Clinicopathologic Features and Lymph Node Metastasis Pattern of the Cervical MiNEN

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Abstract

Purpose: Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN) is a rare type of cervical tumor. Its clinicopathological features, lymph node(LN) metastatic patterns and outcomes are still unclear.

Methods: We have analyzed the clinicopathological information of 26 patients with cervical MiNEN.

Results: The median age of onset for cervical MiNEN was 48 years. Macroscopically, polyps and nodules were the main types. The neuroendocrine components included small cell neuroendocrine carcinoma (SCNEC) (14/26 cases), large cell neuroendocrine carcinoma (LCNEC) (10/26 cases), and typical carcinoid (2/26 cases). Non-neuroendocrine components included adenocarcinoma (AC) (12/26, including one case of AC in situ) and squamous cell carcinoma (SC) (10/26) and adeno-squamous cell carcinoma (ASC) (4/26). Of the 16 AC cases, 15 were human papilloma virus (HPV) -associated AC and onewasHPV-independent AC. Except for the case of MiNEN with HPV-independent AC, all cases were diffusely and strongly positive for p16 protein. The lympho-vascular space invasion (LVSI) was seen in 17/26 cases, and the components that invade lymphatic vessels were mainly neuroendocrine carcinomas (NECs) (15/17), followed by SC (1/17) and AC (1/17). Ten patients developed LN metastases, including six in combined SCNECs (6/14) and four in combined LCNECs (4/10); the metastatic component was pure NEC in eight cases (8/10) and SC or AC in two cases (2/10) .

Conclusions: NEC component is the key factor that determines the clinical behavior and prognosis of cervical MiNEN.

1.introduction

Neuroendocrine neoplasms (NENs) are aggressive malignancies of epithelial or neuronal/neuroectodermal origin [1]. They mainly occur in the lung, gastrointestinal tract, and pancreas; they also occur anywhere within the female genital tract, including the cervix in rare instances [2].

Cervical NENs account for about 1-3.5% of all cervical cancers [3, 4]. The 5th edition of the Female Genital Tumors World Health Organization (WHO) Classification categorizes NENs as a neuroendocrine tumor (NET) grade 1 (formerly known as typicalcarcinoid), NET grade 2 (formerly known as atypical carcinoid), SCNEC, LCNEC, andMiNEN. SCNEC accounts for about 80.4% of cervical NENs and that is more than LCNEC (about 12.0%), followed bytypical carcinoid and atypical carcinoid(about 7.6%) [5, 6].Cervical NENs are often associated with various other types of invasive carcinoma or intraepithelial lesions [7], WHO refers to these tumors as MiNEN carcinoma; HPV-associated ACis the most common non-neuroendocrine component[8].

To date, only case reports and a few small series about cervical MiNENhave been reported[9–14]. Among them, Bermúdezet al reported a case series containing 6 combined cases, half of them developed LN metastasis, with NECs as the main metastatic element [9].Horn et al also reported a case series containing six combined NEC cases. LN metastasis was found in one case, but the metastatic component was unclear [14]. To date,it is still unclear whether neuroendocrine or non-neuroendocrine components are more likely to metastasize due to the rarity of cervical MiNEN.

Here, we presented 26 cases of cervical MiNEN and focused on their clinicopathologic characteristics and patterns of LN metastases. To our knowledge, this is the largest report to date about the LN metastasis pattern of cervix MiNEN.

2.materials And Methods

All the 26 patients with cervix MiNEN were collected from Liaoning Cancer

Hospital and Institute between 2010 and 2021. All cases were diagnosed by two experienced pathologists according to the 5th edition of WHO Classification of Female Genital Tumors. The diagnosis of cervical NEN mainly depends on tumor histological morphology and immunohistochemistry. Specifically, typical organ-like histological morphology and positive immunohistochemical staining for at least one of these neuroendocrine markers such as Synaptophysin (Syn), Chromogranin (CgA), and CD56.Grading of cervical NEN is according to the criteria of NEN for the digestive system[6]. MiNEN is defined as the simultaneous occurrence (to be mixed together or separate)of neuroendocrine and non-neuroendocrine tumors, regardless of the proportion of each component. The updated 2018 International Federation of Gynecology and Obstetrics (FIGO) staging system was used to determine the clinical staging of cervical MiNEN [15].

All specimens were immunostained with Roche Ventana's automatic immunohistochemical staining system (Benchmark XT) or DAKO's autostainer (Link 48). Ready-to-use primary antibodies including cytokeratin (CK) (clone PCK26), CK8/18 (clone B22.1 & 23.1), p40 (clone BC28), Synaptophysin (Syn) (clone SP11), Chromogranin (CgA) (clone LK2H10), CD56 (clone 123C3), Ki67 (clone 30-9), D2-40 (clone GM361907), p16 (clone E6H4)and p53 (IR61661-2CN). Appropriate positive and negative controls were set for each case. The immunohistochemical staining process referred to the primary antibody instructions.

