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Colorectal cancer with invasive micropapillary components (IMPCs) shows high lymph node metastasis and a poor prognosis

A retrospective clinical study

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Abstract

Objects: The present study aimed to identify the clinicopathological characteristics of colorectal cancer (CRC) with invasive micropapillary components (IMPCs) and the relationship between different amounts of micropapillary components and lymph node metastasis.

Methods: A cohort of 363 patients with CRC who underwent surgical treatment in the Second Affiliated Hospital of Zhejiang University School of Medicine between January 2013 and December 2016 were retrospectively reviewed. We compared the clinicopathological characteristics, including survival outcomes and immunohistochemical profiles (EMA, MUC1, MLH1, MSH2, MSH6, and PMS2), between CRC with IMPCs and those with conventional adenocarcinoma (named non-IMPCs in this study). Logistic regression was used to identify the association between IMPCs and lymph node invasion. A multivariate analysis was performed using the Cox proportional hazard model to evaluate significant survival predictors.

Results: Among 363 patients, 76 cases had IMPCs, including 22 cases with a lower proportion of IMPCs (≤5%, IMPCs-L) and 54 cases with a higher proportion (>5%, IMPCs-H). Compared to the non-IMPC group, the IMPC group (including both IMPC-L and IMPC-H) had a lower degree of tumor differentiation (P = .000), a higher N-classification (P = .000), more venous invasion (P = .019), more perineural invasion (P = .025) and a later tumor node metastasis (TNM) stage (P = .000). Only tumor differentiation (P = .031) and tumor size (P = .022) were different between IMPCs-L and IMPCs-H. EMA/MUC1 enhanced the characteristic inside-out staining pattern of IMPCs, whereas non-IMPCs showed luminal staining patterns. The percentage of mismatch repair deficiency (dMMR) in the non-IMPC group was much higher than that in the IMPC group (14.7% vs 4.7%). The overall survival time of patients with IMPCs was significantly less than that of patients with non-IMPCs (P = .002), then that of IMPCs-H was lower than that of IMPCs-L (P = .030). Logistic regression revealed that patients with IMPCs were associated with lymph metastasis, regardless of the proportion of IMPCs. Multivariate analysis demonstrated both IMPCs-L and IMPCs-H as negative prognostic factors.

Conclusions: IMPCs are significantly associated with lymph node metastasis and poor outcome, and even a minor component (≤5%) may render significant information and should therefore be part of the pathology report.

Abbreviations: 1.000 = Reference level, AJCC = American Joint Committee on Cancer, BMI = body mass index, CEA = carcinoembryonic antigen, CI = confidence interval, CRC = colorectal cancer, dMMR = mismatch repair deficiency, HR = hazard ratio, IMPCs = invasive micropapillary components, IMPCs-H = IMPCs-high, >5%, IMPCs-L = IMPCs-less, ≤5%, MMR = mismatch

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repair, Non-IMPCs = conventional adenocarcinoma, OR = odds ratio, OS = overall survival, PDCs = poorly differentiated clusters, pMMR = mismatch repair proficient, pN = pathological node status, pT = pathological invasion level, TNM = tumor node metastasis.

Keywords: colorectal cancer (CRC), invasive micropapillary components (IMPCs), lymph node metastasis, prognostic factor

1. Introduction

Invasive micropapillary carcinoma is a rare histological type of tumor, first described in invasive ductal breast cancer[1] and subsequently in other organs, including urinary bladder,[2] lung,[3] ovary,[4] parotid gland,[5] stomach,[6] and colorectum.[7] Past studies have shown that invasive micropapillary carcinoma is associated with a poor prognosis[2,3,8] amd lymph node metastasis.[9–16] We applied the term "invasive micropapillary components (IMPCs)" because the cases with a lower proportion of IMPCs ($\leq$5%) were contained in the research. Histologically micropapillary components are characterized as small papillary cell clusters surrounded by dense fibrous stroma and lacunar spaces, the clusters lack true fibrovascular cores, the tumor cells display intermediate- to high- grade nuclei and eosinophilic cytoplasm.[7,9,12,17–19] The tumor nests exhibit reverse polarity, leading to a characteristic "inside-out" pattern, the basal surface of cells exhibits properties observed in the apical region, which is probably related to the high invasive potential of these cells.[12,16,20] EMA/MUC-1 staining confirm the "inside-out" pattern of IMPCs, which is primarily strongly and diffusely located on the stroma-facing (basal) surface of the neoplastic cell clusters in patients with IMPCs compared with the color reaction located in the apical part (luminal regions) of normal glandular cells.[9,12,14,16,20,21] To our knowledge, micropapillary components previously reported accounted for at least 5% of the tumor volume.[9–13,15,16] Only 2 studies from a single center reported IMPCs samples with fewer micropapillary patterns, which was a total of 17 cases.[14,20] The association between CRC with fewer micropapillary components and lymph node metastasis or prognosis remains unclear. For this reason, we analyzed the clinicopathological characteristics of colorectal cancers that contain different percentages of IMPCs and evaluated the prognostic significance of IMPCs.

