumor proliferation with heterogeneous cellularity associated with alternating dense and myxoid zones without any particular architecture (Fig. 3A–D). The cellular component consisted of small undifferentiated round cells, eosinophilic cytoplasmic globular cells, and elongated ribbon cells. Under the mucosal epithelium, there was a densification of band tumor cells in places, producing a cambial layer. The stroma was abundant, myxoid, highly vascularized and contained cartilaginous foci. There was also a densification of tumor cells around the vessels. Tumor cells showed myogenin immunolabeling (Fig. 3B). The tumor was classified as clinical stage IIA. The patient was alive without recurrence 18 months after the end of adjuvant treatment.

The 7th case was diagnosed as PNET of the cervix at FIGO stage IVB. The tumor showed a diffuse malignant cell proliferation. The surface of the fragments was the site of extensive ulcers covered with necrotic material, fibrin and isolated tumor cells (Fig. 3E–F). Islets of cervical squamous epithelium were still preserved, thickened and parakeratotic with no epithelial atypia. Tumor proliferation infiltrated the chorion and consisted of diffuse layers of dissociated small lymphocytoid cells, with scant cytoplasm and rounded nuclei that showed regular nuclear contours, dense chromatin and no visible nucleolus. All tumor cells exhibited diffuse immunolabeling with anti-CD99 (Fig. 3F). The patient died of tumor recurrence and distant metastases despite the aggressive therapeutic management involving radical surgery followed by chemotherapy and radiotherapy.

3.2. $p16^{INK4A}$ and TP53 immunostaining and HPV infection

The immunohistochemistry and ISH findings were summarized in Table 2. Among 7 rare primitive tumors, only 3 cases were found to be HPV-associated using the broad-spectrum probe targeting genomic DNA of HPV 6, 11, 16, 18, 30, 31, 33, 35, 45, 51 and 52. The HPV-positive cases included two BSCCs and SCNEC. After HPV typing, we