3.results

The mean age of the 26 cases was 47.3 years at the time of presentation (median, 48 years; range 26 to 73 years). Initial signs were contact bleeding or vaginal bleeding after menopause (23/26), irregular vaginal fluid (2/26), and one patient was asymptomatic (1/26). Most patients were previously healthy (21/26). Among others, one patient had a history of hypertension, one had a history of Hodgkin's lymphoma, three had a history of gynecological surgery (resection of ovarian cyst and/or uterine leiomyoma), and three patients had a history of smoking(Table 1).

3.2Gross and histological findings

Macroscopically, 26 patients with cervical MiNEN presented with an exogenous polypoid (10/26), endogenic nodular (8/26), or ulcerative (8/26) appearance.All of the masses ranged in size from 0.8 cm × 0.5 cm to 5.5 cm × 3 cm.

No masses were observed in the resected uterus and bilateral appendages, in the gastrointestinal tract, lung, or other organs during the preoperative examination.This ruled out the possibility of ACor NEC originating from other organs spreading directly or metastasizing to the cervix, and suggested that the cervix was the primary site ofAC and NEC.

Microscopically, the NEN component was mainly SCNEC (14/26) (accounted for 5%-90% in the tumor), followed by LCNEC (10/26) (accounted for 10-90% in the tumor)or rarely, typical carcinoid (2/26) (accounted for 10% and 90%, respectively).The NEC components in the cervix were mainly arranged in solid, island, trabecular, pseudoglandular, or rosette-like patterns. The main features of the SCNEC were oat-like or short spindle cells with a high N/C ratio, salt and pepper like chromatin, had indistinct nucleoli, abundant pathological mitotic images, and extensive necrosis(Figure1A); In contrast, LCNEC was composed of tumour cells with moderate cytoplasm that was more abundant than SCNEC, relatively low N/C ratio, had larger nuclei with coarse-grained chromatin and prominent nucleoli, although it was also rich in pathological mitosis and necrosis (Figure1B). Typical carcinoid cells werecuboidal, columnar or polygonal, with a variable amount of cytoplasm, uniformchromatin, small nucleoli, no necrosis, and rare mitosis (less than 2/2 mm^2) (Figure 1C).The non-NEN component was either pure SC (10/26)orpure AC (12/26, including one case of AC in situ), as well as ASC (4/26). The types of AC included HPV-associatedin 15 of 16 cases and HPV-independentinone of 16 cases(gastric-typeAC)SC cells were polygonal with abundant cytoplasm, usually arranged in sheets, with inter-cellular bridges and keratinized beads visible in highly differentiated cells (Figure

1D). Tumor cells of HPV-associated ACwerearranged in glandular tubular, cribriform, or papillary structures. The cell feature was obviously apical apoptosis and mitosis(Figure 1E). The gastric-type AC contained cells with abundant clear, foamy cytoplasm, and distinct cell borders;part of these cellswereextremely well differentiatedwith limited desmoplasia while other parts were poorly differentiated and arranged in micropapillary, clusterstructures or single cells(Figure 1F) (Table 2).

The forehead component of infiltration wasNECin 70%(19/26, including one case oftypical carcinoid) in our MiNEN cases, and in the remaining seven cases it was SC (4/7) and AC (3/7). It is worth noting that the proportion of NEC components was less than 20% in all the remaining seven cases.

The incidence of LVSI in combined SCNEC and combined LCNEC was 86% (12/14) and 50% (5/10) respectively (Figure 2A, 2B, 2D). LVSI was not present in either of the two typical carcinoids. Except for two cases of MiNEN that had SC or AC as the component of tumor thrombus in the lympho-vascular spaces (Figure 2C), all the other tumor thrombus were NECs (Table 2).

LN metastasis occurred in 38.5% (10/26) of samples. LN metastasis occurred in 43% (6/14) of combined SCNEC and 40% (4/10) of combined LCNEC. No LN metastasis was observed in the two typical carcinoids. The total number of dissected LNs was 14 to 36. With the number of positive LNs ranging from 1 to 22. The highest LN metastasis rate was 62.8% (case 17, 22/35). NEC was the predominant component in positive LNs, mainly pure NEC (total number of LNs with pure neuroendocrine carcinoma metastasis in all cases/total number of LN metastasis in all cases) was 36/39. The metastatic components were pure SCNEC in four cases, pure LCNEC in four cases, pure SC in one case (case 20), and pure AC in one case (case 26). The proportions of NEC components in the original lesions in the cases with pure SC and pure AC metastasis were relatively low, both accounting for about 10%. Morphologically, the metastatic components in the LNs were similar to the primary cervical tumor, with positive immunoreactivity for CD56, Syn and/or CgAin the neuroendocrine component(Figure 2 E, F) and p40 positive in SC (Figure 2 G, H, Table 2).

Tumor stage (FIGO 2018) was IB in 14 patients (54%), IIA in 2 patients (8%), and IIIC in 10 patients (38%). Early stage (FIGO I~ IIA) and late stage (FIGO IIB ~IV) were 62% and 38%, respectively.

