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# The study of clinicopathologic features of cervical squamous carcinoma with invasive micropapillary like pattern and phenotype

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**Summary.** Invasive micropapillary carcinoma has been reported in the adenocarcinoma of many organs including cervix, and many studies have proved it has more invasive biological behavior. This study, for the first time, reports cervical squamous carcinoma with invasive micropapillary like pattern and phenotype (IMLPP) and further investigates its clinicopathologic features. Cervical squamous carcinoma with IMLPP was selected by histological characteristics and immunohistochemical staining. All patients' clinical information and pathological parameters were collected. Based on histological characteristics and immunohistochemical staining results, 24 cases, out of 104 cases of cervical squamous carcinoma, were identified as having invasive micropapillary like pattern. The staining of all 24 cases with EMA and MUC-1 showed the feature of "reverse polarity like". Meanwhile, patient age at diagnosis ($P$=0.011), maximum invasion depth ($P$=0.001), maximum diameter ($P$=0.015), lymphvascular space invasion ($P$<0.001), pelvic lymph node metastasis ($P$<0.001), metastasis ($P$=0.020), death ($P$=0.025) and FIGO stages ($P$=0.001) were related to the existence of IMLPP, independently of the proportion of IMLPP to the whole tumor in size. Univariate and multivariate disease-free survival analyses (follow-up time >12 months) showed significant statistical difference between cervical squamous carcinoma with or without IMLPP ($P$=0.016, $P$=0.043). Results from our study suggested that IMLPP may be associated with aggressive biological behavior in cervical squamous carcinoma. Therefore, pathologists should pay attention to the existence of it, no matter its proportion with relation to the whole tumor, and bring it to the attention of clinicians.

**Key words:** Cervix, Squamous carcinoma, Invasion, Metastasis, Micropapillae

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## Introduction

Invasive Micropapillary Carcinoma (IMPC) was first described (Siriaunkgul and Tavassoli, 1993) in 1993 as a new subtype of breast carcinoma, which was characterized by small papillary tumor cell clusters that lack fibrovascular cores and was surrounded by clear spaces. These tufts exhibit "reverse polarity" on the outer surface, as shown by positive immunostaining of EMA, MUC-1 or CD10 (Siriaunkgul and Tavassoli, 1993; Toyoda et al., 2016; Stewart et al., 2018). IMPC has been reported in various organs (Munakata et al., 2018), with a majority of studies suggesting that tumors with an invasive micropapillary component showed a significant tendency for vascular invasion and indicated a poor prognosis (Siriaunkgul and Tavassoli, 1993; Amin et al.,1994; Kamiya et al., 2008; Ieni et al., 2016). Until now, only a few dozens of cases of micropapillary adenocarcinoma of cervix have been reported (Kajiyama et al., 2013; Toyoda et al., 2016; Alvarado-Cabrero et al., 2017, 2019; Munakata et al., 2018; Stewart et al., 2018), but there no cervical squamous carcinoma (CSC) with similar morphology has been observed. In this study, we reviewed a series of CSC with invasive micropapillary like pattern and phenotype (IMLPP) and further investigated their clinicopathologic features.

## Materials and methods

### Case selection

104 cases of CSC from December 2013 to July 2019, were selected from Shandong University Qilu Hospital (Qingdao). All the patients had been treated by radical hysterectomy and pelvic lymphadenectomy with available follow-up data. All specimens were fixed in 10% formalin and sliced. The 4 μm thick tissue sections were stained with hematoxylin and eosin (H&E) and were reviewed independently by two experienced pathologists. CSC with IMLPP were identified by H&E and related immunohistochemical staining. Meanwhile, cases of lymphovascular cancer emboli, which is the mimic of invasive micropapillary component, were confirmed by immunohistochemical staining of CD31, D2-40 and excluded. Finally, 24 cases were selected for further study. They were divided into 2 groups according to the proportion of IMLPP to the whole tumor in size, with one group including 17 cases with the areas of IMLPP accounting for more than 5% of the whole tumor, while the other group containing 7 cases with the areas of IMLPP accounting for less than 5% of the whole tumor.

The medical records of all of 104 CSC cases were reviewed for clinical information including the patients' age at diagnosis, 2018 FIGO stages, follow-up date, recurrence/metastasis and survival status. Measured histological parameters included invasion depth, maximum diameter, lower uterine body or vagina involvement, lymphvascular space invasion (LVSI), and pelvic lymph node metastasis (LNM).

### Immunohistochemistry

Immunohistochemical staining of epithelial membrane antigen (EMA), CD10, E-cadherin, cytokeratin 7(CK7), P16, CD31, D2-40 was performed. The Roche autostainer which uses a standard streptavidin-biotin peroxidase complex technique was applied for immunohistochemical studies. Heat-induced epitope retrieval was used as pretreatment for the selected markers. Positive and negative controls were run simultaneously for all the tested markers. Detailed information of these antibodies are listed in Table 1.

