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HPV-associated squamous cell carcinoma and adenocarcinoma in distinct cervical sites: a case report

Huihui Chen[1,2†], Qingyi Wang[2†], Min Kang[2†], Wei Huang[2], Hao Zhang[1], Jiaxin Li[1], Donghan Xu[1], Lin Zhao[1], Bowen Wu[1], Xin Lin[1], Liqi Li[1], Yuhong Zheng[1], Yihao Niu[1], Jiaqi Zhou[5], Donghui Huang[2*] and Peiyu Yan[1,3,4*]

Abstract

Background The synchronous occurrence of adenocarcinoma and squamous cell carcinoma in distinct cervical regions is exceptionally rare. This report highlights a case of HPV-associated adenocarcinoma and squamous cell carcinoma at distinct sites in a patient with primary stage IA1 cervical cancer.

Case presentation A 54-year-old female tested HPV type 18 positive in a routine physical exam. Cervical Liquid - based Cytology Test (LCT) showed Atypical Squamous Cells of Undetermined Significance (ASCUS). Colposcopy-directed biopsies revealed moderately differentiated squamous cell carcinoma at multiple sites (3, 6, 9, 12 o'clock positions and the ECC), with a clinical diagnosis of stage IA1. Preoperative abdominal MRI (including contrast enhancement) showed no lymph node enlargement, and urinary CT urography was normal. Squamous cell carcinoma antigen levels were within the normal range.

Keywords Squamous cell carcinoma, Aadenocarcinoma, Cervical cancer, HPV, Case report

Background

Cervical cancer is one of the more common malignancies worldwide. In 2020, there were approximately 604,000 new cases and 342,000 deaths globally. The incidence and mortality rates of cervical cancer differ significantly across various countries and regions, with 85% of deaths occurring in developing countries. Particularly, countries in sub-Saharan Africa have the highest mortality rate [1].

The incidence of cervical cancer is closely linked to age, most commonly affecting women between 35 and 55 years old. Approximately 84% of cervical cancers are caused by persistent high-risk human papillomavirus (HPV) infection [2], with types 16 and 18 being the primary oncogenic high-risk HPV viruses. HPV induces the transformation of normal epithelial cells into cancer cells mainly through oncogenic E proteins. Currently, the pathological types of cervical cancer are mainly

†Huihui Chen, Qingyi Wang and Min Kang contributed equally to this work.

*Correspondence:
Donghui Huang
13600001163@139.com
Peiyu Yan
pyyan@must.edu.mo
[1]Faculty of Chinese Medicine, Macau University of Science and Technology, Macau, China999780
[2]Zhuhai Hospital of Integrated Traditional Chinese and Western Medicine, 208 Yuehua Road, Xiangzhou District, Zhuhai 519000, Guangdong province, China
[3]State Key Laboratory of Quality Research in Chinese Medicines, Macau 999780, China
[4]Macau University of Science and Technology Zhuhai MUST Science and Tec-hnology Research Institute, Macau 999780, China
[5]HaploX Biotechnology, 8th Floor, Aotexun Electric Power Building, No. 3 Son-gpingsha n Road, Gaoxin North District, Nanshan District, Shenzhen, Guangdong Province 518000, China

squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, and other rare types such as neuroendocrine carcinoma, small cell carcinoma, mucinous carcinoma, clear cell carcinoma, serous carcinoma, and endometrioid adenocarcinoma. Typically, cervical cancer presents as a single type, with mixed types being comparatively rare.

In terms of clinical treatment guidance, the current pathological classification has further categorizes cervical cancers into HPV-associated and HPV-independent types. This is considered one of the most important developments in gynecological pathology over the past 50 years, leading to a shift in the classification system of cervical cancer. In this case, HPV-related adenocarcinoma and HPV-related squamous cell carcinoma occurred at different sites of the cervix, which is a very rare phenomenon. Both types of cancer were HPV-related, and the patient was diagnosed with a single HPV type 18 positive.

Case presentation

A 54-year-old asymptomatic female patient was found to be HPV type 18 positive during a routine physical examination on April 27, 2023. The LCT of the cervix revealed ASCUS. Further colposcopy-guided cervical biopsy revealed moderately differentiated grade squamous cell carcinoma at multiple sites (3, 6, 9, 12 o'clock positions and ECC). The clinical diagnosis was stage IA1. Preoperative imaging, including a full abdominal magnetic resonance imaging (MRI) scan with contrast enhancement and a urinary system CTU, showed no abnormalities (Fig. 1). The SCC antigen level was 1.61 ng/ml, and CEA, AFP, CA199, CA125 were all within the normal range. Her medical history included tubal ligation via laparotomy. She had no chronic illnesses, did not smoke or consume alcohol, and worked in a regular office job, with no history of high-risk occupational exposure. Regarding her family history, her father had been diagnosed with liver cancer, and her brother with colon cancer.