3.3Immunohistochemical staining results

In all ACsamples,CK8/18 was positive (Figures3A, B). In SC, p40 was positive (Figures 3F, G). The NEN components were positive with at least one of the neuroendocrine markers, with a positive rate of 77% (20/26) for CD56 (Figure 3C), 89% (23/26) for Syn (Figure 3H), and 65% (17/26) for CgA(weakly positive); they also expressed CK8/18 (Figure 3B) and CK(weakly positive). Ki-67 had a positive rate of 50-90% in NEC (Figure 3D,I).P16 was diffuse and strong positive in all SC samples (11/11) (Figure 3J), in 25/26 NEC samples (Figures3E, J), and in 15/16 AC samples (Figure 3E). The NEC patient with p16 negative and the AC patient with p16 negative wasactually the same patient (case 26with gastric type AC mixed with SCNEC) (Figure 1s)(Table 3). In this case, p53 was negative in AC and cytoplasmic granular staining in SCNEC (Figure 1s). The immunohistochemical staining result of typical carcinoid mixed with HPV-associated AC in situ was shown in Figure 2s.

3.4Treatment and survival

Except case 9 (typical carcinoid with AC in situ) who received conization, all the others were treated with radical hysterectomy and pelvic lymph node dissection. Five patients received preoperative neoadjuvant therapy; 84% (21 of 25) patients received postoperative chemo-radiotherapy (11/21) or chemotherapy alone (10/21). Four patients were untreated (case 9) or the treatment plan was unknown. The detailed chemotherapy regimensare shown in Table 1.

Eight of the 26 patients were followed up for more than three years (4 LCNE and 4 SCNE), all with IB stage, one of these patients (case 2, LCNEC, IB) died at the 25th month. Three-year overall survival (OS) rates were 87.5% and 100% in combined LCNEC and SCNEC, respectively.

4.discussion

Our data suggested that the average age of cervical MiNEN (47.3 years) was slightly younger than that of SC (51 years) [16]. In tumors consisting of mixed components, the incidence of AC was slightly higher than that of SC with 12/26 (46%) and 10/26 (38%) respectively (the remaining four cases were mixed adeno-squamouscarcinoma).Giventhat SC is muchmorecommonthan AC inthecervix, thisindicatesthat AC is muchmorelikelytobeassociatedwithaneuroendocrinecomponent. The SCNEC (54%, 14/26) was slightly higher than that of LCNEC (38%, 10/26), and the typicalcarcinoidwas the lowest (8%, 2/26). These results are consistent with the literature [5, 6, 8].

It is well known that the incidence of cervical SC, AC, and NEN is closely related to high-riskHPV infection [17–20]. The E6 and E7 proteins encoded by high-risk HPV bind to the tumor suppressor p53 protein and the retinoblastoma protein family (Rb) protein to induce their inactivation and lead to overexpression of cyclin-dependent kinase (CDK) inhibitor 16 (p16); therefore, overexpression of p16 protein is the main basis for the diagnosis of HPV-associated cervical carcinoma [7, 21]. In our cases, the expression rate of p16 was 100% in SC (14/14), LCNEC (10/10) and typicalcarcinoid(2/2), 94% in AC (15/16) and 93% in SCNEC (13/14). Our findings are fairly consistent with the literature [7, 22–23], suggesting that the occurrence of cervical MiNEN may also be closely related to HPV infection. Notably, although rare, we did find one case of HPV-independent MiNEN (case 26) (see supplementary materials for Figure1s). The p16 protein was negative in the AC and SCNECcomponents, suggesting itwasaHPV-independentcervicalMiNEN.Cavalcanti et al. also reported a case of cervical HPV-independentMiNEN [24] in which both the mesonephric AC and the high-grade NEC components were HPV negative. Literature confirms that cervical HPV-independentAC is mainly associated with TP53 mutation followed by STK11, GNAS, and KRAS mutations [25]. We also found a TP53 mutation in our HPV-independentMiNEN case, in whichthep53 was negative in AC and cytoplasmic granular staining in SCNEC (both staining patterns suggested that TP53 was mutated) [26]. Cavalcanti et al. also found a set of molecular alterations in their case of HPV-independent MiNEN, including MYCN amplification, GATA3 mutation, and U2AF1 mutation [24]. The above instances suggest that the etiology of cervical HPV-independentMiNENmay also be associated with mutations of key molecular factors (such as TP53) in classic signal pathways. Due to the limited number of HPV-independentMiNEN cases, the etiology of this kind of carcinoma is not entirely clear. We believe that as this kind of carcinoma begins to receive progressively greater attention, its etiology will be gradually revealed.