2. Materials and methods

2.1. Patients

This study recruited 363 colorectal cancer patients consecutively, including 150 women and 213 men, who had all been treated surgically as the initial treatment at the Second Affiliated Hospital of Zhejiang University School of Medicine between January 2013 and December 2016. Patients with pathological stages I-III underwent curative resection, and primary tumor resection was performed for patients with at least pathological stage IV cancers. All clinical data were collected from a retrospective database. All samples were pathologically diagnosed as CRC. The evaluated clinicopathological parameters of the patients included age at diagnosis, sex, tumor size, preoperative serum carcinoembryonic antigen (CEA), preoperative body mass index (BMI), tumor location, histological type, tumor differentiation, invasion depth (pT classification), lymph node metastasis (pN classification), venous invasion, perineural invasion, tumor deposits, tumor node metastasis (TNM) stage, preoperative hemorrhage or perforation or bowel obstruction (fecal occult blood test positive and abdominal computed tomography examination confirmed), preoperative metastasis, data from the most recent follow-up and survival status. Patients were staged using the eighth edition of the American Jiont Committee on Cancer (AJCC) TNM staging system.[22] Information on follow-up was provided by the patient follow-up database of the Second Affiliated Hospital of Zhejiang University School of Medicine and was conducted until May 31, 2017. This study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine.

2.2. Micropapillary component analysis

All surgically resected specimens were fixed in 10% formalin, serially cut into 5-7-mm-thick slices, and embedded in paraffin. There were a total of 1878 paraffin-embedded tissue blocks. The presence of IMPCs was pathologically evaluated according to hematoxylin and eosin (H&E) and immunohistochemical analysis. IMPCs were found in 267 paraffin-embedded tissue blocks and the corresponding tissue blocks were sampled. The proportion of the IMPCs in the entire tumor was recorded for each case. To exclude poorly differentiated clusters (PDCs), the histopathological micropapillary features were characterized by tight neoplastic cell clusters with papillary morphology surrounded by cleft-like spaces. The tumor nests exhibit reverse polarity with an outer common border. The tumor cells had eosinophilic cytoplasm and pleomorphic nuclei (Fig. 1A), as previously described.[7,9,10,20] They were often observed at the invasive front of the tumor.[10,14,19,20] Then PDCs are composed of $\geq$5 cancer cells lacking any glandular differentiation, which are located in the stroma and found at the invasive margin and within the tumor (Fig. 1B).[23] PDCs surrounded by lacunar spaces display a morphological similarities with micropapillary component, but the spaces are less prominent.[24,25] A reversed pattern of MUC1 and EMA expression displaying focal and partial were also observed in PDCs, but not always identified.[25,26] The pathologic parameters were blindly and independently evaluated by 2 pathologists. In case of a discrepancy, a diagnostic consensus was reached by both pathologists at a multiheaded microscope to review the slides again.

Immunohistochemical staining for all markers, including EMA (GP1.4 clone; ZM-0095; ZSGB-BIO, Beijing, China; dilution 1:200), MUC1 (EP85 clone; ZA-0656; ZSGB-BIO, Beijing, China; dilution 1:200), MLH1 (ES05 clone; ZM-0154; ZSGB-BIO, Beijing, China; dilution 1:50), MSH2 (RED2 clone; ZA-0622; ZSGB-BIO, Beijing, China; dilution 1:100), MSH6 (EP49 clone; ZA-0541; ZSGB-BIO, Beijing, China; dilution 1:200), and PMS2 (EP51 clone; ZA-0542; ZSGB-BIO, Beijing, China; dilution 1:50), were performed on the arrayed blocks containing IMPC and non-IMPC samples. All immunostainings were performed in the Benchmark XT automatic immunostaining device (Ventana Medical System, Tucson, Ariz) using formalin-fixed, paraffin-embedded tissue sections. Four-micrometer-thick sections were obtained by microtome, transferred onto adhesive slides, and dried at 62°C for 30 minutes.