On May 22, 2023, the patient underwent a radical hysterectomy and bilateral adnexectomy, pelvic lymphadenectomy, and para-aortic lymphadenectomy under general anesthesia. During the surgery, the uterus was found to be of normal size, with a smooth surface and good mobility. Both ovaries and fallopian tubes appeared normal, with no abnormalities noted. Additionally, no enlarged lymph nodes were detected in the pelvic or retroperitoneal regions. Exploration of the upper abdomen revealed no apparent abnormalities involving the intestines, peritoneum, or omentum. The liver, gallbladder, and appendix were also examined and found to be unremarkable.

A complete uterine specimen was excised during surgery, revealing no notable macroscopic lesions in the cervix (Fig. 2). Postoperative pathology of the surgical specimen indicated: I. (Total uterus) Tumor location: cervix. Tumor size: at 1 o'clock, 3–4 o'clock, and 11–12 o'clock positions of the cervix. Histological type: HPV-related squamous cell carcinoma of the cervix (at 11–12 o'clock positions) and HPV-related cervical adenocarcinoma (at 1 o'clock and 3–4 o'clock positions). Cervical infiltration depth: The squamous cell carcinoma had a maximum width of 0.4 cm and a maximum infiltration depth of 0.2 cm under microscopy; the adenocarcinoma had a maximum width of 1 cm and a maximum infiltration depth of 0.4 cm. The invasion of the cervix was less than 1/2 (cervical wall thickness was about 2 cm). Involvement at the uterine junction: none, serosal involvement: none, cervical stromal involvement: none,

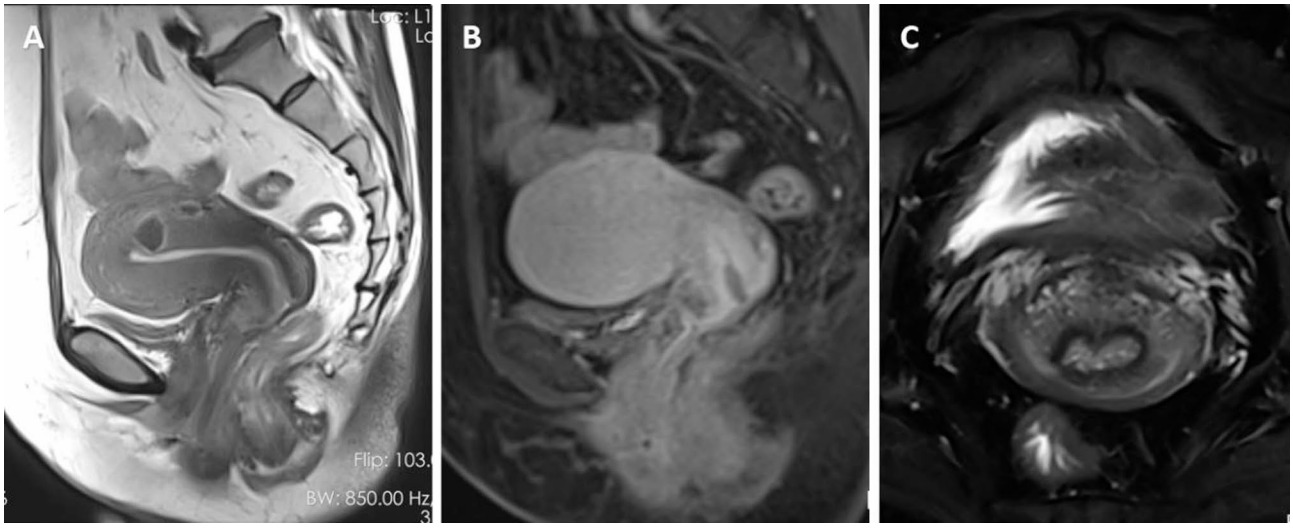

Fig. 1 Preoperative magnetic resonance imaging. **(A)** Preoperative T2-weighted sagittal imaging. **(B)** Preoperative T1-weighted contrast-enhanced sagittal imaging. **(C)** Preoperative fat-suppressed T2-weighted axial imaging