Emersonet al. performed LOH and X-chromosome inactivation analysis for eight cases of cervical NEC which were combined with the SC or AC component. They found 63% (5/8) cases showed identical LOH and 50% (4/8) cases demonstrated an identical pattern of nonrandom X-chromosome inactivation in both components[7], suggesting that the two components had a common clonal origin. Recently, Cavalcanti et al. also found overlapping molecular alterations in both the mesonephric and neuroendocrine components of a case of HPV-independentMiNEN [24], suggesting that the two components originated from a common precursor. Here, we found four mixed tumors with multiple components (a combination of NEN, AC, and SC) from 26 cervical MiNEN (19%); it is obviously unreasonable to explain such a high incidence of cervical MiNEN by using the theory (collision tumor) of incidental events. Therefore, our data also provide support for the common origin theory of cervical MiNEN.Due to the rarity of typicalor atypical carcinoid inMiNEN, there is not yet sufficient data about their histological origin. It is thought that they are

also derived from the common precursor stem cells [11, 27, 28], but there is not enough molecular genetic evidence to confirm this.

The poor overall survival of cervical NEC is significantly associated with LVSI and lymph node status [9, 29]. LVSI is the earliest manifestation of metastasis. Patients with cervical SCNEC were evidenced of approximately 80% LVSI [8]. Among our cases, LVSI occurred in 71.4% (10/14) of combined SCNEC, which is consistent with rates reported in literature. We also found that SCNEC had a higher frequency of LVSI than LCNEC which occurred in50% (5/10) in our cases. This may be related to the relatively small number of cases.Of the 17 cases with LVSI, the tumor thrombocytic component was pure NEC in 15 cases and SC and AC in the other two cases (case 20 and 26), respectively. In both cases, NEC accounted for a small proportion of all tumor components, about 10%.Patients with NEC in cervix were evidenced of 45–57% of the positive lymph nodes [9, 30, 31].From published case reports and several small case series [9, 32], it was found that the lymph node metastasis rate of cervical MiNEN was also depressing. In Bermúdez et al'sreport, it reached 50% (3 out of 6) [9]. In our cases, the proportion was approximately 41%, including 43% (6/14) of combined SCNEC and 40% (4/10) of combined LCNEC, which were slightly lower than that of cervical pure NEC. This may be the reason why the prognosis of cervical MiNEN is better than that of pure NEC as described in the literature. When it comes to the metastatic components in lymph nodes, among the three patients with lymph node metastasis reported byBermúdez et al, two patients developed pure neuroendocrine carcinoma metastasis, and one patient had mixed NEC metastasis [9]. In our 10 positive cases, eight of the metastatic components were pure NEC. Interestingly, we found that in almost 70% of our MiNEN cases, the deepest infiltrating component in the cervical wall was NEC (including one typical carcinoid); our results are consistent with the literature [14], suggesting that the NEC component is a key factor that determines the clinical behavior and prognosis of cervical MiNEN. We also noticed that, in the definition of digestive system MiNEN, WHO clearly proposed that the proportion of each component should reach at least 30% [33], but for the definition of the corresponding neoplasm in cervix, WHO did not put forward a clear proportion limit. In Bermúdez'sreport[9], the proportion of metastatic NEC in the primary lesion was 20% and in our case it was as low as 10%.We speculate thatthe prognosis of cervical MiNEN also depends on the ratio of the neuroendocrine components.Larger series and multicenter studies are needed to determine how the percentage of components in mixed cervical neuroendocrine tumors should be defined. In addition, the methodology to accurately assess the percentage of neuroendocrine components in the cervix should be strictly regulated to avoid sampling bias. It is recommended that all tumor specimens should be sampled and non-neuroendocrine tumors with unrecognized neuroendocrine cell morphology and scattered expression of neuroendocrine markers should be excluded [34].

The survival of cervical MiNEN has been poorly studied and the results are controversial. Bermúdez's study showed that the five-year survival rate was 19%, worse than 54% in patients with pure NEC of the same stage [9]. However, in Wang's study [17], the prognosis between combined and pure NEC was not significantly different. Our results show that the three-year OS of the combined SCNEC was 100%, it was better than that of pure SCNEC of the same stage (60%)[35]. So, which tumor has a worse prognosis still needs to be further explored.

Cisplatin/carboplatin and etoposide (EP/C) is the most commonly used treatment scheme, but there is still no unified treatment scheme at present [8]. Only 24% (6/25) of patients in our study received EP/C chemotherapy regimens. Given the low incidence of this neoplasia, the most effective chemotherapy regimen remains to be further studied.

5.conclusions

This is the first study to systematically describe the pattern of lymph node metastasis in MiNEN of the cervix, suggesting that NEC components may determine the clinical behavior and prognosis of cervical MiNEN. Large-scale

prospective studies of cervical MiNEN lymph node metastases are needed to further define the precise definition of this kind of tumor.

Declarations

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Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Liaoning Cancer Hospital and Institute (Ethics Committee Approval Document: 20200815YG, 2020.8.19- 2021.8.18).

