Clinical Impact of Poorly Differentiated Cluster at the Invasive Front in Colorectal Cancer Invading beyond the Muscle Layer

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Abstract
Introduction: The clinicopathological significance of poorly differentiated cluster (PDC) at the invasive front in colorectal cancer (CRC) has been reported. We analyzed whether PDC reflects malignant findings in patients with CRC invading beyond the muscle layer.

Patients and methods: Sixty-eight patients who underwent surgery between January 2015 and June 2016 for CRC invading beyond the T3 (median observation period: 32.2 months) were enrolled. The relationship between PDC and clinicopathological factors was analyzed. PDC was graded based on the criteria described in a report by Ueno H et al.

Results: Tumor location was at the proximal colon in 26 cases, distal colon in 34 cases, and rectum in eight cases. The number of cases with ly2,3 and v2,3 was 24 and 38, respectively. Thirty-eight cases had node positive and 11 cases had distant metastases, including 10 cases with hemogenous metastasis and four cases with peritoneal metastasis. The number of cases with stages II, III, and IV was 28, 28, and 12, respectively. The number of cases with PDC grades 1 (G1), 2 (G2), and 3 (G3) was 48, 15, and 5, respectively. A PDC G2 or G3 is a risk factor for lymph node and distant metastases. Cases with PDC G2 or G3 had significantly poor overall survival (OS) (p < 0.0001). In cases with curability (cur) A resection for stage II or III, disease-free survival (DFS) and OS were significantly poorer in cases with PDC G2 or G3 (p = 0.0022 and p = 0.0049, respectively).

Conclusion: Analyses concerning PDC at the invasive front in cases with CRC invading beyond the muscle layer were performed. As the stage progresses, cases with PDC G2 and G3 increased significantly. In cases with PDC G2 and G3, the DFS and OS were significantly poorer. These results suggest that PDC is a malignant predictor in patients with CRC invading the T3 or deeper.

Keywords: poorly differentiated cluster, malignant predictor, colorectal cancer

Histological grading has been used to assess the malignant potential of CRC. The most widely accepted histological grading is based on the degree of tumor differentiation³. Recently, Ueno et al. reported that the grading of poorly differentiated clusters (PDCs), which are defined as tumor cells without glandular formation in the invasive front reflected in the prognosis. PDCs were classified as grade 1 (G1), grade 2 (G2), and grade (G3) based on their count⁴. The prognosis of patients with CRC with PDC G3 is more unfavorable than those with PDC G1 or G2⁵-⁹.

In this study, we classified patients who underwent resection of CRC tumors invading the T3 or deeper into their respective PDC grades to investigate the clinical significance. Here, we report the importance of PDCs in clinical practice for patients with CRC tumors invading beyond the T3.
Patients and Methods

Patients
One hundred ten patients with CRC who consecutively underwent surgery at Saiseikai Kurihashi Hospital between January 2015 and June 2016 were enrolled as a cohort for this study. From this cohort, 68 patients were chosen with a pathological diagnosis of T3 or deeper tumor invasion. Clinicopathological findings were described according to the Japanese Classification of Colorectal Carcinoma.  

Definition of PDC
PDCs are cancerous clusters composed of five or more cells without glandular formation. To assess the grading of PDCs, the most frequent area of PDCs was identified using a low-power magnification view. The number of PDCs was counted using an X20 objective lens. PDCs were classified according to Ueno’s criteria. In brief, tumors with <5, 5–9, and >10 PDCs were defined as G1, G2, and G3, respectively. The grading of PDCs in this cohort was judged by one investigator (K.Y.) who was blinded to all clinical information.

Statistical analyses
Statistical analyses were performed using JMP Pro (version 13; SAS institute Inc., Cary, NC, USA). The relationship between PDCs and clinicopathological findings was analyzed using the chi-squared test and Fisher’s exact test. The overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan–Meier method. Significant differences were assessed using the log-rank test. P values less than 0.05 were considered statistically significant.

Ethical approval
The protocol of this retrospective study was assessed and approved by the institutional review board of Saiseikai Kurihashi Hospital (approval no. 79-6).

Results

Patient’s demographics (Table 1)
In this study, the median age of the study cohort was 68.5 (range, 40–90 years). Fifty-three were men and 15 were women. The primary tumor location was at the proximal colon in 26 cases, distal colon in 34 cases, and rectum in eight cases. Lymph node metastasis was observed in 38 cases, including 24 cases with ly 2, 3 and 38 cases with v 2, 3. Regarding stage distribution, 28 patients had stage II, 19 had stage IIIa, nine had stage IIIb, and 11 had stage IV, including 12 cases with hematological metastasis and four cases with peritoneal metastasis. The median number of PDCs was 3. Regarding PDC grading, 48 cases had G1, 15 cases had G2, and 5 cases had G3.

Relationship between PDC grade and clinicopathological factors (Table 2)
Lymph node metastasis including N1, N2, and N3 was significantly observed more in cases with PDC G2 or G3 (p = 0.0046). Moreover, highly lymphatic and venous invasion were significantly found more in cases with PDC G2 or G3 (p = 0.0022 and p = 0.0206, respectively). The significant relationship between histology and PDC grade was also elucidated. Additionally, distant metastasis significantly occurred more in cases with PDC G2 or G3 (p = 0.0029). Therefore, PDC grade significantly increased as the stage progressed (p = 0.0006).