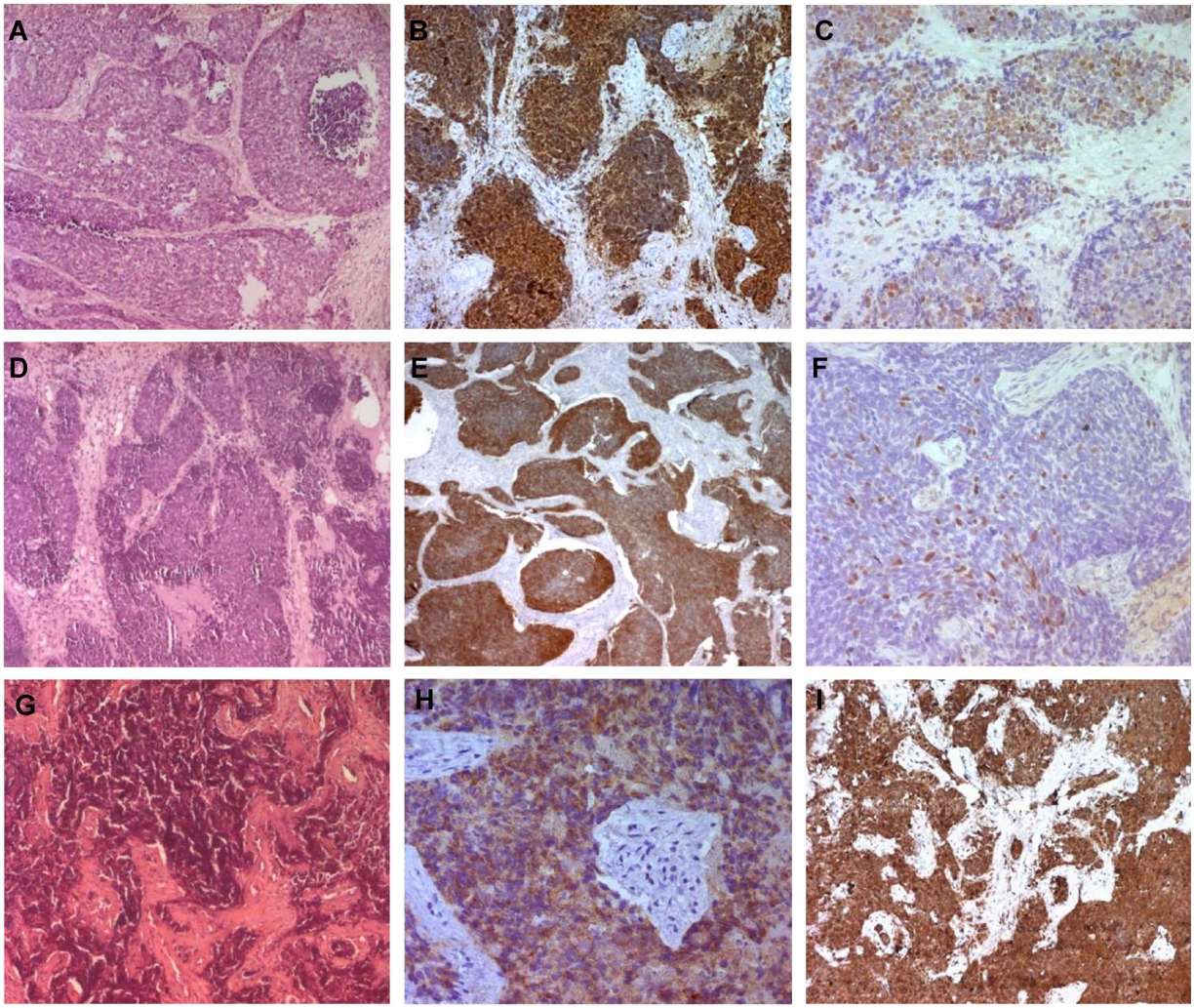

Fig. 1. Expression of p16 ^{INK4A} and TP53 in rare epithelial cancers of the cervix (Mx200).
(A–F) Basaloid type squamous cell carcinoma (Case 1 (A–C) and case 2 (D–F)). Hematoxylin and eosin (A and D). Diffuse and intense, nuclear and cytoplasmic p16^{INK4A} overexpression (B and E). Overexpression (C) and focal expression (F) of TP53 protein.
(G–I) Small cell neuroendocrine carcinoma (case 3). Hematoxylin and eosin (G). Positive CD56 expression (H). Diffuse and intense, nuclear and cytoplasmic p16^{INK4A} overexpression (I).

detected HPV16/18 in BSCCs and SCNEC. We found no HPV31/33 in all cases. The remaining tumors were HPV-negative.

p16^{INK4A} overexpression was observed in the SCNEC and the two BSCCs (score 15). These positive tumor cases exhibited strong and diffuse p16^{INK4A} immunostaining in the nucleus and the cytoplasm of all tumor cells (Fig. 1B/E/I). However, only focal and scattered p16 ^{INK4A} immunoreactivity (score 2 and 3) was observed in the remaining rare cancers of the cervix including GS, LMS, PNET and BE-RMS (Figs. 2C/F and 3C/G).

TP53 overexpression was observed in case 1 (BSCC) and case 4 (GS) exhibiting more than 60% of tumor cells indicating *TP53* missense mutation (Figs. 1C and 2D). No TP53 immunostaining was detected in SCNEC indicating *TP53* null mutation. The remaining cases exhibited wild-type TP53 (staining in 1–60% of tumor cells, Figs. 1F and 2G and 3D/H).