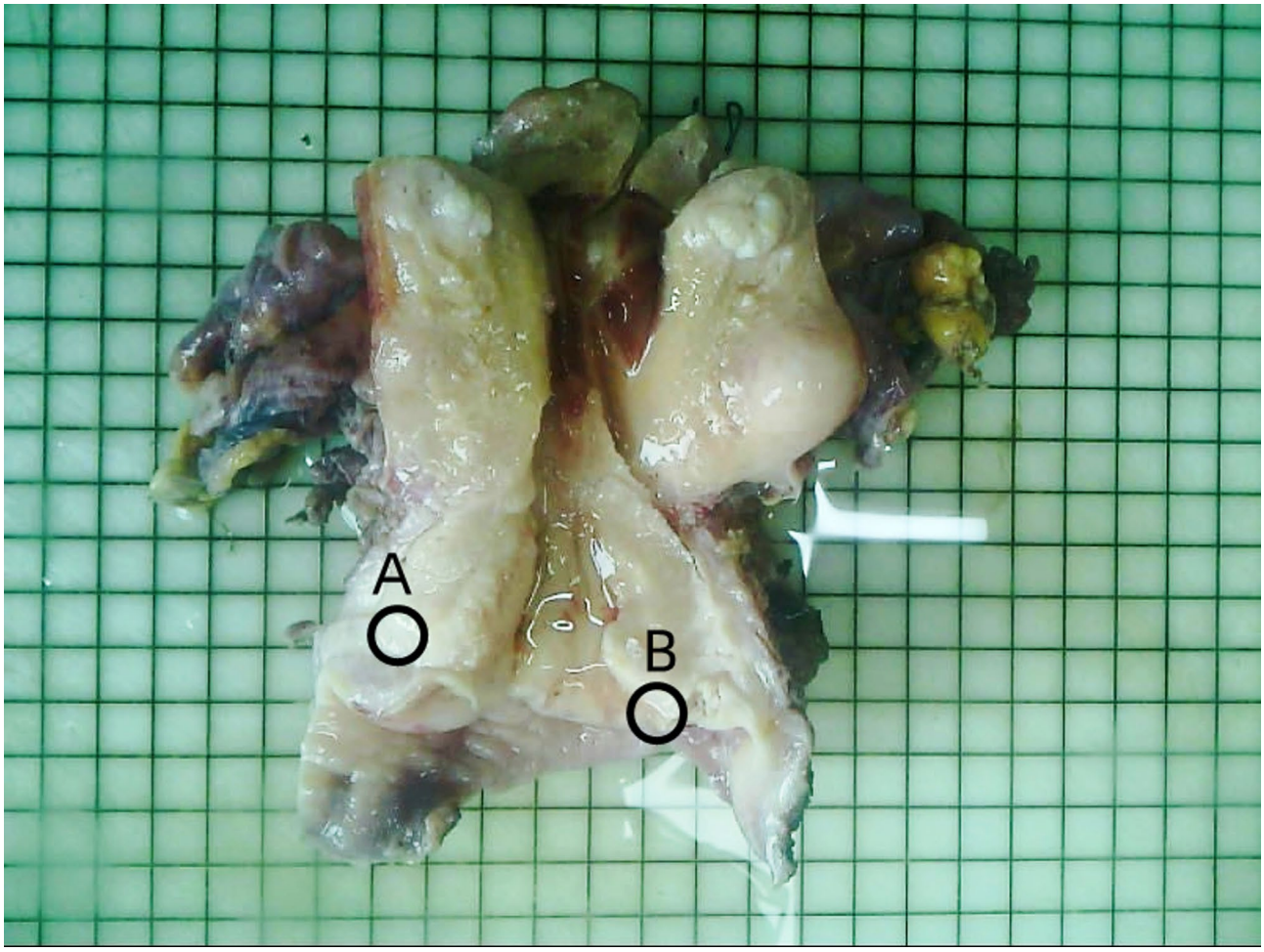

Fig. 2 Postoperative whole uterus specimen. **(A)** HPV-related squamous cell carcinoma of the cervix (at 11–12 o'clock positions); **(B)** HPV-related cervical adenocarcinoma (at3-4 o'clock positions)

involvement of other tissues/organs: none. Margins: No cancer invasion was found in the vagina, vaginal margin, or bilateral parametrium. No lymphovascular, blood vessel, or nerve invasion was observed.Immunohistochemistry: Adenocarcinoma: Ki-67(90%+),P16(diffuse+), MLH1(+), MSH2(+), MSH6(+), PMS2(+), P53(-, mutant type), P40(-), CK7(+), CEA(+), ER(-), PR(-); Squamous cell carcinoma: P40(partial+), P63(+), ki-67(90%+), CK7(+), P16(diffuse+). Detailed in the Figs. 3 and 4.