Prognosis and PDC grade
The OS in 68 cases was significantly divided between the cases with PDC G1, G2, and G3 (p < 0.0001) (Fig. 1a, 1b).

Fig. 1 No poorly differentiated clusters (PDCs) are found in (a). It is assessed as PDC G1. More than 10 PDCs (arrows) are detected in (b), and they are classified as PDC G3.
As for the curative cases in stages II and III, the DFS and OS in cases with PDC G2 and G3 were significantly poorer than those in cases with PDC G1 ($p = 0.0022$ and $p = 0.0049$, respectively) (Fig. 2b, 2c).

**Discussion**

PDCs are often observed in the center of a tumor; however, their molecular characteristics are different from the PDCs at the invasive front\(^1\). Bertoni L et al. reported that the PDCs at the invasive front of CRC showed a similar expression pattern of two epithelial-mesenchymal-transition (EMT)-related proteins\(^1\). EMT is an early stage driver of the cancer metastasis pathway including cell migration and lymphovascular invasion\(^1\). Therefore, we focused on the PDCs at the invasive front of CRC tumors invading the T3 or deeper to investigate their impact on such CRC in which we should treat.

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Table 2 Relation between PDC grade and clinicopathological factors

|                | G1 | G2 | G3 |
|----------------|----|----|----|
| Lymph node metastasis\(^1\) | N0 | 26 | 4  | 0  |
|                | N1 | 14 | 3  | 4  |
|                | N2, N3 | 8 | 8  | 1  | $p=0.0046$ |
| Lymphatic invasion\(^2\) | ly0, ly1 | 36 | 8  | 0  |
|                | ly2, ly3 | 12 | 7  | 5  | $p=0.0022$ |
| Venous invasion\(^2\) | v0, v1 | 26 | 4  | 0  |
|                | v2, v3 | 22 | 11 | 5  | $p=0.0206$ |
| Histology\(^2\) | tub1, tub2 | 46 | 14 | 3  |
|                | pol, muc | 2  | 1  | 2  | $p=0.0139$ |
| Distant metastasis\(^2\) | M0 | 44 | 10 | 3  |
|                | M1a | 4  | 2  | 0  |
|                | M1b | 0  | 3  | 2  | $p=0.0029$ |
| Peritoneal metastasis | No | 48 | 12 | 4  |
|                | Yes | 0  | 3  | 1  | $p=0.0061$ |
| Hematological metastasis | No | 44 | 11 | 3  |
|                | Yes | 4  | 4  | 2  | $p=0.0546$ |
| Stage\(^2\) | II | 23 | 3  | 0  |
|                | IIIa | 15 | 1  | 3  |
|                | IIIb | 4  | 5  | 0  |
|                | IV | 4  | 6  | 2  | $p=0.0006$ |

: according to Japanese Classification of Colorectal Carcinoma

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2a). As for the curative cases in stages II and III, the DFS and OS in cases with PDC G2 and G3 were significantly poorer than those in cases with PDC G1 ($p = 0.0022$ and $p = 0.0049$, respectively) (Fig. 2b, 2c).

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Fig. 2 The overall survival (OS) curves of poorly differentiated cluster (PDC) G1 and PDC G2 and G3 in 68 cases with CRC tumors invading beyond T3 (a). The disease-free survival (DFS) curves (b) and OS curves (c) of cases with PDC G1, G2, and G3 among patients with stages II and III who underwent cur A resection.
Clinicopathological parameters were assessed relative to the PDC grade. Lymph node metastasis and PDC grade were significantly correlated. Additionally, the number of cases with PDC G2 or G3 increased as the grade of lymph node metastasis progressed. Among the cases with a highly lymphatic invasion, the number of cases with PDC G2 or G3 increased. Similar results were reported using cohorts consisting of 239 patients with pT2–T3 CRC\(^\text{[11]}\) and pT1 CRC\(^\text{[12]}\).

Among the cases with distant metastasis including both peritoneal and hematological metastasis, the number of cases with PDC G2 or G3 increased among cases with highly venous invasion. To metastasize to different organs, tumor cells should move and invade lymphatic or vascular vessels. Therefore, transformation into small clusters shaped like spheroids is needed. This phenomenon reflects the morphological change of EMT because a key event in promoting stationary tumor cells to migrate and invade is the EMT program\(^\text{[12]}\). These results suggest that PDC represents a morphologically hallmark EMT\(^\text{[14]}\).

Moreover, the prognostic value of the PDC grades was assessed. In the total cohort of this study, the OS in cases with PDC G2 or G3 was significantly worse than that in those with PDC G1. In the curative cases including stages II and III, the DFS and OS in cases with PDC G2 or G3 were also significantly worse. These results confirm that the number of PDCs is a prognostic factor.

In conclusion, the number of cases with PDC G2 and G3 significantly increased as the stage progressed. In cases with PDC G2 and G3, the DFS and OS were significantly poor. Our reproducible results indicate that the number of PDCs at the invasive front of CRC has great clinical efficacy.

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