### HPV typing

89 cases of CSC had HPV genotyping, and 22 cases were with IMLPP. This test used the Roche linear array testing kit (Roche Molecular Diagnostics) which can identify 37 HPV genotypes, including 14 high-risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The hybridization and the color reaction were performed automatically in an AutoLiPA device. The results of hybridization were assessed visually by comparing to the standard grid.

### Statistical analysis

Statistical analyses were performed by using Statistical Package for the Social Sciences Statistical analysis (version 17.0, SPSS, Inc., Chicago, IL). All numerical variables were normal distributions and were presented by mean ±SD. Classified variables were scored as absolute values.

Analysis on the differences between histological subgroups were operated by student t-test for normally distributed continuous variables, $\chi^2$ test, continuity correction $\chi^2$ or Fisher exact test for classified variables. Besides, the differences between order-classified variables were assessed by Mann-Whitney U test.

Disease-free survival (DFS) curves were drawn by the Kaplan–Meier method, and the time-period was from the date of surgery to the date of recurrence or metastasis. Cox regressive analysis and Cox regressive analysis with time-dependent variables were used for multiple variables survival analysis. P-value was computed by log-rank test and $P<0.05$ was considered of statistical significance.

## Results

### Clinical Findings

The age at diagnosis of the usual CSC ranged from 30 to 77 years, while that of CSC with IMLPP was 30 to

**Table 1.** Detailed information of antibodies used in study.

| Antibody | Clone | Source | Vendor |
|---|---|---|---|
| EMA | E29 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |
| MUC-1 | EP85 | Rabbit monoclonal | ZSGB-BIO |
| CD10 | MX002 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |
| E-cad | MX020 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |
| CK7 | OV-TL 12/30 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |
| P16 | E6H4 | Mouse monoclonal | VenTANA |
| CD31 | MX032 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |
| D2-40 | D2-40 | Mouse monoclonal | Fuzhou Maixin Biotech CO.Ltd |

All of the reagents used above were ready-to-use antibodies.

67 years. The mean age at diagnosis was 51.74±9.92 and 45.88±8.73 years for the usual CSC and CSC with IMLPP, respectively ($P$=0.011).

All patients were assessed by 2018 FIGO system, and this detailed information is listed in Table 2. The mean rank order was 48.14 and 67.02 for the usual CSC and CSC with IMLPP, respectively ($P$=0.001). The analysis of the differences of FIGO stages between histological subgroups are listed in Table 3.

By the end of July 2021, 15 patients experienced local recurrence of disease (15/104, 14.42%), 8 patients developed metastatic disease (8/104, 7.69%) and 3 patients died of disease (3/104, 2.88%). The rate of metastasis was 3.75% (3/80) and 20.83% (5/24) for the usual CSC and CSC with IMLPP, respectively ($P$=0.020). Meanwhile, the mortality was 0.00% (0/80) and 12.50% (3/24) for the usual CSC and CSC with IMLPP, respectively ($P$=0.025). But there were no differences in recurrence rates between histological subgroups, which can be seen in Table 3.

Data in Table 4 showed that there was no statistically significant differences in the clinical factors described above between groups with different proportion of IMLPP with relation to the whole tumor.

### *Histological findings*

Twenty-four (24/104, 23.10%) cases were confirmed by H&E and immunohistochemistry. Morphologically, these confirmed that IMLPP consisted of small neoplastic squamous cell slices or tufts without fibrovascular cores and often lying in clear spaces (Fig. 1). In cases with IMLPP, the maximum invasion depth and diameter ranged from 3 to 28mm (mean 12.67±5.67) and 6 to 100mm (mean 40.88±22.23), respectively, compared with 0 to 25mm (mean 8.08±5.80) and 1 to 75mm (mean 30.48±16.65), for the two parameters respectively in the usual CSC cases (invasion depth: $P$=0.001; maximum diameter: $P$=0.015).

LVSI was identified in 55 (55/104, 52.88%) cases of all patients. Among them, there were 21(21/24, 87.50%) cases of CSC with IMLPP and 34(34/80, 42.50%) cases of the usual CSC ($P$<0.001).

Pelvic LNM was demonstrated in 19 cases (19/104, 18.27%). CSC with IMLPP and the usual CSC accounted for 13 (13/24, 54.7%) and 6 (6/80, 7.50%) cases of the metastasis positive cases ($P$<0.001) respectively. Besides, micropapillary like architecture could also be observed in lymph node metastatic component in 2 cases.

However, analysis of all of the above histological factors between CSC with IMLPP>5% group and <5% group did not show statistically significant differences (Table 4). In addition, there were no statistically significant differences observed between histological subgroups or between different IMLPP proportion groups in the aspect of lower uterine body or vagina involvement (Tables 3, 4).