To further confirm the patient's genomic profiling of tumors, we performed targeted sequencing on her peripheral blood leucocytes, paraffin sections of her cervical cancer sample using the HapOnco StarPanel NGS Assay (HaploX Biotechnology, Shenzhen).Genetic testing revealed 3 somatic mutations, including 1 mutation related to targeted therapy and 1 mutation related to immunotherapy. The gene mutations related to targeted therapy include an activating NRAS mutation p.Q61K (mutation rate % copy number 3.06), and EP300 (mutation rate % copy number 6.26). Genes not detected include: ALK, BRAF, BRCA1, BRCA2, EGFR, PIK3CA, FGFR2, FGFR3, ERBB2(HER2), KIT, KRAS, MET, IDH1, etc.

For immunotherapy, PD-L1 TPS < 1%, CPS < 1 was detected, and KEAP1 (mutation rate % copy number 4.46). Microsatellite instability (MSI) suggests microsatellite stability (MSS). The tumor mutation burden (TMB) is 0.66 Muts/Mb. Both HLA-I and HLA-II are heterozygous. Viral sequence detection indicates HPV type 18 positive.

No mutations were detected in homologous recombination repair (HRR) related genes; Additionally, testing for pathogenic germline mutations indicates no genetic predisposition to familial cancer.Detailed information regarding the specific mutations is presented in Table 1.

The patient recovered well after surgery and received three cycles of TC regimen chemotherapy. Postoperative tumor markers including SCC, CEA, AFP, and CA125 were all within the normal range. A full abdominal MR scan and enhancement were performed three months