4. Discussion

The role of genital HPV infection in cervical carcinogenesis has been well investigated during last two decades. HrHPVs were involved in the development of the majority of cervical cancers [5,6]. However, in rare primitive cancers of the cervix, data on their relationship with hrHPV are limited [4,19]. Thus, the causative role of HPV infection remains unclear due to their low frequency and unusual localization. BSCC, arising mainly in the head and neck region, is an uncommon and aggressive variant of SCC of the cervix with different morphological and biological features [20,21]. Cervical BSCC is a neglected and under recognized entity that is neither recognized nor included as a specific histologic subtype in the current World Health Organization classification of the uterine cervix tumors [4]. In other genital and anal regions, BSCCs were associated with HPV infection [22,23]. In the current investigation, we found that BSCCs were HPV-infected and were, more specifically, associated to HPV16/18. Moreover, these cases exhibited a diffuse and strong nuclear and cytoplasmic p16^{INK4A} overexpression in all tumor cells similar to that described in frequent SCC of the cervix [9–11]. These results indicate that, although their different morphological and biological features, BSCCs are HPV-induced cancers exhibiting p16^{INK4A} overexpression similar to that described for common SCC of the cervix.

As previously shown, the TP53-staining patterns correlate with the mutational status of TP53 [18]. Mutated *TP53* is described in BSCC (case 1) as reported in cervical SCCs and adenocarcinomas [16,24]. Recently, a very high mutation rate of the *TP53* gene has been described in the nucleus and the cytoplasm of cervical cancers [24]. Nuclear mutated TP53 protein expression was significantly associated with better overall survival of patients possibly due to better therapy