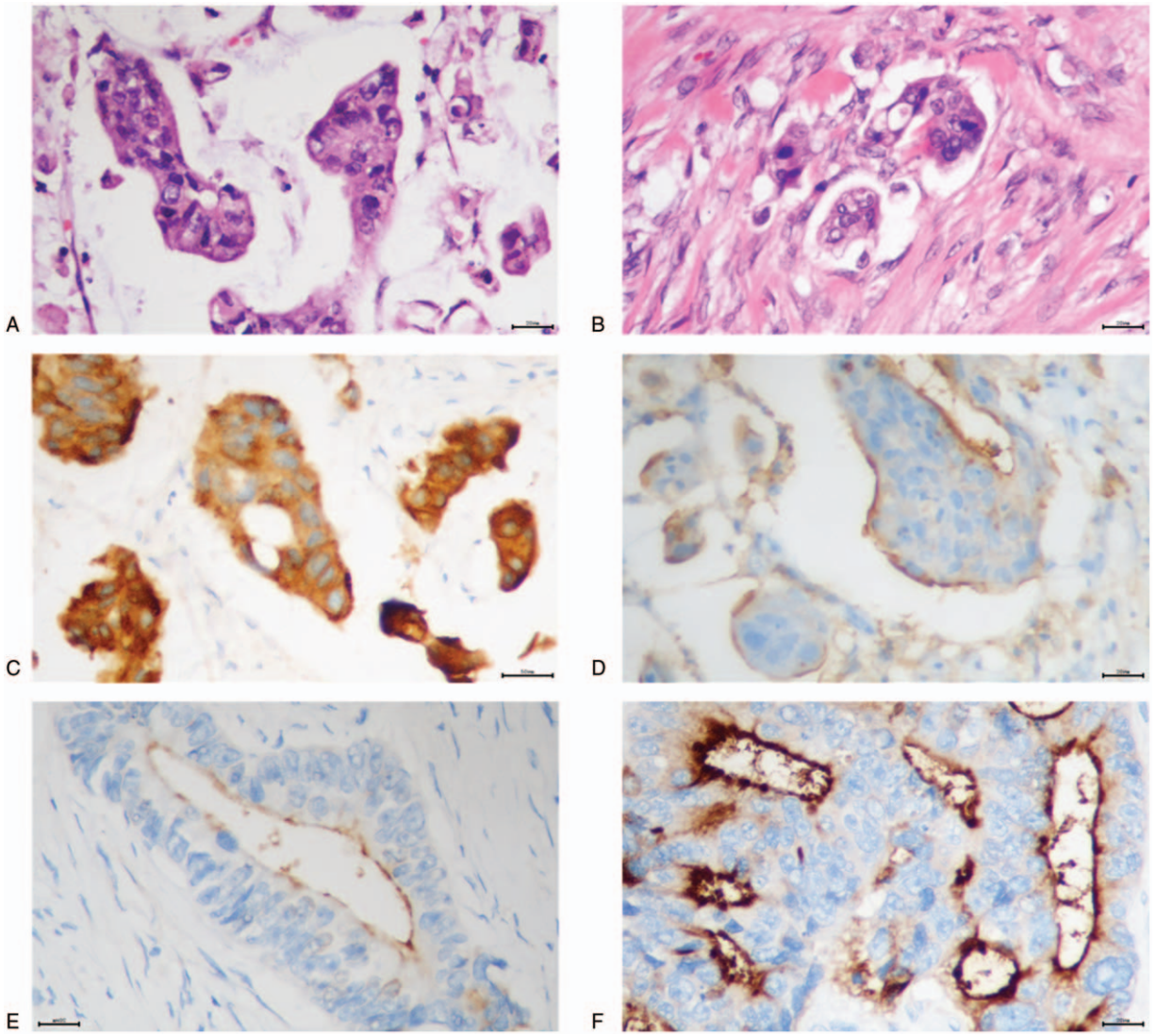

Figure 1. Tight neoplastic cell clusters are surrounded by cleft-like spaces and show eosinophilic cytoplasm and pleomorphic nuclei in the IMPCs (A) (H&E: 400×). PDCs in colorectal cancer are composed of ≥5 cancer cells lacking any glandular differentiation (B) (H&E: 400×). EMA (C)/MUC1 (D) expression is present on the stroma-facing (basal) surface of the neoplastic cell clusters, indicating an inside-out pattern in the IMPCs (400×). EMA (E)/MUC1 (F) immunoreactivity is expressed in the luminal regions in the non-IMPCs (400×).