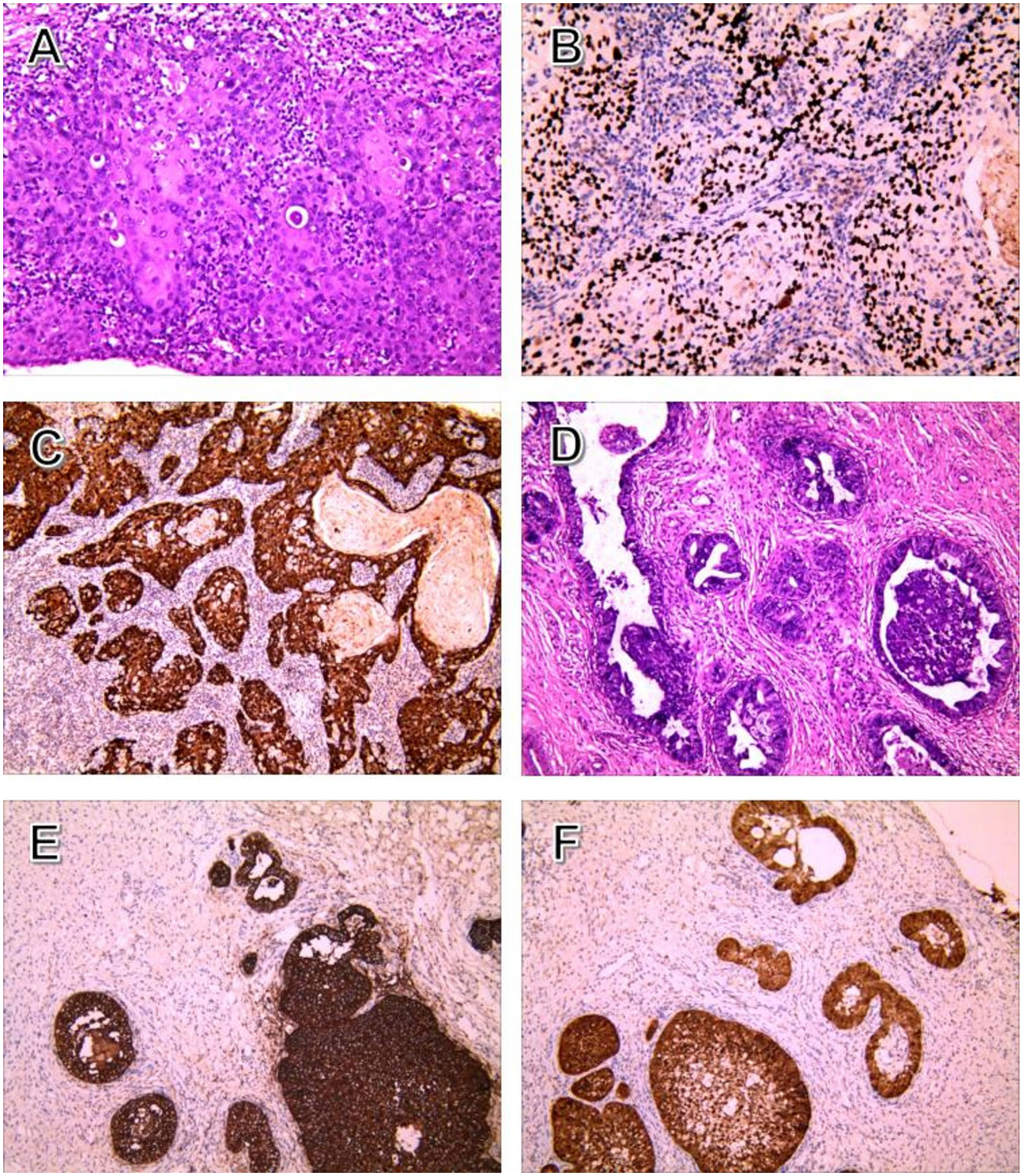

Fig. 3 Immunohistochemistry of the specimen. **(A)** Cervical squamous cell carcinoma, with cancer cell nests infiltrating the cervical stroma, and single cell keratinization observed (HE×200). **(B)** Cervical squamous cell carcinoma, Immunohistochemistry P63. **(C)** Cervical squamous cell carcinoma, Immunohistochemistry P16 (IHC×100). **(D)** Cervical adenocarcinoma, with glandular and sieve-like cell nests infiltrating the cervical stroma (HE×100). **(E)** Cervical adenocarcinoma, Immunohistochemistry CEA (IHC×100).**(F)** Cervical adenocarcinoma, Immunohistochemistry P16 (IHC×100)