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Tables

Table 1. Clinical characteristics, treatment and outcome of patients with cervical MiNEN

NO.	Age	Symptom	Sign	Size (cm)	FIGO Stage	Treatment	Follow up (months)	Outcome	Chemotherapy regimens
1	37	CB	Nodular	2.0x2.0	IB	S+CT	96	AWD	TC
2	41	CB	Polyps	2.0x2.0	IB	S+CT+RT	25	DOD	TC
3	73	VBAM	Polyps	2.0x2.1	IIA	S+adjuvant RT+CT	92	AWD	DC
4	43	CB	Polyps	2.5x2.0	IB	S+CT	59	AWD	TC
5	62	VBAM	Nodular	2.4x1.1	IB	S	50	AWD	unknown
6	43	CB	Polyps	1.5x1.0	IB	S+CT	55	AWD	DC
7	26	CB	Ulcer	2.0x1.5	IIIC	S+CT	48	AWD	TC
8	52	VBAM	Nodular	3.5x3.0	IIIC	S+adjuvant CT+RT	lost	AWD	TC
9	34	WNS	Nodular	0.8x0.8	IB	S(conization)	33	AWD	NOT
10	57	CB	Ulcer	0.8x0.5	IB	S+CT	41	AWD	TP
11	40	CB	Polyps	3.0x1.6	IB	S+CT	36	AWD	EP
12	38	CB	Polyps	2.0x1.5	IIIC	S+adjuvant CT	20	AWD	TP
13	48	CB	Ulcer	5.0x2.5	IB	S	lost	AWD	unknown
14	32	IVF	Nodular	2.0x2.0	IIA	S+adjuvant CT	14	AWD	DP+TP
15	37	CB	Ulcer	3.5x2.5	IIIC	S+CT+RT	12	AWD	DP
16	48	IVF	Polyps	4.0x3.5	IB	S+CT+RT	14	AWD	TP
17	50	VBAM	Polyps	4.0x3.0	IIIC	S+adjuvant RT+CT	10	AWD	EP
18	49	CB	Ulcer	4.0x3.0	IIIC	S	3	AWD	unknown
19	55	CB	Ulcer	1.5x1.0	IB	S+CT	12	AWD	EC
20	49	VBAM	Polyps	5.5x3.0	IIIC	S+CT+RT	6	AWD	EC+TP
21	66	VBAM	Polyps	2.0x1.5	IIIC	S+CT+RT	6	AWD	TP
22	49	CB	Ulcer	2.0x1	IB	S+CT	8	AWD	DP
23	60	VBAM	Nodular	3.5x1.5	IB	S+CT+RT	7	AWD	EP
24	41	CB	Nodular	2.5x2	IB	S+CT	1	AWD	TC
25	30	CB	Ulcer	4x2.5	IIIC	S+adjuvant CT	1	AWD	EP
26	70	VBAM	Nodular	3.5x3	IIIC	S	1	AWD	unknown

CB: Contact bleeding; IVF: Irregular vaginal fluid; VBAM: Vaginal bleeding after menopause; WNS: with no symptom; CT: chemotherapy; RT: radiotherapy; S: surgery; DOD: dead of disease; AWD: alive with disease; EC: Etoposide combined with carboplatin; EP: Etoposide combined with cisplatin; DP: Docetaxel combined with cisplatin; DC: Dotaxel combined with carboplatin; TC: Taxol combined with carboplatin; TP: Taxol combined with cisplatin.

Table 2 Pathological characteristics of patients with MiNEN

NO.	Tumor pathol	Subtypes of AC	Differentiation of non-NEN	Percent of NEN	Pathol of LVSI	Pathol of lymph nodes	No. of positive nodes/total nodes
1	SCNEC+SC	-	M	80%	SCNEC	-	-
2	LCNEC+AC+SC	HPV-associated	H	80%	-	-	-
3	typical carcinoid +SC	-	M	10%	-	-	-
4	LCNEC+AC+SC	HPV-associated	L	85%	-	-	-
5	LCNEC+AC	HPV-associated	L	90%	SCNEC	-	-
6	LCNEC+AC	HPV-associated	L	30%	-	-	-
7	SCNEC+AC	HPV-associated	L	90%	SCNEC	SCNEC	2/19
8	LCNEC+AC	HPV-associated	L	80%	LCNEC	LCNEC	1/20
9	typical carcinoid + AC in situ	HPV-associated	H	90%	-	-	-
10	SCNEC+AC	HPV-associated	H	70%	-	-	-
11	LCNEC+AC	HPV-associated	M	85%	-	-	-
12	SCNEC+AC	HPV-associated	H	70%	SCNEC	SCNEC	2/26
13	SCNEC+AC	HPV-associated	M	60%	SCNEC	-	-
14	SCNEC+SC	-	L	40%	SCNEC	-	-
15	SCNEC+SC	-	L	20%	SCNEC	SCNEC	2/14
16	SCNEC+SC	-	M	20%	SCNEC	-	-
17	LCNEC+SC	-	H	80%	LCNEC	LCNEC	22/35
18	LCNEC+SC	-	M	70%	LCNEC	LCNEC	3/36
19	SCNEC+AC+SC	HPV-	M	75%	SCNEC	-	-

		associated					
20	SCNEC+SC	-	M	10%	SC	SC	1/31
21	LCNEC+AC	HPV-associated	L	10%	LCNEC	LCNEC	2/29
22	LCNEC+AC	HPV-associated	H	30%	LCNEC	-	-
23	SCNEC+SC	-	L	10%	-	-	-
24	SCNEC+AC+SC	HPV-associated	H	5%	-	-	-
25	SCNEC+SC	-	H	40%	SCNEC	SCNEC	2/22
26	SCNEC+AC	HPV-independent	M	10%	AC	AC	2/24

LCNEC: large cell neuroendocrine carcinoma; SCNEC: small cell neuroendocrine carcinoma; AC: adenocarcinoma; SC: squamous carcinoma; H: High differentiation; M: medium differentiation; L: low differentiation; No.: number; LVSI: invade the lympho-vascular space; NEC: neuroendocrine carcinoma; -: not applicable.