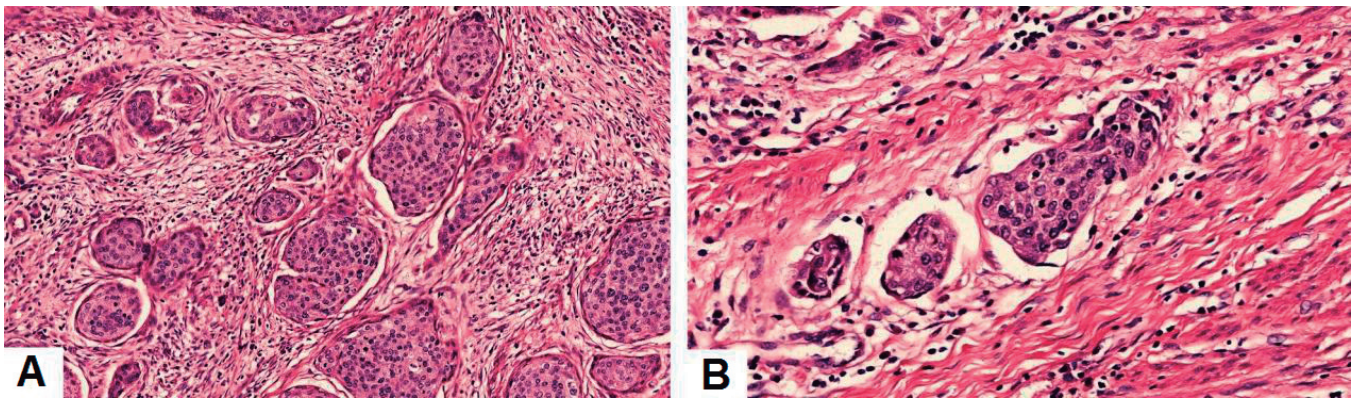


**Fig. 1.** Small neoplastic squamous cell slices or tufts without fibrovascular cores scattered in cervical stroma and often surrounded by clear spaces. H&E **(A)**. Clear spaces which surrounded tumor slices or tufts can be observed more clearly at high magnification. H&E **(B)**. A, x 100; B, x 200

**Table 2.** 2018 FIGO stages.

| | I stage | | | | | II stage | | III stage | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IA1 | IA2 | IB1 | IB2 | IB3 | IIA1 | IIA2 | IIIA | IIIC1 | IIIC2 |
| All CSC | 8 | 5 | 8 | 30 | 19 | 6 | 9 | 1 | 16 | 2 |
| CSC with IMLPP | 0 | 1 | 1 | 4 | 5 | 0 | 1 | 0 | 11 | 1 |
| Normal CSC | 8 | 4 | 7 | 26 | 14 | 6 | 8 | 1 | 5 | 1 |

### Immunohistochemical staining

All of the 24 cases of CSC with IMLPP showed stronger positive staining of EMA on peripheral cells of micropapillary squamous cell clusters than on inner cells of the clusters ("reverse polarity like") (Fig. 2A). In some cases, the existence of IMLPP in LNM lesions was also confirmed by EMA staining (Fig. 2B). There were 17 cases exhibiting the same "reverse polarity like" staining pattern in IMLPP with MUC-1 (Fig. 2C). Though the expression of E-cadherin was observed on the intercellular cell membrane of IMLPP in all cases, there was a loss or decreased expression of E-cadherin on the membranes of IMLPP facing towards mesenchyme (Fig. 2D).

In addition to EMA and MUC-1, CD10 is another Immunostaining marker for IMPC in many organs. However, in our study, stained CD10 in all 24 cases of CSC with IMLPP only resulted in 4 cases with "reverse polarity like" staining, with another 20 cases shown as negative. Furthermore, those with CD31 and D2-40 staining were operated in all 24 cases to ensure that the micropapillary like components weren't lymphovascular cancer embolis (Fig. 2E,F).

Strongly positive expression for P16 was observed in 103 cases. One usual CSC was negative for P16 staining.

CK7 was stained in all CSC cases, but no statistically significant differences were found between histological subgroups or between different IMLPP proportion groups (Tables 3, 4).

### HPV typing

HPV typing was detected in 89 cases. HPV 16 infection was detected in 86.36% (19/22) of CSC patients with IMLPP and 62.69% (42/67) of usual CSC patients (P=0.038). HPV18 was separately found in 9.09% (2/22) of CSC patients with IMLPP and 13.43% (9/67) of usual CSC patients. Besides, in CSC with IMLPP group, there was 1 case of HPV 59(+) and 1 case of HPV 56(+), while in the usual CSC group, there was 1case of HPV 11(+), 4 cases of HPV 31(+), 3 cases of HPV 39(+), 1 case of HPV 44(+), 2 cases of HPV 45(+), 1 case of HPV 51(+), 4 cases of HPV 52(+), 3 cases of HPV 58(+), 1 case of HPV 59(+), 1 case of HPV 68(+),