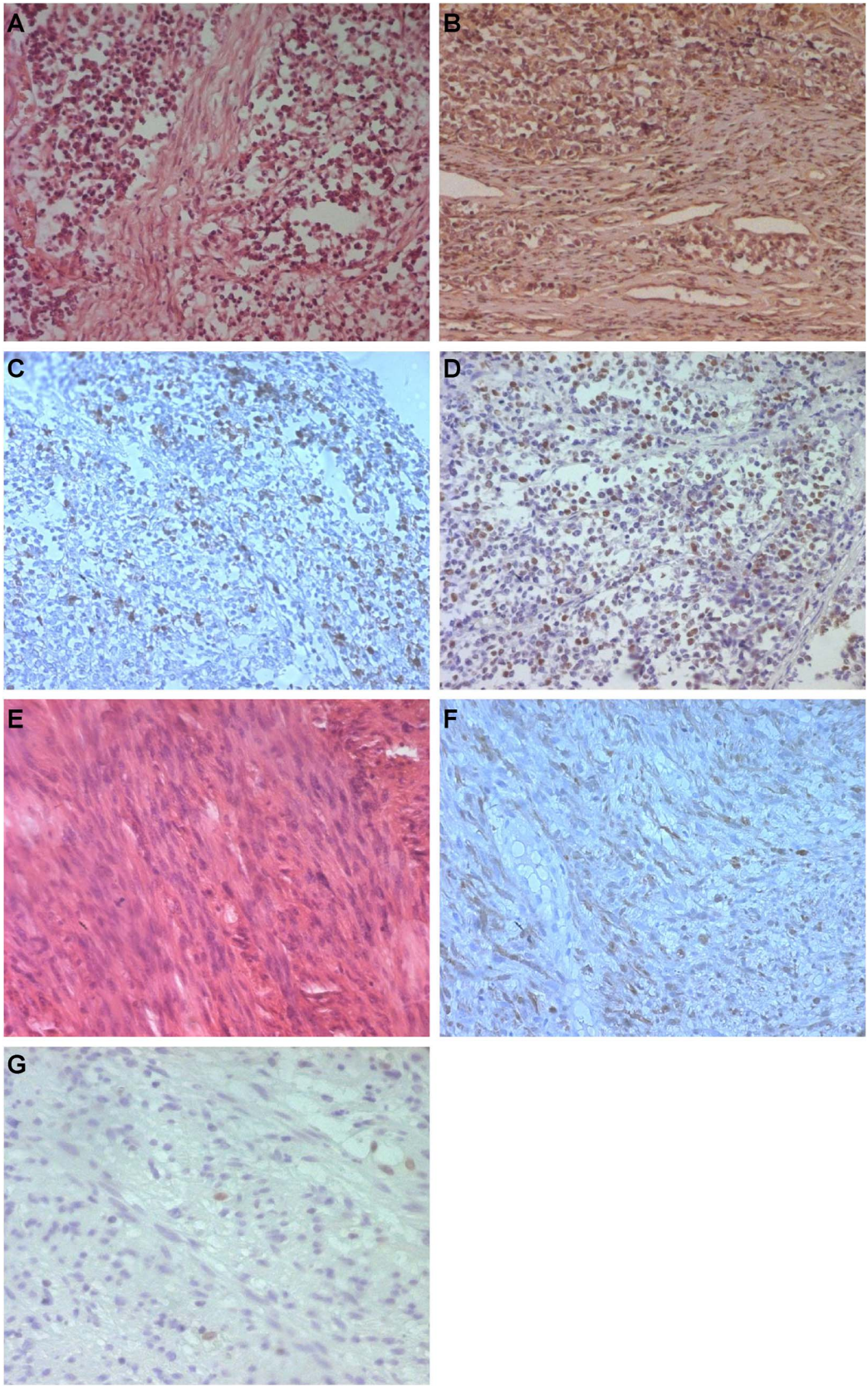

Fig. 2. Expression of $p16^{INK4A}$ and TP53 in granulocytic sarcoma and leiomyosarcoma of the cervix (Mx200).
(A–D) Granulocytic sarcoma (case 4). Hematoxylin and eosin (A). Positive myeloperoxydase expression (B). Focal and scattered $p16^{INK4A}$ expression (C). TP53 overexpression (D).
(E–G) Leiomyosarcoma (case 5). Hematoxylin and eosin (E). Focal and scattered $p16^{INK4A}$ expression (F). Focal TP53 expression (G).