Table 3 The immunohistochemical information of 26 patients with MiNEN

	Neuroendocrine component						non-Neuroendocrine component			
NO.	Tumor pathol	CD56	Syn	CgA	p16	Ki-67	Tumor pathol	p40	CK8/18	p16
1	SCNEC	+	+	-	+	60%	SC	+	-	+
2	LCNEC	+	+	+	+	70%	AC+SC	SC+	AC+	+
3	typical carcinoid	+	-	+	+	1%	SC	+	-	+
4	LCNEC	+	+	+	+	70%	AC+SC	SC+	AC+	+
5	LCNEC	+	+	+	+	65%	AC	-	+	+
6	LCNEC	-	+	+	+	40%	AC	-	+	+
7	SCNEC	+	+	+	+	90%	AC	-	+	+
8	LCNEC	-	+	+	+	80%	SC	+	-	+
9	typical carcinoid	+	+	+	+	2%	AC in situ	-	+	+
10	SCNEC	+	+	+	+	90%	AC	-	+	+
11	LCNEC	-	+	+	+	60%	AC	-	+	+
12	SCNEC	+	+	-	+	80%	AC	-	+	+
13	SCNEC	+	+	+	+	60%	AC	-	+	+
14	SCNEC	+	+	-	+	20%	SC	+	-	+
15	SCNEC	+	+	-	+	90%	SC	+	-	+
16	SCNEC	+	-	+	+	90%	SC	+	-	+
17	LCNEC	-	+	+	+	60%	SC	+	-	+
18	LCNEC	+	+	-	+	80%	SC	+	-	+
19	SCNEC	+	+	+	+	80%	AC+SC	SC+	AC+	+
20	SCNEC	-	+	-	+	80%	SC	+	-	+
21	LCNEC	+	-	-	+	50%	AC	-	+	+
22	LCNEC	+	+	+	+	80%	AC	-	+	+
23	SCNEC	-	+	-	+	90%	SC	+	-	+
24	SCNEC	-	+	+	+	90%	AC+SC	SC+	AC+	+
25	SCNEC	+	+	+	+	80%	SC	+	-	+
26	SCNEC	+	+	-	-	80%	AC	-	+	-

LCNEC: large cell neuroendocrine carcinoma; SCNEC: small cell neuroendocrine carcinoma; AC: adenocarcinoma; SC: squamous carcinoma;