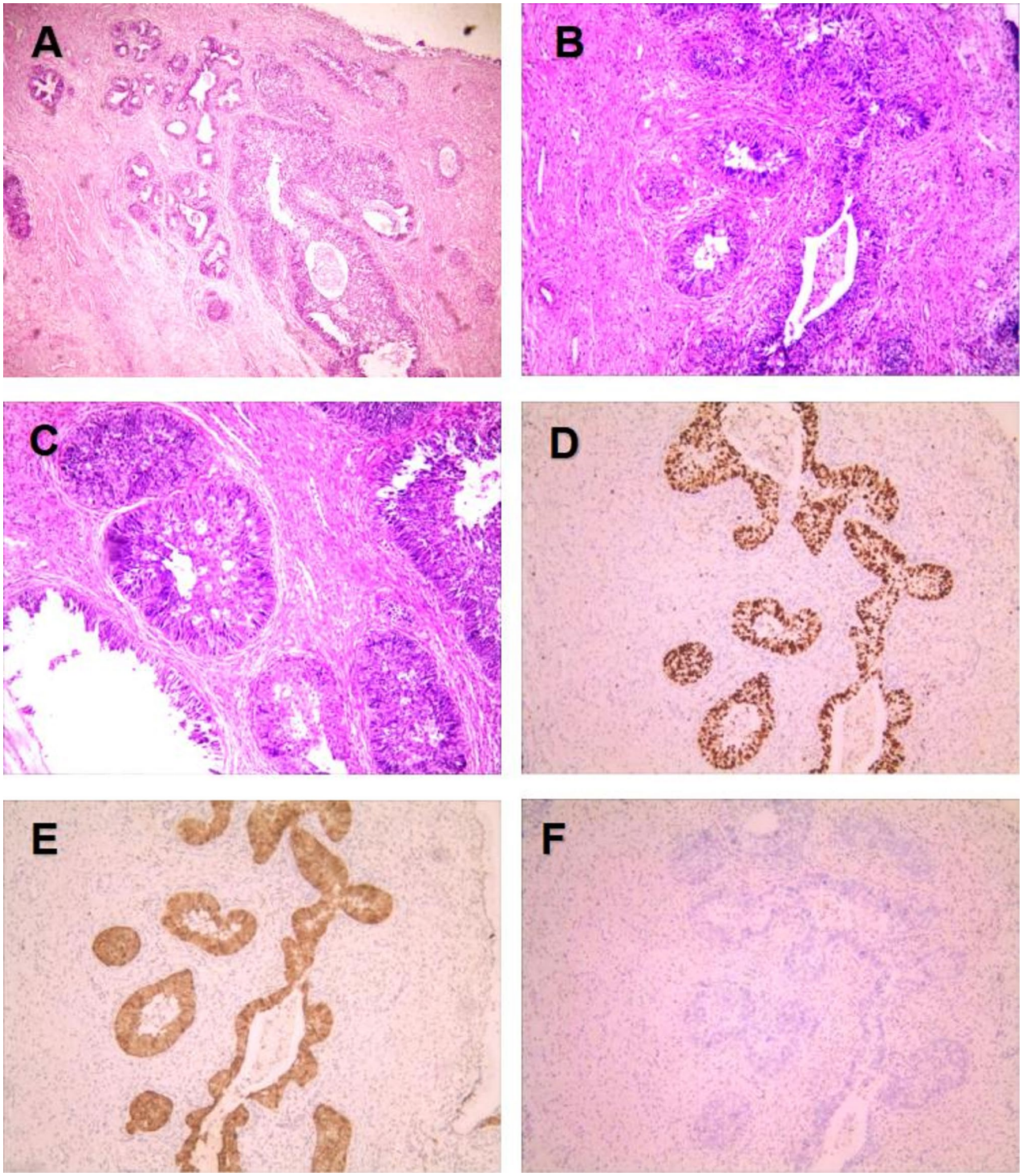

Fig. 4 H&E and immunohistochemistry for cervical adenocarcinoma. **(A)** Cervical adenocarcinoma with multiple invasive foci.(HE×40) **(B)** Cervical adenocarcinoma with destructive infiltration(HE×100).**(C)** Cervical adenocarcinoma with destructive infiltration.Mode C in the Silva pattern (HE×100).**(D)** Cervical adenocarcinoma, Immunohistochemistry, Ki67(90%),(HE×100). **(E)** Cervical adenocarcinoma, ImmunohistochemistryP16.**(F)**Cervical adenocarcinoma, Immunohistochemistry P53(-, mutant type)(IHC×100)

Table 1 The mutations detected in surgical specimen

Type	Gene	Detection target	Result
Target	NRAS	p.Q61K	3.06 (mutation rate % copy number)
	EP300	Exon31	6.26(mutation rate % copy number)
	ALK	Fusions、G1202R	not detected
Immune-related gene	PD-L1	-	TPS < 1%,CPS < 1
	KEAP1	Exon2, P.A191T	4.46(mutation rate % copy number)
The tumor mutation burden (TMB)	-	-	0.66Muts/Mb
human leucocyte antigen(HLA)	HLA-I, HLA-II	HLA-A, HLA-B, HLA-C, HLA-DPB1,HLA-DQB1,HLA-DRB1	hybrid subtype
Viral sequence detection	HPV	HPV-16/18/58/52/33/31/45/6b/11	HPV type 18 positive
pathogenic germline mutation testing	-	-	no familial cancer genetic risk

Genes detected include: NRAS, EP300, KEAP1.Genes not detected include: ALK, BRAF, BRCA1, BRCA2, EGFR, PIK3CA, FGFR2, FGFR3, ERBB2(HER2), KIT, KRAS, MET, IDH1, etc. No mutations were detected in HRR

after the surgery, as shown in Fig. 5. On August 13, 2023, the HPV test result was negative.The patient remains under routine follow-up care.

Discussion and conclusions

In this case, the patient was diagnosed with both adenocarcinoma and squamous cell carcinoma at distinct sites of the cervix following infection with HPV type 18, a phenomenon considered rare.The pathological types of cervical cancer are divided into squamous cell carcinoma (accounting for about 70-90%),adenocarcinoma (accounting for about 10-20%), and rare types of cancer (accounting for about 2-5%), including mixed carcinoma, small cell carcinoma, etc [3].

The surface of the cervix consists of stratified squamous epithelium and columnar epithelium, which covers the cervical canal and the region surrounding the cervical os. The junction of these two epithelial types is known as the transformation zone, a site particularly vulnerable to HPV infection and a frequent locus for tumor development [4]. The histological origin of cervical cancer is still unclear. In recent years, with the rise of the cancer stem cell theory, some scholars believe that cervical cancer stem cells may be the histological origin of cervical cancer [5], Cervical stem cells, located within the cervical transformation zone, exhibit high proliferative and differentiation potential, enabling transitions between

