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Original

Relationship between Poorly Differentiated Clusters and Clinicopathological Factors in Colorectal Cancer

Takuzo HASHIMOTO[1], Michio ITABASHI[1,*], Shinpei OGAWA[1], Tomoichiro HIROSAWA[1], Yoshiko BAMBA[1], Sanae KAJI[1], Satoru SHIMIZU[2], Noriyuki SHIBATA[3] and Shingo KAMEOKA[1]

[1]Department of Surgery II, Tokyo Women's Medical University
[2]Tokyo Women's Medical University Medical Research Institute
[3]Department of Pathology, Tokyo Women's Medical University
*Corresponding author

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We investigated the expression of poorly differentiated clusters (PDC) in 330 cases of colorectal cancer patients who underwent Curability A resection. PDC are defined as clusters consisting of five or more cancer cells with no gland formation. We counted the number of PDC, defined Grade 1 as having fewer than 4, Grade 2 (5～9), and Grade 3 (more than 10). The PDC Grade tended to increase with stage progression. There was a significant difference in the disease-free 5-year survival between PDC Grades 1 and 2 and PDC Grade 3. There was a significant relationship between PDC and invasive growth pattern (INF). We investigated the disease-free 5-year survival based on PDC appearance. In the lymph nodes metastasis positive group, there were no significant differences between PDC Grades 1 and 2 and PDC Grade 3, however, in the lymph nodes metastasis negative group, there were significant differences between PDC Grades 1 and 2 and PDC Grade 3. In Stage II, PDC Grades 1 and 2 in depth of tumor invasion T3 had a tendency to have good prognosis than any PDC Grade in depth of tumor invasion T4, and PDC Grade 3 in depth of tumor invasion T3.

Key Words: clinicopathological factors, colorectal cancer, poorly differentiated clusters

Introduction

Several studies[1)2)] have reported that dedifferentiation at the front of carcinoma is an important factor when determining the prognosis in colorectal cancer. However, there are currently no standard diagnostic criteria. Recently, the significance of tumor budding in prognosis has been pointed out. Budding is defined as an isolated single cancer cell or a cluster composed of fewer than 5 cancer cells at the invasive front of the tumor[1)3)]. In particular, tumor budding is said to be useful as an indicator of lymph node metastasis depending on the submucosa (SM) depth of colorectal cancer[4)]. On the other hand, poorly differentiated clusters (PDC) are defined as clusters consisting of five or more cancer cells with no gland formation[5)]. It has been reported that PDC is associated with lymph node metastasis and SM depth of the cancer as well as budding[6)], and reported that even in the case of advanced cancer with poor PDC expression, patients have had excellent prognosis[7)]. Here, we investigated the expression of PDC in colorectal cancer, in addition to examining the relationship of PDC expression and disease-free 5-year survival based on clinicopathological factors.

Patients and Methods

1. Patients

We conducted a consecutive survey of 330 cases of colorectal cancer patients who underwent Curability A resection in our institution between 2003 and 2006. According to the Japanese Classification of Colorectal Carcinoma Second English Edition[8)],

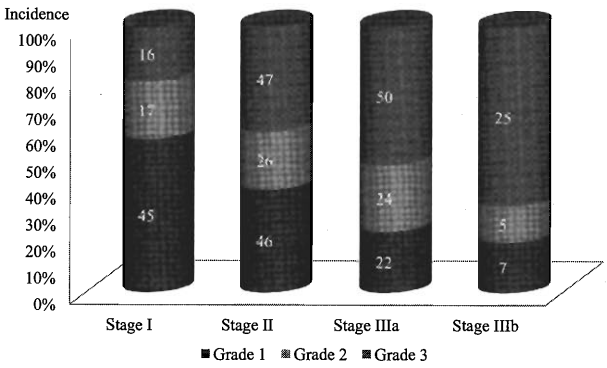

Fig. 1 PDC appearance according to Stage progression
The PDC grade tended to increase with stage progression ($p<0.0001$).