**Table 3.** Clinicopathological variables in patients of CSC with or without IMLPP.

| | CSC with IMLPP(N=104) | | P value |
|---|---|---|---|
| | Yes (N=24) | No (N=80) | |
| Age (mean±SD) | 45.88±8.73 | 51.74±9.92 | 0.011 |
| CK7 | | | |
| (+) | 13 | 50 | 0.464 |
| (-) | 11 | 30 | |
| Maximum invasion Depth (mean±SD) | 12.67±5.68 | 8.08±5.80 | 0.001 |
| Maximum diameter (mean±SD) | 40.88±22.23 | 30.48±16.65 | 0.015 |
| Lower uterine body or vagina Involvement | | | |
| Yes | 4 | 17 | 0.841 |
| No | 20 | 63 | |
| LVSI | | | |
| 0-4 vessels | 11 | 64 | <0.001 |
| 5-19 vessels | 8 | 15 | |
| ≥20 vessels | 5 | 1 | |
| Pelvic LNM | | | |
| Yes | 13 | 6 | <0.001 |
| No | 11 | 74 | |
| Recurrence | | | |
| Yes | 3 | 12 | 1.000 |
| No | 21 | 68 | |
| Metastasis | | | |
| Yes | 5 | 3 | 0.020 |
| No | 19 | 77 | |
| Death | | | |
| Yes | 3 | 0 | 0.025 |
| No | 21 | 80 | |
| FIGO stages | | | |
| I | 11 | 59 | 0.001 |
| II | 1 | 14 | |
| III | 12 | 7 | |

**Table 4.** Value of clinicopathological variables in patients with different proportions of IMLPP.

| | proportion of IMLPP(N=24) | | P value |
|---|---|---|---|
| | ≥5% (N=17) | <5% (N=7) | |
| Age (mean ± SD) | 45.18±7.92 | 47.57±10.97 | 0.553 |
| CK7 | | | |
| (+) | 10 | 3 | 0.659 |
| (-) | 7 | 4 | |
| Maximum invasion Depth (mean ± SD) | 13.59±6.23 | 10.43±3.46 | 0.223 |
| Maximum diameter (mean ± SD) | 39.94±20.70 | 43.14±27.27 | 0.756 |
| Lower uterine body or vagina Involvement | | | |
| Yes | 2 | 2 | 0.552 |
| No | 15 | 5 | |
| LVSI | | | |
| 0-4 vessels | 8 | 3 | 0.706 |
| 5-19 vessels | 6 | 2 | |
| ≥20 vessels | 3 | 2 | |
| Pelvic LNM | | | |
| Yes | 9 | 4 | 1.000 |
| No | 8 | 3 | |
| Recurrence | | | |
| Yes | 1 | 2 | 0.194 |
| No | 16 | 5 | |
| Metastasis | | | |
| Yes | 3 | 2 | 0.608 |
| No | 14 | 5 | |
| Death | | | |
| Yes | 1 | 2 | 0.194 |
| No | 16 | 5 | |
| FIGO stages | | | |
| I | 8 | 3 | 0.746 |
| II | 1 | 0 | |
| III | 8 | 44 | |

1 case of HPV 81(+), 1 case of HPV 82(+), and 7 cases negative. There was other 6 patients were positive for HPV detection in other hospital, but we did not get the detailed HPV typing. All of these HPV positive cases exhibited diffuse block type immunoreactivity with p16.

*Disease-free survival curves*

The follow-up periods of all patients ranged from 1 to 89 months (mean 36.18). We divided them into two groups, the follow-up time of the patients in group 1 was equal or lesser than 12 months (21/104), and that in group 2 was more than 12 months (83/104).

In the former group, there was no significant difference between CSC with or without IMLPP groups for DFS (P=0.847) (Fig. 3A). However, CSC with IMLPP had shorter DFS than CSC without IMLPP (*P*=0.016) (Fig. 3B) in the latter group, and the DFS rates were 77.78% (14/18) and 93.85% (61/65) respectively.