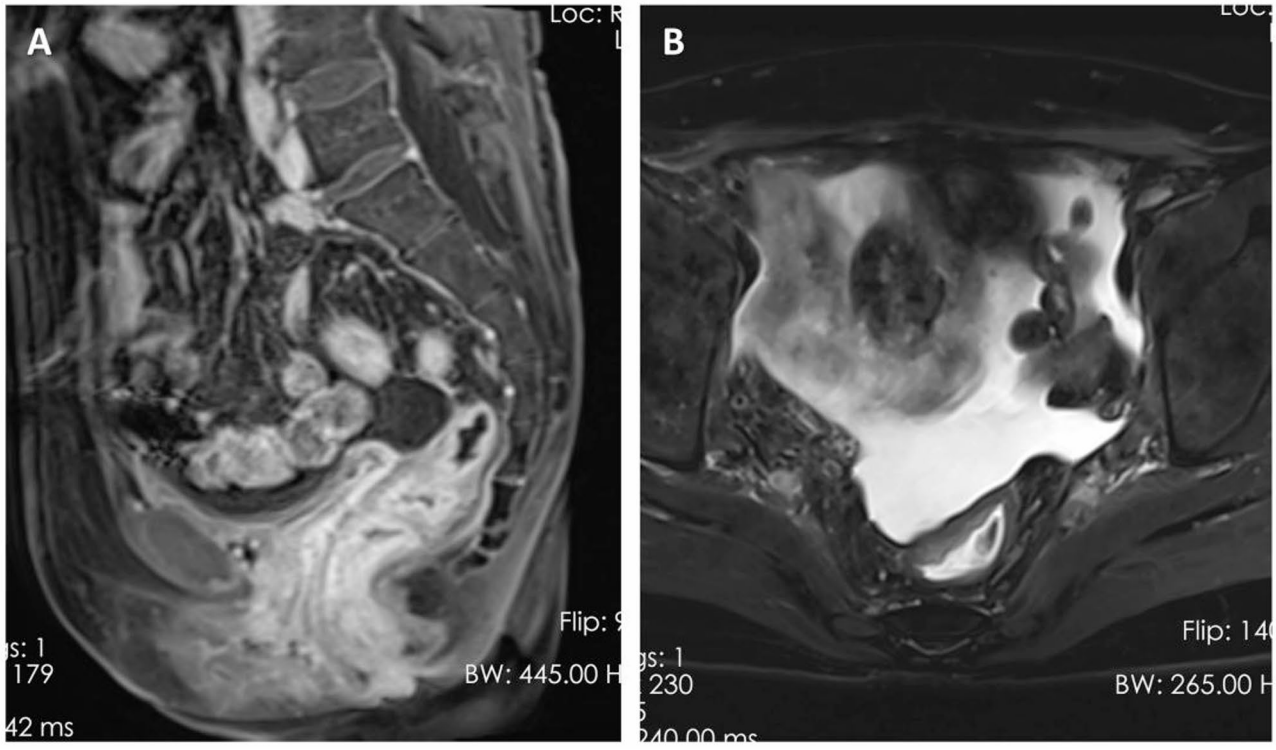

Fig. 5 Postoperative magnetic resonance imaging. **(A)** Postoperative sagittal T1 enhancement. **(B)** Postoperative axial fat-suppressed T2-weighted imaging

squamous epithelium and columnar epithelium within this zone. When these cervical stem cells undergo persistent infection with HPV, they may proliferate and differentiate abnormally under the influence of HPV oncogenes and cellular alterations, ultimately leading to the formation of squamous cell carcinoma, adenocarcinoma, or other cancer types [6, 7]. HPV infection is the primary cause of cervical cancer, with HPV types 16 and 18 responsible for approximately 70% of cases [8], HPV types 18 is the second most carcinogenic HPV type after HPV types 16, accounting for about 12% of SCC and 37% of ADC globally [9]. The mechanism by which HPV causes cervical cancer is related to the oncogenic E proteins. The E6/E7 genes can integrate into the DNA of cervical epithelial cells, disrupting the regulation of proliferation and differentiation, and ultimately leading to transformation [10].

The histological differentiation of human papillomavirus (HPV) infected cells is not yet explained. Research by Yao et al. [5]. found that the oncogenic E6/E7 genes in HPV can integrate into the DNA of cervical stem cells, disrupting the regulation of cell proliferation and differentiation, and affecting Notch signaling, causing cancerous cervical stem cells to differentiate into different pathological types.Khelil et al. [11]. performed RNA sequencing and immunofluorescence mapping on 279 cases of HPV-related cervical cancer and found three molecular subtypes of cervical cancer, with cervical reserve cell tumors being the most common, and the phenotype of cervical reserve cells being a proliferative phenotype.Kusakabe et al. [12] performed genomic and transcriptomic analysis on 42 cases of HPV-positive cervical cancer tissues, and through phylogenetic analysis and evaluation of HPV integration sites, they found that cancer cells of different histological types have a common cellular origin, especially HPV18-positive cancer tissues retain the immune-cold component of stem cell characteristics, and are more likely to develop mixed cancers.However, the mechanisms driving the coexistence of both adenocarcinoma and squamous cell carcinoma components remain unclear.