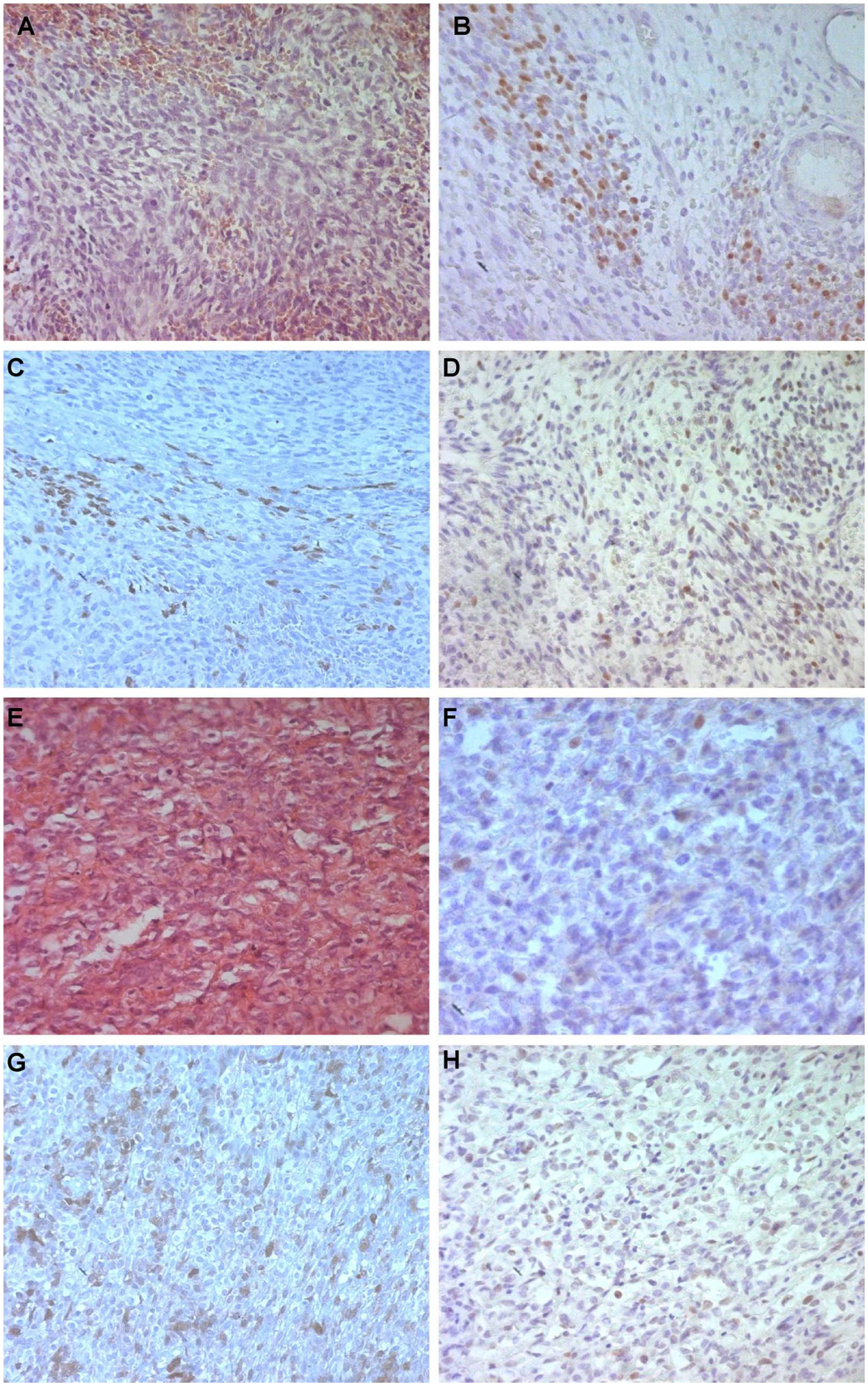

Fig. 3. Expression of $p16^{INK4A}$ and TP53 in botryoid-type embryonic rhabdomyosarcoma and primitive neuroectodermal tumor of the cervix (Mx200).
(A–D) Botryoid-type embryonic rhabdomyosarcoma (case 6). Hematoxylin and eosin (A). Positive myogenin expression (B). Focal and scattered $p16^{INK4A}$ expression (C). Focal TP53 expression (D).
(E-H) Primitive neuroectodermal tumor (case 7). Hematoxylin and eosin (E). Positive CD99 expression (F). Focal and scattered $p16^{INK4A}$ expression (G). Focal TP53 expression (H).

Table 2
HPV infection and $p16^{INK4A}$ and TP53 expression in rare primitive cancers of the cervix.

Cases	Histological diagnosis	HPV infection	HPV16/18	HPV31/33	Immunostaining	
					TP53	$p16^{INK4A}$ score
1	Basaloid type squamous cell carcinoma	+	+	–	Overexpressed	15
2	Basaloid type squamous cell carcinoma	+	+	–	Wild-type	15
3	Small cell neuroendocrine carcinoma	+	+	–	Absent	15
4	Granulocytic sarcoma without acute myeloid leukemia	–	–	–	Overexpressed	3
5	Leiomyosarcoma	–	–	–	Wild-type	3
6	Primitive neuroectodermal tumor	–	–	–	Wild-type	2
7	Botroid-type embryonic rhabdomyosarcoma	–	–	–	Wild-type	3

response [24]. Interestingly, in our study, the first patient with mutated *TP53* was alive 10 years after the first diagnosis, in contrast to the second patient, diagnosed with BSCC expressing wild-type *TP53*, who died only 4 months after the first diagnosis during treatment.