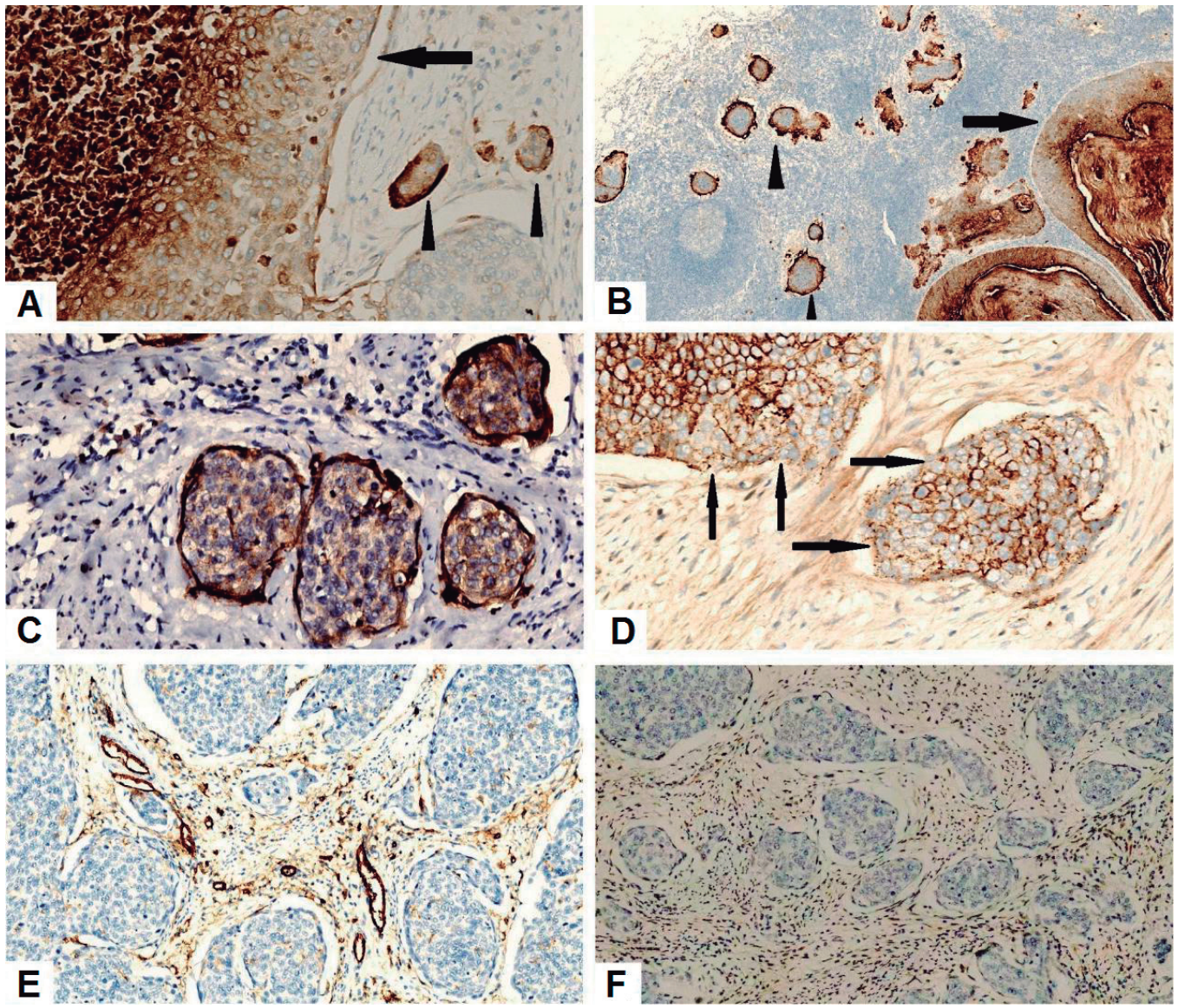


**Fig. 2.** EMA shows stronger positive staining on peripheral cells of IMLPP than on inner cells of it (arrow head), while the stronger positive staining of EMA in the area without IMLPP was located in the inner cell of cancer nest (arrow). SP **(A)**. The component of the usual CSC (arrow) and IMLPP (arrow head) can be observed in lymph node metastasis lesion. SP **(B)**. MUC-1 demonstrates the same staining pattern as EMA. SP **(C)**. E-cadherin is expressed on the intercellular cell membranes of IMLPP. There is lost or decreased E-cadherin expression on the ourtercellular cell membranes of IMLPP facing towards mesenchyme (arrow). SP **(D)**. CD31 **(E)** and D2-40 **(F)** staining confirm that these IMLPP are not lymphovascular cancer embolis. SP. A, C, D, x 200; B, x 40; E, F, x 100.

*Cox regressive analysis*

Multivariate DFS analysis by using Cox regressive analysis was used to analyze the asscociation of IMLPP, maximum diameter, infiltration depth, LVSI, pelvic LNM, FIGO stages and patient age with prognoses in CSC patients. No significantly independent predictor of survival was found in equal or lesser than 12 months follow-up time group, while IMLPP ($P$=0.043) and patient age ($P$=0.014) were significantly independent predictors of survival in more than 12months follow-up time group.

## Discussion

Invasive Micropapillary Carcinoma (IMPC), as a subtype of breast carcinoma, was first reported (Siriaunkgul and Tavassoli, 1993) in 1993. IMPC was subsequently observed in various organs such as lung, digestive tract, pancreas, thyroid, urinary tract and ovary (Munakata et al., 2018).