2.3. Statistical analysis

Continuous variables were reported as a median with interquartile range when the data distribution was skewed and then compared between different groups using the A comparison of means was performed using the unpaired Student *t* test or the Mann–Whitney test. Comparisons between categorical variables were assessed using Pearson Chi-Squared tests or Fisher exact test as appropriate (two-tailed). Logistic regression analysis was performed to identify factors associated with lymph node involvement. Survival curves were generated using the Kaplan–Meier method and statistically compared using the log-rank test. A multivariate analysis was performed using the Cox proportional hazard model to evaluate significant survival predictors. All data for the included patients were retrospectively analyzed. The data were analyzed using SPSS Statistics 22.0 software (IBM, Armonk, NY, USA). A two-tailed $P < .05$ was defined as the threshold of significance.

3. Results

3.1. Clinicopathological characteristics of IMPCs and non-IMPCs

Out of 363 patients, 76 (20.9%) were found with IMPCs, and 287 patients were conventional adenocarcinomas (named non-IMPCs in this study). The clinicopathological findings of tumors with IMPCs and non-IMPCs are compared in Table 1.

The average preoperative CEA was 24.33 ± 63.29 ng/ml for patients with IMPCs and 23.01 ± 32.55 ng/ml for patients with non-IMPCs ($P = .002$). Significantly more patients suffered preoperative bowel obstruction ($P = .006$) in the IMPC group compared with the non-IMPC group. Seventeen IMPCs (22.4%) suffered preoperative distant metastases (liver, n = 8; peritoneum, n = 3; lung, n = 3; paraaortic lymph, n = 2; bone, n = 1), and 37 non-IMPCs (12.9%) suffered distant metastases before surgery ($P = .046$). For histological grade, poor differentiation accounts for 32.9% in IMPCs and 8.4% in non-IMPCs ($P = .000$). More

Table 1

Clinicopathological characteristic of patients with and without IMPCs.

Variable	IMPCs (n = 76)	Non-IPMCs (n = 287)	*P*
Sex (male:female)	49:27	164:123	.295
Age (years)	64.63 ± 12.17	64.77 ± 12.77	.931
Tumor size (cm)	4.27 ± 3.78	4.11 ± 1.58	.573
Preoperative CEA (ng/ml)	24.33 ± 63.29	23.01 ± 32.55	.002
Preoperative BMI (kg/m^2)	22.84 ± 3.37	23.01 ± 3.27	.725
Location (%)			.394
Right colon	21 (27.6)	63 (22.0)	
Left colon	26 (34.2)	121 (42.2)	
Rectum	29 (38.2)	103 (35.9)	
Histological type (%)			.123
Ulcerative	58 (76.3)	188 (65.5)	
Uplifting	16 (21.1)	94 (32.8)	
Infiltrating	2 (2.6)	5 (1.7)	
Tumor differentiation (%)			.000
Poor	25 (32.9)	24 (8.4)	
Moderate	46 (60.5)	219 (76.3)	
High	5 (6.6)	44 (15.3)	
pT classification (%)			.628
T1-2	13 (17.1)	59 (20.6)	
T3-4	63 (82.9)	228 (79.4)	
pN classification (%)			.000
N0	27 (35.5)	174 (60.6)	
N1	23 (30.3)	77 (26.8)	
N2	26 (34.2)	36 (12.6)	
Venous invasion (%)	42 (55.3)	114 (39.7)	.019
Perineural invasion (%)	23 (30.3)	52 (18.1)	.025
Tumor deposits (%)	7 (9.2)	36 (12.5)	.550
AJCC TNM stage (%)			.000
I-II	23 (30.3)	160 (55.7)	
III-IV	53 (69.7)	127 (44.3)	
Preoperative hemorrhage (%)	36/61 (59.0)	136/262 (51.9)	.323
Perforation (%)	1/62 (1.6)	1/262 (0.4)	.347
Bowel obstruction (%)	18 (23.7)	35 (12.2)	.006
Preoperative metastasis (%)	17 (22.4)	37 (12.9)	.046
Mismatch repair deficiency (%)	2/42 (4.7)	21/142 (14.7)	.144
Death case (%)	23 (30.3)	26 (9.1)	.000
Overall survival (month)	14.14 ± 13.24	25.48 ± 10.79	.002

AJCC = American Joint Committee on Cancer, BMI = body mass index, CEA = carcinoembryonic antigen, IMPCs = invasive micropapillary components, Non-IPMCs = conventional adenocarcinoma, pN = pathological node status, pT = pathological invasion level, TNM = tumor node metastasis.

Table 2

Clinicopathological characteristic of patients with IMPCs.