Cervix SCNEC is a highly aggressive tumor with early recurrence and a very low survival rate [25,26]. In our study, the patient died after only 2 months of diagnosis date and the aggressive course of the disease was characterized by the development of widespread hepatic metastases. The molecular mechanisms that underlie the development of SCNEC remain largely unknown. Some studies have suggested that hrHPVs might have an important role [19,27,28]. In fact, a high proportion of SCNECs exhibited staining for hrHPV or contained hrHPV DNA sequences and associated to HPV16 and/or 18 [25–33]. In our study, the SCNEC was HPV16/18 infected. Using ISH, Stoler et al. [25] described for the first time that HPV18 is associated to cervix SCNECs. Other studies also have demonstrated that these tumors contain HPV18 and HPV16 DNA [29–33]. Masumoto et al. [31] found that all analyzed cases were HPV-associated and the majority were HPV18-positive yielding a punctuate signal representing the integrated form. Among 31 cases, Wang et al. [33] observed that HPV viral infection was absent in 8 cases and HPV18 was detected in 17 cases. By contrast, additional case reports have described HPV16 as the most frequent HPV type in these tumors [27,29]. Thus, our results and all these previous studies have documented that hrHPVs, in particular HPVs 16 and 18, are involved in the oncogenic process of cervical SCNEC.

Although the earlier study of Wistuba et al. [30] reporting that the allelic loss of 9p21 (encoding for $p16^{INK4A}$) was the second most frequent deletion in cervical neuroendocrine tumors, $p16^{INK4A}$ expression was described latterly in the majority of SCNECs [27,31–35]. In the study of Horn et al. [27], all 9 SCNECs showed strong cytoplasmic and nuclear $p16^{INK4A}$ immunoreactivity within the tumor cells. Recently, Ganesan et al. [35] described $p16^{INK4A}$ expression in all 23 cases. $p16^{INK4A}$ expression was nuclear or cytoplasmic in 5 cases among 6 SCNECs reported by Li and Zhu [34]. Previously, Masumoto et al. [31] considered that Rb protein inactivation by HPV18 E7 oncoprotein may be associated with the malignant transformation of these tumors similar to the described pathway in SCC carcinogenesis. Herein, strong and diffuse $p16^{INK4A}$ immunostaining of the nucleus and the cytoplasm was observed in all tumor cells of the SCNEC probably caused by the HPV16/18 infection. All these results taken together suggest an unmethylated, undeleted, and unmutated genetic status of *p16^INK4A^* gene in SCNEC and support the hypothesis that $p16^{INK4A}$ overexpression is a hrHPV infection consequence similar to that described in common cancers of the cervix.

TP53 expression has been reported in almost 35% of SCNECs [27]. Although few studies have investigated genetic alterations in these malignancies, TP53 inactivating mutations have been described as in SCNEC of other anatomic localizations [36–38]. Wang et al. [38] described undetectable TP53 levels in all SCNEC cases. Straughn et al. [39] found a trend towards poorer survival for patients whose tumors did not express TP53. Interestingly, herein, no TP53 staining was detected in SCNEC and the patient died only two months after diagnosis.

Sarcomas of the cervix are distinctly uncommon and heterogeneous tumors accounting for less than 1% of all cervical malignancies [40,41]. Although substantial progress has been achieved in the diagnosis and treatment of uterine sarcomas, cervix sarcomas are still not well-characterized and standardized concepts for their management are lacking due to their rarity, histopathological diversity and unusual localization [40,41]. Moreover, because the number of reported cases in the literature is so small, few studies have investigated genetic alterations in these rare malignancies. As a result, their molecular and pathogenic mechanisms remain still unknown. In the current study, we reported 4 mesenchymal tumors of the cervix including GS, LMS, PNET and BE-RMS. The HPV analysis showed that all cases were HPV-negative, suggesting the limited causal role of HPV infection in the carcinogenesis of mesenchymal tumors of the cervix. In addition, only a focal and scattered $p16^{INK4A}$ immunostaining was observed in all these mesenchymal tumors in comparison to the strong and diffuse, cytoplasmic and nuclear immunoreactivity either observed here in BSCC and SCNEC cases or reported previously in SCC and adenocarcinoma of the cervix [9–13]. The molecular mechanism of $p16^{INK4A}$ expression in these tumors is largely unclear and presumably HPV-independent as described in some other HPV-negative cancers [42,43]. $p16^{INK4A}$ expression may result from a deregulation in the Rb signaling pathway unrelated to HPV infection, as described in small-cell lung cancer and lymphomas [42,43].