Though the cause of IMPC is still unclear, it has the following characteristics: (1) formation of a micropapillary structure without fibrovascular axis in the tumor cells; (2) there is a gap between the micropapillary structure and the mesenchyme; (3) the micropapillary structure has the characteristic of "reverse polarity" by immunohistochemical staining. That is, in micropapillary structure, the antibody EMA, MUC-1 and CD10 which should stain the cell surface facing towards the luminal cavity now exhibit positive staining on the cell surface facing towards the mesenchyme instead (Siriaunkgul and Tavassoli, 1993; Toyoda et al., 2016; Stewart et al., 2018). Since the report of IMPC, dozens of cervical adenocarcinomas have been reported with IMPC (Kajiyama et al., 2013; Toyoda et al., 2016; Alvarado-Cabrero et al., 2017, 2019; Munakata et al., 2018; Stewart et al., 2018). However, CSCs with IMLPP have not been reported. In this study, we investigated the clinicopathological features of CSC with IMLPP through 24 cases to explore whether CSC with IMLPP has the same invasiveness and prognosis as IMPC of other organs (Siriaunkgul and Tavassoli, 1993; Amin et al.,1994; Kamiya et al., 2008; Ieni et al., 2016).

In this study, 104 specimens were collected from patients who had undergone complete hysterectomy and pelvic lymphadenectomy. Following examination with H&E, 34 samples with micropapillary like pattern were selected. They are squamous carcinomas characterized with small atypical squamous cells arranged in clusters in micropapillary like pattern, the center of which lacks fibrovascular cores, and there are lumen-like gaps between the micropapillary like pattern and the mesenchyme. Before diagnosis of IMLPP, budding should be excluded, characterized by single or small cluster of cancer cells usually surrounded by fibroblast cells and inflammatory cells instead of lumen-like gap (Fig. 4A). In our study, the cells in tumor budding did not exhibit the "reverse polarity like" staining pattern of EMA and MUC-1 in IMLPP (Fig. 4B,C). The E-cad expression was absent or decrease in the cell membrane facing towards mesenchyme in IMLPP, but not in tumor budding (Fig. 4D). All of these differences between IMLPP and budding can help us identify and exclude them. Then, by immunohistochemical staining using CD31and D2-40, lymphovascular cancer emboli also characterized with micropapillary structure were excluded, resulting in 24 cases of CSC with IMLPP for further study. These cases were divided into two groups as IMLPP ≥5% or IMLPP <5%, for which the size of IMLPP accounts for 5% or more of the tumor or less than 5% of the tumor.

EMA and MUC-1 showed "reverse polarity like" staining in all 24 cases, indicated by the darker staining at the edge of IMLPP. By contrast, CD10 showed "reverse polarity like" by focal staining in only 4 cases. In addition, Stewart et al. (2018) reported that CD10 staining only identified 2 out of 8 cases as positive, and the research by Alvarado-Cabrero et al. (2019) showed that CD10 staining could not identify any positive cases out of 40 cases. The above results also suggested that CD10 may not be a suitable immunological marker for micropapillary structures in cervical tumors.

In all 24 cases, the expression of E-cadherin was

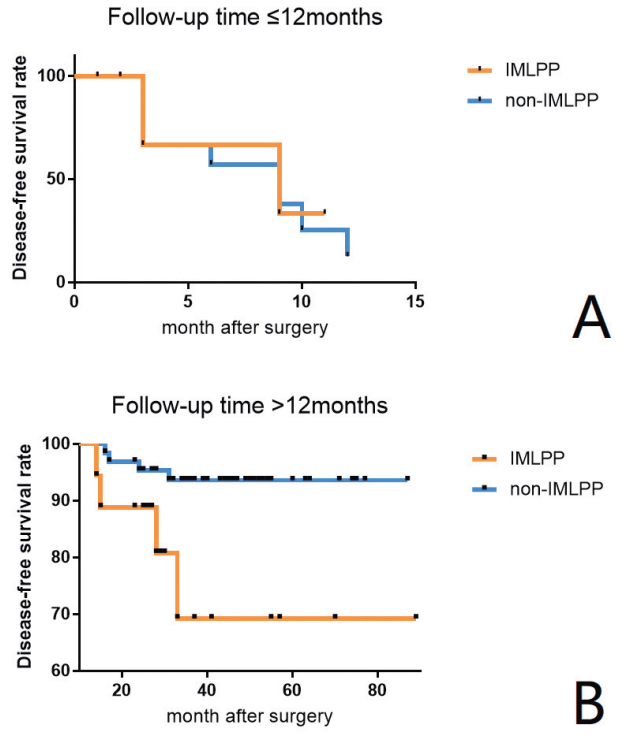


**Fig. 3.** DFS curves between CSC with or without IMLPP groups (follow-up time ≤12 months) **(A)**. DFS curves between CSC with or without IMLPP groups (follow-up time >12months) **(B)**.

either lost or decreased to various level on the outer membrane of IMLPP, but was positive on the membrane of IMLPP inside the carcinoma cells.