Variable	IMPCs-L (≤5%) (n = 22)	IMPCs-H (>5%) (n = 54)	*P*
Sex (male:female)	12:10	37:17	.295
Age (years)	62.83 ± 11.32	64.79 ± 12.31	.709
Tumor size (cm)	2.92 ± 0.92	4.39 ± 3.9	.022
Preoperative CEA (ng/ml)	7.97 ± 8.45	25.84 ± 65.95	.072
Preoperative BMI (kg/m^2)	21.96 ± 2.29	22.93 ± 3.46	.426
Location (%)			.188
Right colon	8 (36.4)	13 (24.1)	
Left colon	9 (40.9)	17 (31.5)	
Rectum	5 (22.7)	24 (44.4)	
Histological type (%)			.446
Ulcerative	18 (81.8)	40 (74.1)	
Uplifting	4 (18.2)	12 (22.2)	
Infiltrating	0 (0)	2 (3.7)	
Tumor differentiation (%)			.031
Poor	3 (13.6)	22 (40.7)	
Moderate	16 (72.7)	30 (55.6)	
High	3 (13.6)	2 (3.7)	
pT classification (%)			.602
T1-2	3 (13.6)	10 (18.5)	
T3-4	19 (86.4)	44 (81.5)	
pN classification (%)			.302
N0	8 (36.4)	19 (35.2)	
N1	9 (40.9)	14 (25.9)	
N2	5 (22.7)	21 (38.9)	
Venous invasion (%)	11 (50.0)	31 (57.4)	.616
Perineural invasion (%)	6 (27.3)	16 (21.0)	.717
Tumor deposits (%)	2 (9.1)	5 (9.3)	1.000
AJCC TNM stage (%)			1.000
I-II	7 (31.8)	16 (29.6)	
III-IV	15 (68.2)	38 (70.4)	
Preoperative hemorrhage (%)	8 (36.4)	28 (51.9)	.461
Perforation (%)	0 (0)	1 (1.9)	.601
Bowel obstruction (%)	7 (31.8)	11 (20.4)	.469
Preoperative metastasis (%)	4 (18.2)	12 (22.2)	.767
Death case (%)	4 (18.2)	19 (35.2)	.295
Overall survival (month)	26.92 ± 15.14	11.45 ± 11.48	.030

AJCC = American Joint Committee on Cancer, BMI = body mass index, CEA = carcinoembryonic antigen, IMPCs = invasive micropapillary components, IMPCs-H = IMPCs-high, >5%, IMPCs-L = IMPCs-less, ≤5%, pN = pathological node status, pT = pathological invasion level, TNM = tumor node metastasis.

patients were infected with lymph node metastasis in the IMPC group than in the non-IMPC group (64.5% vs 39.4%, $P = .000$). Venous invasion was observed in 55.3% of IMPC patients and 39.7% of non-IMPC patients ($P = .019$). Perineural invasion was observed in 30.3% of IMPC patients and in only 18.1% of non-IMPC patients ($P = .025$). Carcinoma with IMPCs compared with non-IMPCs revealed a higher percentage of AJCC TNM stage III and IV (69.7% vs 44.3%, $P = .000$).

There was no significant difference in age, sex, tumor size, preoperative BMI, tumor location, histological type, pT classification, tumor deposits, preoperative hemorrhage, and perforation between the 2 groups ($P > .05$).

3.2. Clinicopathological characteristics of IMPCs-H and IMPCs-L

Micropapillary components ranged from 2% to 90% of the tumor area in histologic sections. Cases with micropapillary components comprising more than 5% of the tumor were grouped as IMPCs-high (>5%,IMPCs-H) (n = 54), and the remaining was grouped as IMPCs-less (≤5%, IMPCs-L) (n = 22). Clinicopathological findings of tumors with IMPCs-L or IMPCs-H are compared in Table 2.

For patients with IMPCs-H, the tumor size was larger than that of patients with IMPCs-L, 4.39 ± 3.9 cm vs. 2.92 ± 0.92 cm ($P = .022$). In addition, carcinoma with IMPCs-H had a higher percentage of poor-differentiation tumors, 40.7% vs 13.6% ($P = .031$). There was no difference in pN classification between the 2 groups ($P = .302$), showing an equal trend in lymph invasion. No other difference was found regarding other clinicopathological characteristics between these 2 groups, $P > .05$.