GS is an uncommon solid extramedullary tumor composed of immature leukocytes and commonly associated with AML [44,45]. In the female genital organs, GS is uncommon and exceedingly rare [44,45]. In our study, the GS was not associated with AML and overexpressed TP53 protein, supporting the possible role of *TP53* gene mutations in the development and progression of cervical GS.

Cervix LMS is an exceedingly aggressive rare malignant smooth muscle tumor with local recurrence and metastasis [46]. In the present study, although the LMS was diagnosed at early clinical stage, the patient died due to a tumor recurrence 8 months after chemotherapy. Recently, Whitcombe et al. [47] reported cervix LMS in a gravid patient exhibiting $p16^{INK4A}$ expression and TP53 staining in few scattered nuclei as described in our study.

RMS was a highly malignant soft tissue sarcoma arising from embryonic muscle cells and localized more frequently in the head and neck. Cervix RMS is one of the least common sites for RMS in the genitourinary tract [48]. Herein, we reported BE-RMS of the cervix uteri in 51-year-old women. The patient was alive without recurrence 18 months after the adjuvant chemotherapy end. Only focal TP53 staining was reported indicating a wild-type TP53. Previously, Semczuk et al. [49] described TP53 expression in more than half of the neoplastic cells of sarcoma botryoides of the cervix. A point mutation in exon 6 of the *TP53* tumor suppressor gene was found, suggesting that *TP53* gene alterations may play a role in the development and progression of these tumors [49].

Cervix PNET is extremely rare cancer and only a limited number of cases have been reported in the literature to date [50–53]. Therefore, there are no universally accepted standard treatment guidelines and

multimodal therapies have been adopted to treat patients with cervix PNET [50–53]. In our study, the patient received neoadjuvant chemotherapy and neoadjuvant radiotherapy. She died after bone metastases 8 years after first diagnosis. The pathogenesis mechanism remains not established due to their extremely rarity. Herein, PNET case expressed wild-type TP53 protein. Previous molecular genetic analyses identified characteristic chromosomal translocation t(11;22)(q24;q12), resulting in *EWS–FLI1* fusion, in approximately 85% of PNET cases resulting in the formation of a chimeric fusion of the *EWS-FLI1* gene [50,53]. Most of the remaining cases showed fusion of the *EWS* gene to *ERG gene* as a result of the chromosomal translocation t(21;22)(q22;q12) [50,53].

To our knowledge, this is the first study reviewing simultaneously six different rare primitive cancers of the cervix and analyzing the etiopathologic role of HPV infection and expression of $p16^{INK4A}$ and TP53 in the pathogenesis of these malignancies. Rare cervix cancers are aggressive, diagnosed at advanced clinical stage and associated with unfavorable prognosis and lower survival. The current analysis supports the presence of two distinct pathogenic groups in rare primitive tumors of the cervix. The carcinogenesis of epithelial tumors, including BSCC and SCNEC, is hrHPV-induced and $p16^{INK4A}$ overexpression can serve as a sensitive surrogate marker for hrHPV infection similar to that already described for common cervix cancers. By contrast, cervical mesenchymal tumors are etiologically unrelated to hrHPV infection. The focal $p16^{INK4A}$ expression observed in these malignancies is HPV-independent and may be Rb protein-related as described in other cancers. In addition, *TP53* mutations seem to be not negligible genetic event in rare cancers of the uterine cervix. More multicenter studies, using much larger series, will be necessary to further confirm these findings.

Conflict of interest

None of the authors have any conflict of interest to disclose.

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Disclaimer

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