Figures

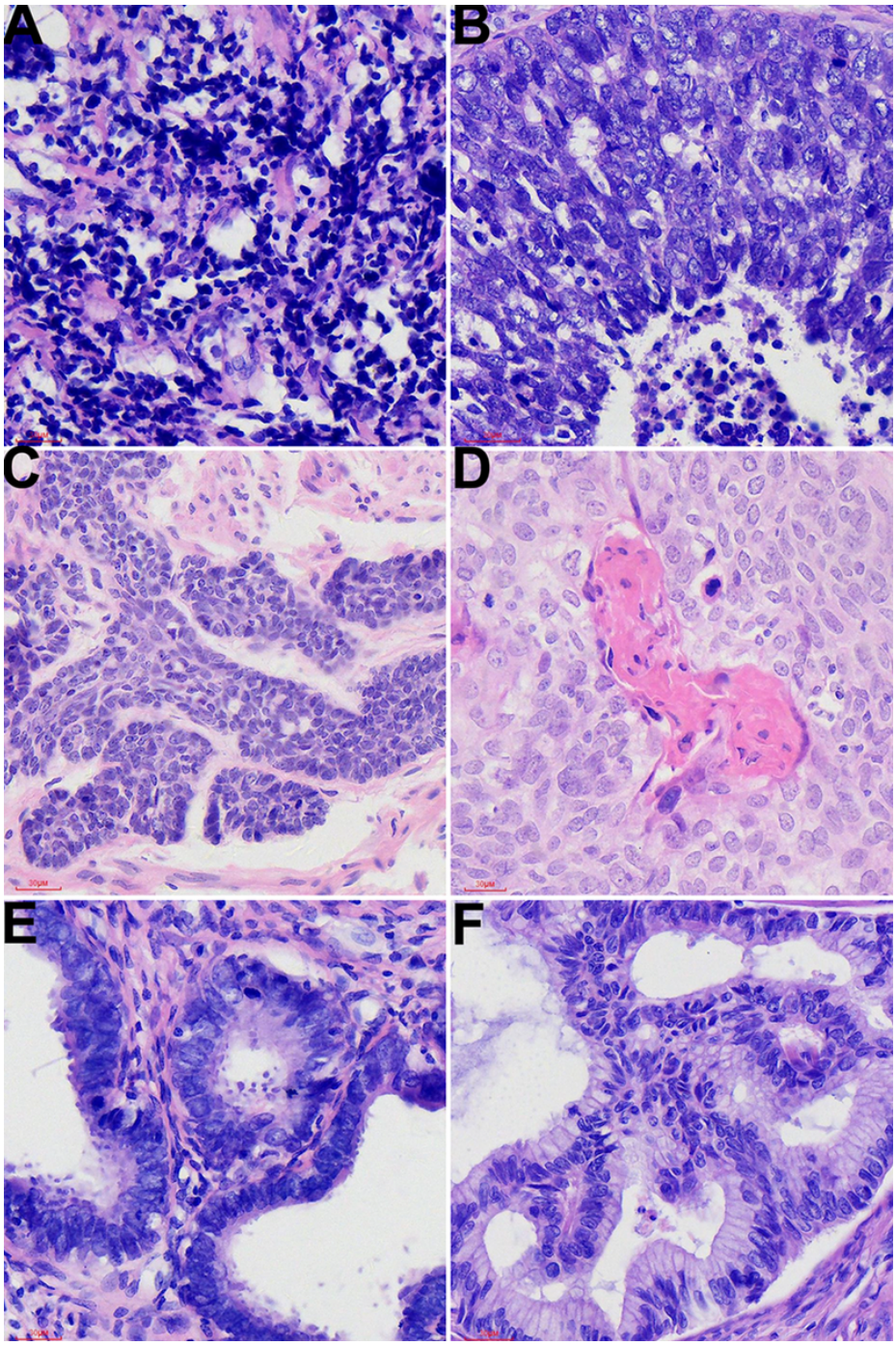

Figure 1

Various tumor components in cervical MiNEN. (400x Hematoxylin and eosin staining (HE) staining for SCNEC (A), LCNEC (B), typicalcarcinoid (C), SC(D), HPV-associated AC (E), HPV-independent AC (gastric-type endocervical AC)(F).