In genetic testing analysis of tumor specimens, we identified an EP300 gene mutation. EP300 (E1A-binding protein p300) serves as a crucial histone acetyltransferase that regulates target gene transcription through H3K27 acetylation (H3K27ac).EP300-mediated acetylation is enriched in HPV-positive cervical cancers, particularly those with HPV18 subtype, and is closely associated with malignant phenotypes (e.g., migration, invasion). Mechanistically, EP300 may enhance downstream oncogenic pathways (e.g., FOSL2 signaling) via acetylating modifications (e.g., FOSL2-K222), thereby promoting proliferation of HPV18-positive cervical cancer cells [13]. Genetic testing also revealed NRAS gene mutation. Aberrant NRAS expression may contribute to cervical cancer malignant transformation by modulating cellular proliferation, apoptosis, and invasive potential. The transcript stability of NR4A1 (an NRAS-associated nuclear receptor) could indirectly influence cervical cancer progression [14]. While no direct evidence currently links NRAS overexpression with specific histopathological subtypes of cervical cancer, its regulation of key signaling pathways may indirectly impact tumor heterogeneity.The genetic testing report additionally indicated low expression of the KEAP1 gene, which serves as a critical negative regulator of NRF2 (nuclear factor erythroid 2-related factor 2) and mediates oxidative stress responses through the KEAP1-NRF2 pathway. Currently, no evidence exists to demonstrate an association between KEAP1 and the pathogenesis or progression of cervical cancer.This observation suggests that these epigenetic regulatory mechanisms may be associated with HPV18 infection, which could potentially influence cervical cancer proliferation and differentiation by modulating multiple gene expressions and signaling pathways. However, whether these epigenetic alterations differentially contribute to distinct histological subtypes of cervical cancer remains to be further investigated. Regrettably, due to financial constraints preventing repeated genetic testing, homologous cell lineage analysis between the adenocarcinomatous and squamous cell carcinoma sites could not be performed in this case, leaving their clonal relationship undetermined.

In conclusion, this case demonstrates the exceptionally rare synchronous presentation of HPV18-associated adenocarcinoma and squamous cell carcinoma at distinct cervical loci, suggesting HPV18's potential etiological role in this phenomenon. Epidemiologically, HPV18 exhibits significantly higher detection rates in adenocarcinomas and adenosquamous carcinomas compared to pure squamous cell carcinomas [15]. However, the prevalence of HPV18 in mixed histology carcinomas and its tissue-specific tropism remain poorly characterized. The limitation of this study lies in that patients could not afford the cost of two genetic tests, so it lacked lineage tracing to confirm a common precursor for both carcinomas.It need more further clinicopathological studies incorporating molecular analyses to elucidate the mechanisms underlying HPV18-driven histopathological heterogeneity in the future.

Author contributions

Huihui Chen; Data collection, Manuscript writing and editing, and Protocol. Qingqi Wang; Manuscript editing. MinKing; Project development, Manuscript editing. Wei Huang; Data collection; Hao Zhang, Jiaxin Li, Donghan Xu, Lin Zhao, Bowen Wu, Xin Lin, Liqi Li, Yuhong Zheng, Yihao Niu; Project development and Manuscript editing. Peiyu Yan and Donghui Huang; Project development and Manuscript editing.Jiaqi Zhou were employed by company HaploX Biotechnology.All authors read and approved the final manuscript.

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Data availability
The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA007815) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Declarations

Ethics approval and consent to participate
This study was approved by the institutional ethics committee of Zhuhai Hospital of Traditional Chinese and Western Medicine (Ethic code:20190412001). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee, and the consent for participate was approved by the ethics committee.

Consent for publication
The case were conducted in accordance with the local legislation and institutional requirements. The participant provided her written informed consent to participate in this case. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Competing interests
The authors declare no competing interests.

Raw data processing and alignment
Raw sequencing data were preprocessed by fastp V.0.18.0; preprocessing included adaptor trimming, removal of the reads in which the N base reached a certain percentage (default length of 5 bp) and reads that contained low-quality bases (default quality threshold value ≤ 20) above a certain portion (default 40%), and sliding window trimming [16]. Clean reads were aligned to the hg19 genome (GRCh37) using Burrows-Wheeler Aligner V.0.7.15–r1140 with the default settings [17]. GenCore V.0.12.0 was used to remove duplicate reads [18]. Samtools V.0.1.19 was applied to generate pileup files for properly paired reads with mapping quality ≥ 60 [19].

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