3.3. Immunohistochemical evaluation

In patients with IMPCs, EMA/MUC1 expressions were strongly and diffusely present on the stroma-facing (basal) surface of the

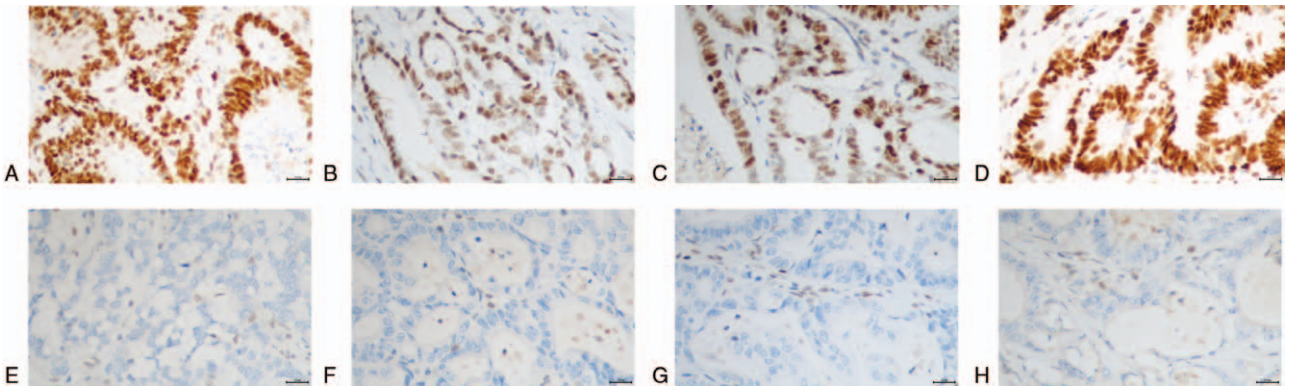

Figure 2. Positive nuclear staining of tumor cells for MLH1 (A), MSH2 (B), MSH6 (C) and PMS2 (D) (400×). No nuclear staining of tumor cells for MLH1 (E), MSH2 (F), MSH6 (G) and PMS2 (H) (400×).

cancer cell clusters, indicating an inside-out pattern (Fig. 1C-D). In conventional adenocarcinoma cells, EMA/MUC1 was expressed in the luminal regions (Fig. 1E-F).

A total of 184 carcinomas (42 cases with IMPCs, 142 cases with non-IMPCs) underwent immunohistochemical evaluation to test mismatch repair (MMR) status. MMR deficiency (dMMR) was defined as loss of expression (no nuclear staining of tumor cells) for at least one of MLH1, MSH2, MSH6, and PMS2,[27] and retained MLH1, MSH2, MSH6 and PMS2 expression (convincing any nuclear staining of tumor cells) was regarded as MMR proficient (pMMR) (Fig. 2A-H). For patients with IMPCs, 2 cases were detected as dMMR and 40 cases were detected as pMMR. For patients with non-IMPCs, 21 cases were detected as dMMR, and the remaining 121 cases were pMMR. The percentage of dMMR in the non-IMPC group was much higher than that in the IMPC group, but it was no significant difference (14.7% vs 4.7%, $P=.144$, Table 1).

3.4. Factors associated with lymph node metastasis

The results of the logistic regression analysis demonstrated that when compared to patients with non-IMPCs, patients with IMPCs are associated with lymphatic invasion, regardless of the proportion of IMPCs. Moreover, right colon carcinoma, poor differentiation, T3 classification, T4 classification, venous invasion, and distant metastasis were more likely to result in lymph node metastasis (Table 3).

Table 3

Logistic regression analysis of factors associated with lymph node metastasis.

Variable	OR	95%CI	*P*
Venous invasion	1.819	1.123–2.944	.015
pT classification			
T3	3.387	1.026–11.184	.045
T4	4.001	1.040–15.390	.044
Right colon	0.439	0.226–0.856	.016
Poor differentiation	2.653	1.223–5.757	.014
Distant metastasis	2.058	1.036–4.001	.045
IMPCs			
≤5%	3.272	1.243–8.616	.016
>5%	2.260	1.102–4.637	.026

CI = confidence interval, IMPCs = invasive micropapillary components, OR = odds ratio, pT = pathological invasion level.

3.5. Survival analysis

The median follow-up duration for the 363 patients was 27.6 months (range: 1–60 mo). 30.3% of patients with IMPCs died in the follow-up duration, and 9.1% of patients with non-IMPCs died during the follow-up period ($P=.000$). The overall survival (OS) time of patients with IMPCs was significantly less than that of patients with non-IMPCs, 14.14 ± 13.24 months vs 25.48 ± 10.79 months, $P=.002$ (Table 1, Fig. 3). For patients with IMPCs, 18.2% of IMPCs-L group died during the follow-up, and 35.2% of IMPCs-H group died in the follow-up duration, $P=.295$. The overall survival (OS) time of patients with IMPCs-H was less than that of patients with IMPCs-L, 11.45 ± 11.48 months vs 26.92 ± 15.14 months, $P=.030$, Table 2.