Epithelial-mesenehymal transition (EMT) is a reversible biological process in which epithelial cells are transformed into mesenchymal phenotype cells through specific procedures including changes in cell morphology and behavior (Thiery et al., 2009). Studies have shown that tumor cells which have undergone EMT exhibit reduced level of cell adhesion molecules and keratin expression, but increased levels of vimentin expression. Such changes lead to tumor cells losing epithelial characteristics and obtaining mesenchymal features, which weakens their adhesion to interstitial structures, while it enhances their mobility (Polyak and Weinberg, 2009). In addition, EMT can also induce stemness in cancer cells (Brabletz et al., 2005; Ieni et al., 2016). Due to the above processes, EMT plays an important role in the development, invasion, metastasis and drug-resistance of cervical cancer (Lee and Shen, 2012). The main epithelial markers of EMT include E-cadherin (E-cad), Cytokeratin, and α-catenin. The main interstitial markers include Vimentin and α- Smooth muscle actin (α-SMA), N-cadherin, Snail protein, E-box binding zinc finger protein 1 and 2 (ZEB1 and 2) (Lamouille et al., 2014). In this study, CSC with IMLPP showed decreased or lost expression of adhesion molecules while the characterizations of invasion and metastasis of tumor cells was enhanced. Together with a previously reported study which confirmed the correlation between colonic IMPC and EMT (Gonzalez et al., 2017), we speculated that the EMT process also occurs in IMLPP to increase its ability to invade and metastasize, and both in squamous carcinoma and adenocarcinoma, the existence of IMLPP may prompt the EMT of tumor cells. But all of these speculations