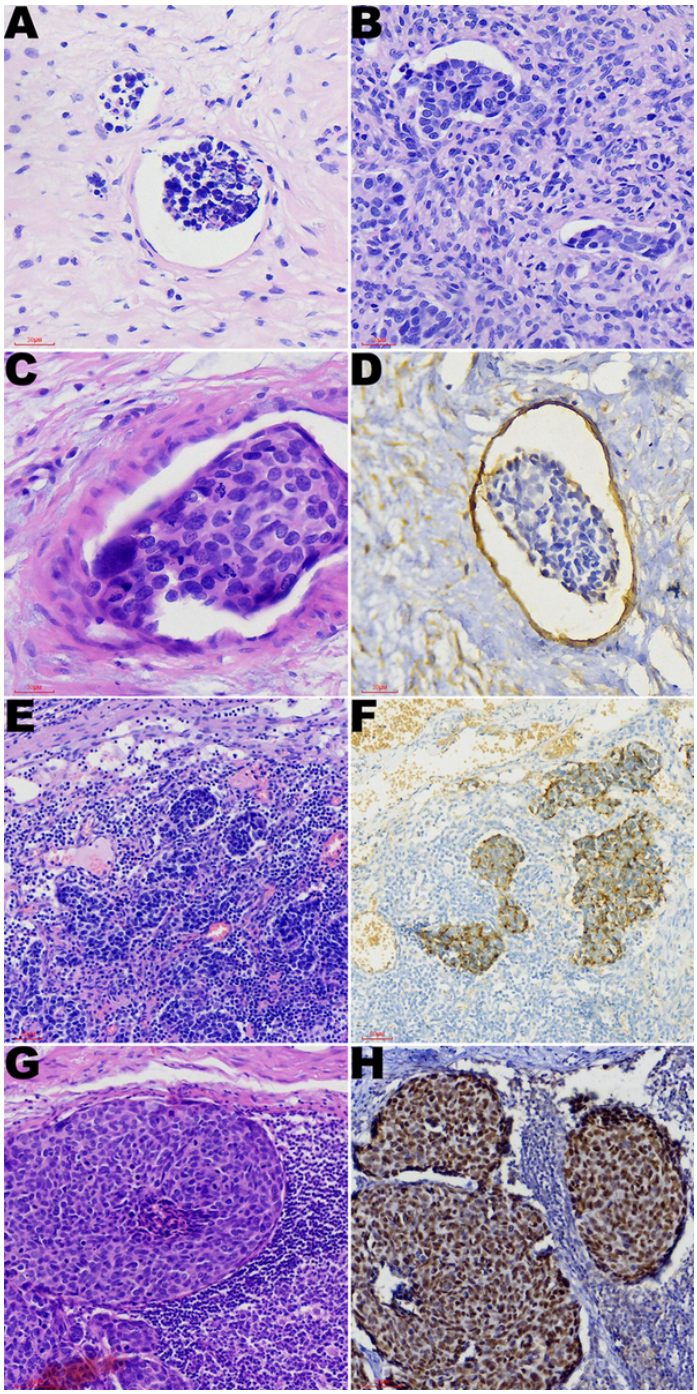

Figure 2

LVSI and LN metastasis of NEC or non-NEC components. (A-D: 400x, E-H: 200x) HE staining for LVSI of SCNEC (A)、LCNEC (B) and SC (C). Immunohistochemical staining of D2-40 showed positive of lymphatic endothelial cells (D). HE staining showed LN metastasis of NEC (E). Positive immunohistochemical staining for CgA showed NEC in LN metastasis (F). HE staining showed LN metastasis of SC (G).Positive immunohistochemical staining for p40 showed SC in LN metastasis (H).

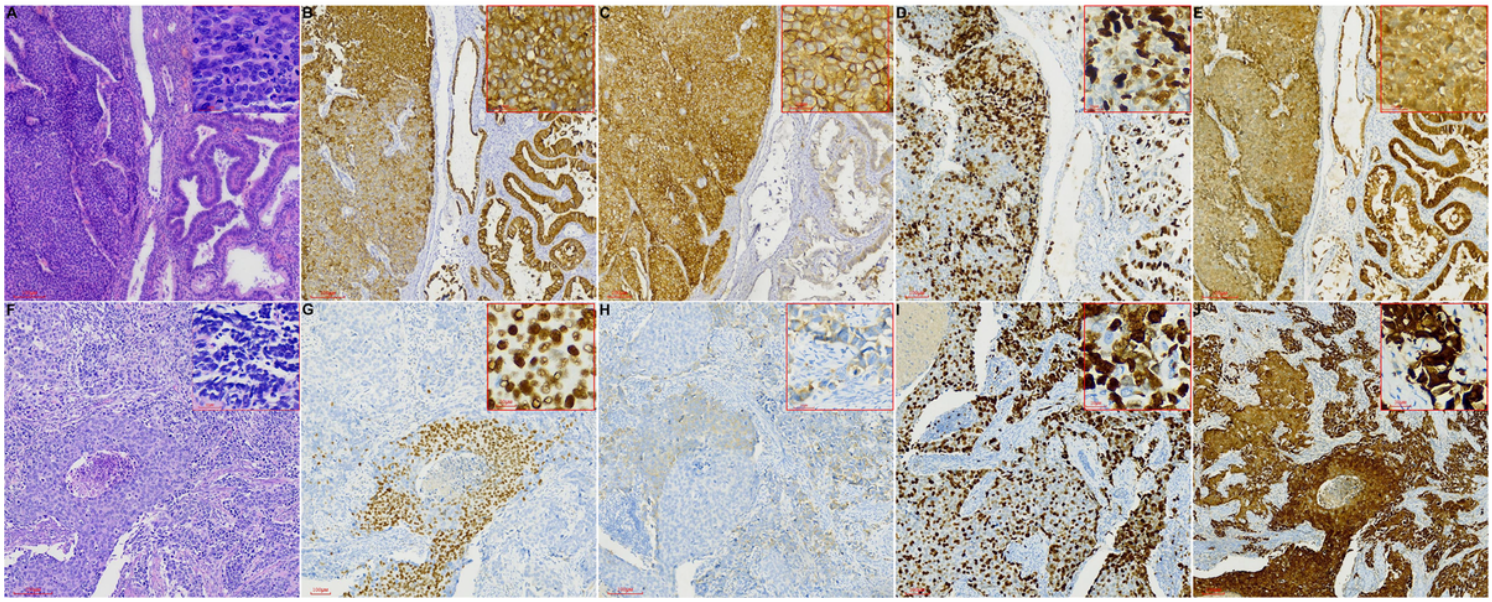

Figure 3

Immunohistochemical staining resultsofcervicalMiNENs.（100x） HE staining for mixed AC-LCNEC (A).The LCNEC components showed positive immunoreactivity for CK8/18 (B, left), CD56 (C, left), Ki-67 (positive rates were 70%; D, left)and p16 (E, left); The AC component showed positive immunoreactivity for CK8/18 (B, right), Ki-67 (positive rates were40%; D, right) and p16 (E, right), but negative for CD56 (C, right).HE staining for mixed SC-SCNEC (F). The SCNEC components showed positive immunoreactivity for Syn (H, upper right), Ki-67 (positive rates were 80%; I, upper right), p16 (J, upper right), but negative for p40 (G, upper right); the SC components showed positive immunoreactivity for p40 (G, left lower), Ki-67 (positive rates were 40%; I, left lower), p16 (J, left lower), but negative for Syn (H, left lower).We show the enlarged image of NEC component in the upper right corner of Figure A-F and H-J, and the enlarged image of p40 staining for SC in the upper right corner of Figure G.

Supplementary Files

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- Figure1S.tif
- Figure2S.tif