3.6. Prognostic factors for survival

Multivariate Cox proportional hazard regression analysis identified some independent factors associated with survival. A

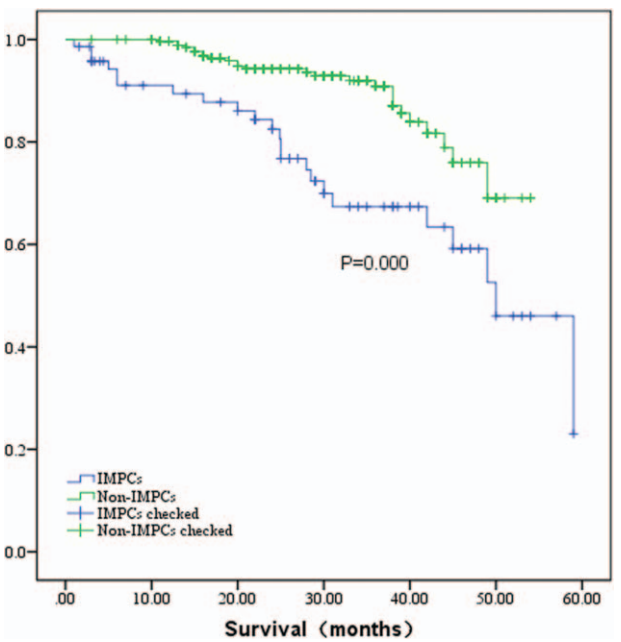

Figure 3. Kaplan–Meier curves show overall survival of patients with IMPCs and non-IMPCs.

Table 4

Multivariate analysis for prognostic factors associated with overall survival.

Variable	HR	95%CI	P
Age (years)			
≤65	1.000		
>65	2.684	1.266–5.690	.010
pT classification			
T1	1.000		
T2	0.266	0.022–3.190	.296
T3	1.256	0.164–9.588	.826
T4	1.184	0.144–9.758	.875
Location			
Right colon	1.368	0.635–2.949	.424
Left colon	0.628	0.288–1.367	.241
Rectum	1.000		
Distant metastasis			
No	1.000		
Yes	10.442	5.078–21.472	.000
Perineural invasion			
No	1.000		
Yes	1.979	1.057–3.706	.033
Preoperative CEA (ng/ml)			
≤5	1.000		
>5	2.282	1.373–3.794	.001
IMPCs			
0	1.000		
≤5%	0.398	0.146–1.085	.045
>5%	7.972	0.731–5.302	.037

1.000 = reference level, CEA = carcinoembryonic antigen, CI = confidence interval, HR = hazard ratio, IMPCs = invasive micropapillary components, pT = pathological invasion level.

higher risk of death was associated with IMPCs, age >65 years, distant metastasis, perineural invasion, preoperative CEA >5 ng/ml (Table 4). The micropapillary component (proportion more than 5% or not) was an independent prognostic factor for CRC.

4. Discussion

In recent years, IMPCs have received increasing attention, but there are few reports about IMPCs in CRC. There are 9 large case series,[9–16,20] and 14 case reports[7,17–19,28–37] on CRC with IMPCs in English papers, and more than 300 cases have been reported thus far. Our research found that 20.9% of cases contain IMPCs, the incidence rate much higher than previous reports (other studies have reported an incidence of 4%[16] to 19%[10]). This difference is mainly due to the presence of IMPCs as criteria in our research.

Compared with the non-IMPC group, the IMPC group had a later N classification and a higher lymph node metastasis rate ($P=.000$), which was consistent with most previous reports.[9–16] However, Katarzyna et al[20] reported that the lymph node metastasis rate and number of the IMPC group were higher than those of the non-IMPC group, but this difference was not statistically significant ($P=.087$, $P=.094$). The total number of IMPC cases in this study was small, with only 5 cases. Therefore, the IMPC group had a stronger lymph node invasion in general. The higher the content of IMPCs was, the higher the rate of lymph node metastasis was, and this result was statistically significant ($P=8.49*10^{-9}$, $P<.0001$) according to Verdú et al.[12] and Lee et al.[15] Neither of the 2 studies included cases of IMPCs <5%. Therefore, we further conducted research on the IMPC group. Compared with the IMPC-L group (≤5%), the IMPC-H group (>5%) had both a higher N2 ratio and lymph node metastasis rate, with no significant difference ($P=.302$). The appearance of IMPCs is more prone to lymph node metastasis, regardless of its proportion, which is also verified through logistic regression analysis of factors associated with lymph node metastasis. We believe that the presence of IMPCs is more important than its proportion, which also reminds pathologists to report the presence of IMPCs. In addition, we were surprised to find that IMPCs-L implies a higher risk for lymph node metastasis than IMPCs-H (OR 3.272 vs 2.260). The reason may be that there is not enough sample size (only 22 IMPC-L cases), which leads to data deviation. So we need to expand the sample size for further research.