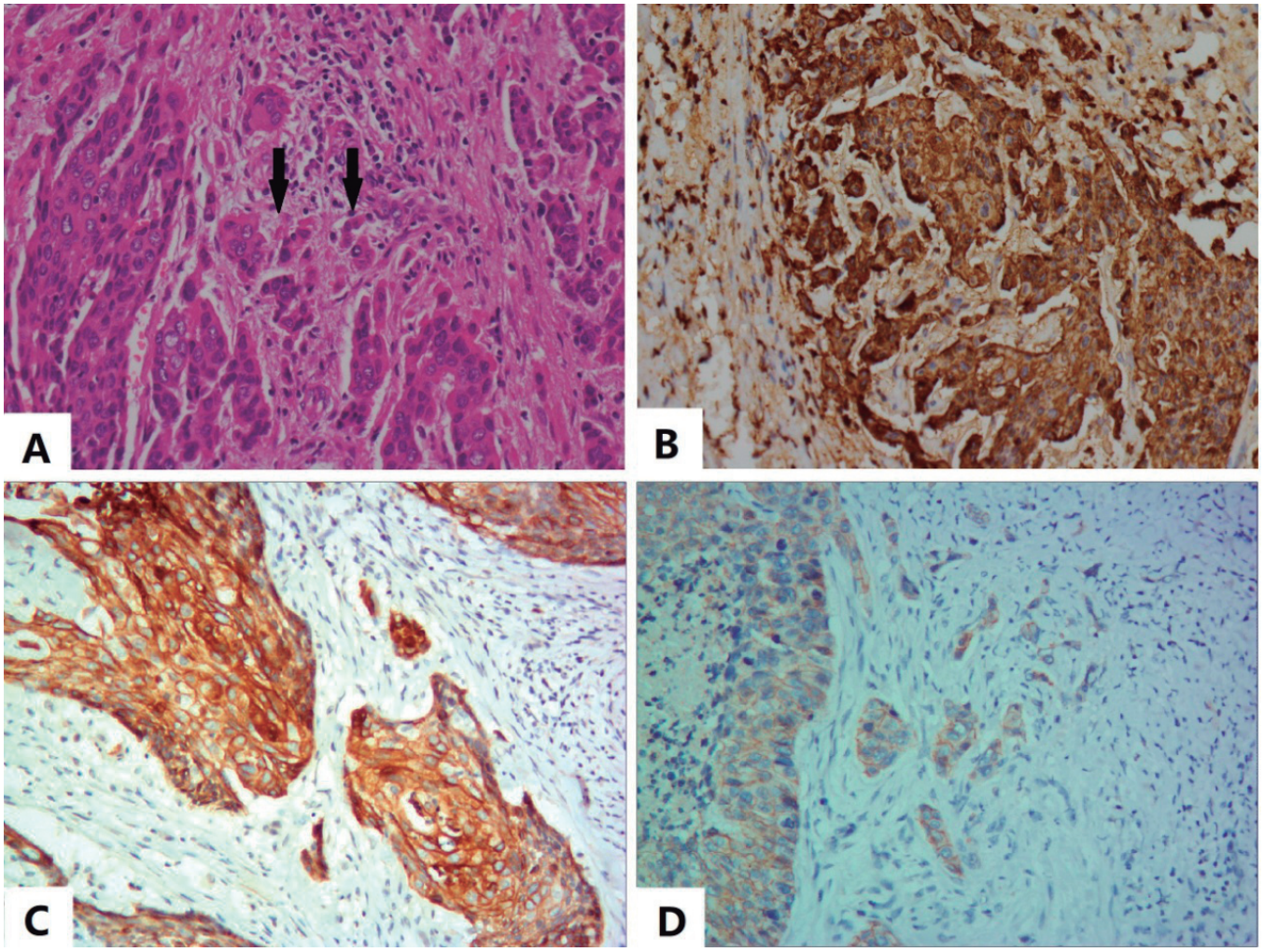


**Fig. 4.** Budding(arrow) consists of single or small cluster of cancer cells, and usually surrounded by fibroblast cells and inflammatory cells. SP **(A)**. Budding shows same stain intensities of EMA **(B)** and MUC-1 **(C)** in both inner and peripheral cells, which is different with "reverse polarity like" staining pattern in IMLPP. SP. Budding shows E-cad expression on the cell membranes facing towards mesenchyme. SP **(D)**. x 200.

need futher confirmation.

Though HPV16 and 18 virus infections are closely related to cervical squamous epithelial lesions and glandular epithelial lesions, they exhibit different infection rates for these two types of lesions. The infection rate of HPV18 in cervical adenocarcinoma and adenocarcinoma in situ is about 40-50%, higher than that in squamous cell carcinoma, which has a higher HPV16 infection rate of about 50% (Zaino, 2002). In this study, the infection rate of HPV16 was higher than that of HPV18 in both types of CSC, consistent with previously reported findings. Also, the infection rate of HPV16 in cases with IMLPP was higher than which in cases without IMLPP, and this may suggest that the infection of HPV 16 may have some relationship with the incidence of IMLPP.

The squamocolumnar junction (SCJ) is a prevalent occurrence site for cervical squamous cell carcinoma. In recent years, there have been a series of reports on SCJ immunolabels (Herfs et al., 2012), with CK7 as the most commonly used one. Positive expression of CK7 suggested that tumor cells originate from SCJ. In this study, through staining of CK7 in 104 cases of CSC, we investigated whether CSC with or without IMLPP have different histological origins. Statistical analysis did not show any significant difference, indicating that two types of CSC have the same histological origins. Thus, the further investigation is still needed to confirm what has led to the histological variation of the two types of CSC.

Invasion and metastasis are the key mechanisms leading to the mortality of cancer patients, and tumors with micropapillary structures in various organs show higher invasiveness and tendency of lymphatic invasion (Siriaunkgul and Tavassoli, 1993; Amin et al.,1994; Kamiya et al., 2008; Chen et al., 2014; Ieni et al., 2016). In this study, IMLPP was observed in lymph node metastases, suggesting that IMLPP is involved in the metastasis of CSC to the lymph node. In addition, further analysis of LVSI and pelvic LNM in all cases also confirmed that CSC with IMLPP had a higher tendency of vascular invasion and lymph node metastasis. Microscopic observation revealed that IMLPP was distributed mainly in the peripheral part of the tumor, suggesting that the infiltration of CSC could be through IMLPP into the surrounding mesenchyme, and that IMLPP has higher local invasiveness compared with ordinary CSC cells. However, regarding the aspect of lower uterine body or vagina involvement, there were no statistically significant differences between histological subgroups. This may indicate that there are still other factors influencing the ability of invasiveness in CSC apart from IMLPP. At the same time, compared with CSC without IMLPP, CSC with IMLPP had a lower age of onset, greater depth of invasion, larger maximum tumor diameter, higher metastasis and death rate as well as higher FIGO stage. Furthermore, this study showed that the above clinical pathological features were not statistically significantly different between the IMLPP $\geq$ 5% group and the IMLPP <5% group. So pathologists should pay attention to the presence of IMLPP no matter the proportion involved.

Besides, we demonstrated that, when the follow-up time was less than 12 months, there was no significant difference between IMLPP and non-IMLPP groups on DFS rates. But for those people whose DFS was more than 12 months, the existence of IMLPP and ages would be important influencing factors on the DFS rates. We speculated that IMLPP components may possess some drug resistance. Therefore, IMLPP might become a more prominent influencing factor on DFS rates after long-term drug treatment which has weakened other components of the tumor. However, these hypotheses need further study to confirm.

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*Conflict of interest statement.* The authors declare that they have no conflicts of interest to this work.

*Authors' contributions.* H.W, Q.L: writing, slide review; K.Y: data analysis; W.S, X.L: literature search; K.F: sorting data for CSC, imaging; H.S, S.L: technical performance of IHC staining; H.J: the article review. All persons read the manuscript and wrote the part for which they were responsible

*Ethics approval and consent to participate.* This is a retrospective study in which patients were de-identified. The findings did not result in a change in treatment, cost to patients or patient harm therefore no ethics committee approval was sought

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