IMPCs often predict a poor prognosis. Multivariate analysis showed that the IMPC was an independent predictor of the poor prognosis. The OS of IMPCs was significantly lower than that of non-IMPCs, and the OS in the IMPC-H group was lower than that of the IMPC-L group, indicating that the higher the content of IMPCs was, the worse the prognosis was Lee et al[15] also found that the IMPC was an independent poor prognostic factor. In contrast, Xu et al[11] indicated that in the subgroup analysis, only patients with stage I-II carcinoma in the IMPC group had a lower 5-year survival rate than that of those with conventional adenocarcinoma ($P<.0001$), and there was no significant difference between the 2 groups of patients with stage III-IV carcinoma ($P=.7223$). Studies by Silva et al[13] showed that the 3-year survival of patients with IMPCs was significantly lower than that of the conventional adenocarcinoma group in stage III-IV colonic cancer (86.7% vs 93.3%, $P=.035$), but in multivariate analysis, the IMPC was not a predictor ($P=.927$). Raul et al[16] found that patients with colorectal cancer containing IMPCs usually had later AJCC TNM stage but a similar prognosis compared with the conventional adenocarcinoma group. The differences in the above research require us to have longer follow-up studies, larger sample sizes, and more comprehensive statistical analysis.

We selected EMA and MUC1 as immunohistochemical staining markers and confirmed that the cancer cells in the IMPCs showed a significant "inside-out" pattern, as reported in previous studies.[7,12–14] The remaining tumor components showed a classic luminal staining pattern. This pattern led to characteristic intercellular fissures around the tumor nest in IMPCs, with invasive potential. Furthermore, Raul et al[16] found the focally loss of membranous E-cadherin expression was in most of their cases. These also were found in the PDCs foci.[24,25] IMPCs and PDCs may reflect the loss of epithelial properties in tumor cells and possibly indicate the epithelial–mesenchymal transition (EMT),[16,24,25] which may be the potential mechanism for lymph node metastasis and other undesirable histological parameters.

By MMR immunohistochemistry test, we also concluded that the incidence of dMMR was lower (4.7%) in the IMPC group. Previously, Raul et al[16] found that 21 cases of colorectal cancer with IMPCs were also MMR, while the proportion of colorectal cancer without IMPCs was 82/294 (28%) ($P=.003$). Verdú et al[12] also found that the incidence of dMMR was lower, and cases with a high frequency of dMMR were rare, and few RER phenotypes occurred in CRC with IMPCs, suggesting that the presence of micropapillary carcinoma in tumors was related to the classical pathogenesis of chromosomal instability and that the prognosis of colorectal cancer with micropapillary carcinoma was poor. Moreover, Katarzyna et al[20] found that colorectal

cancer with IMPCs was not significantly associated with the presence of inflammatory infiltration in the invasive front of the tumor ($P = .098$), which was also consistent with the fact that dMMR was rare in the IMPC group in our study.

Additionally, we also found that the tumor differentiation of the IMPC group was worse, mainly moderate-poor differentiation, while the IMPC-H group mainly showed poor differentiation, accounting for 40.7%. Therefore, we believe that the higher the proportion of IMPCs is the worse the tumor differentiation is, which is also a manifestation of the high degree of malignancy of CRC with IMPCs. At the same time, the venous invasion and perineural invasion rate was significantly higher than that of the non-IMPC group, and the AJCC TNM stage was later and the preoperative metastasis was more, all of which were also factors of the poor prognosis of the IMPC group. In addition, there were more patients with preoperative bowel obstruction in the IMPC group, which was statistically significant, suggesting that we should pay more attention to the detection of IMPCs in these patients.

5. Conclusion

In summary, IMPCs are highly invasive, with high lymph node metastasis rates and poor prognosis. Reporting on CRC with IMPCs is very important, although there is just a minor component ($\leq 5\%$). And that poor prognosis may not depend on the proportion but on the presence of this component. Now our study cannot provide the data on tumor buddings. It is worthy of further study of the association between IMPCs, PDCs and tumor buddings. And, the main limitation of this study is its retrospective nature and short follow-up time. The influence of IMPCs on prognosis requires long-term follow-up, and their molecular formation mechanisms and tumor treatment strategies still require further research.

Author contributions

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