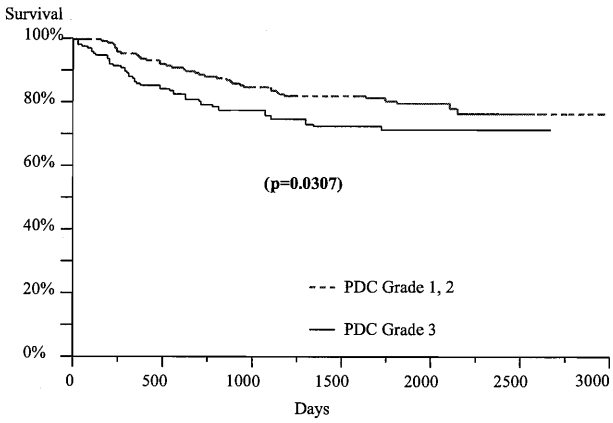

Fig. 2 PDC Grade and disease-free 5-year survival
PDC Grades 1 and 2 had significantly good prognosis than PDC Grade 3 ($p=0.0307$).

the distribution of tumor location was as follows: 212 colon, 46 rectosigmoid portion, and 72 rectum cases. The distribution according to histological staging was stage I, 78 patients; stage II, 119 cases; stage IIIa, 95 cases; stage IIIb, 37 patients.

2. PDC Grading

We examined the occurrence of PDC in the invasion front of carcinoma and the relationship between PDC and disease-free 5-year survival based on clinicopathological factors. We performed PDC grading in accordance with the 75th Japanese Society for Cancer of the Colon and Rectum (JSCCR) web site[5]. PDC are defined as clusters consisting of five or more cancer cells with no gland formation. We made no distinction about PDC existence within or outside the lymphatic and venous vessels. We excluded cancer fragments by degeneration and necrosis with inflammatory cell infiltration and cluster suspended in mucinous lake. We observed the entire tumor with cut surface typical of the tumor using a low-power microscope and selected the most intensive region. We then observed in microscopic field of 20 times an objective area. We counted the number of PDC, and defined Grade 1 as having fewer than 4, Grade 2 (5～9), and Grade 3 (more than 10).

3. Statistics

The χ^2 test, Mann–Whitney U test, and Wilcoxon test were used for statistical univariate analysis and logistic regression analysis or model and Cox's proportional hazard regression model were used for statistical multivariate analysis. A p value of less than 0.05 was taken to indicate a statistical significance (JMP® statistics system).

4. Ethical matters

This study was carried out in compliance with the Declaration of Helsinki and the Ethical Guidelines for Clinical Research (No. 459 of the Ministry of Health, Labour and Welfare Notification, July 31, 2008).

Results

1. PDC appearance according to Stage progression

For Stage I, 45 cases (57.7%) were classified as PDC Grade 1, 17 cases (21.8%) were PDC Grade 2, and 16 cases (20.5%) were PDC Grade 3. For Stage II, 46 cases (38.7%) were PDC Grade 1, 26 cases (21.9%) were PDC Grade 2, and 47 cases (39.5%) were PDC Grade 3. For Stage IIIa, 22 cases (22.9%) were PDC Grade 1, 24 cases (25.0%) were PDC Grade 2, and 50 cases (52.1%) were PDC Grade 3. For Stage IIIb, 7 cases (18.9%) were PDC Grade 1, 5 cases (13.5%) were PDC Grade 2, 25 cases (67.6%) were PDC Grade 3. The PDC Grade tended to increase with stage progression ($p<0.0001$) (Fig. 1).

2. Relationship between PDC appearance and clinicopathological factors

We examined the relationship between PDC appearance and disease-free 5-year survival. There was no significant difference in the disease-free 5-year survival between PDC Grade 1 and PDC Grade 2 and 3 ($p=0.1936$). However, PDC Grades 1

Table Relationship between PDC appearance and clinicopathological factors

		PDC Grade 1, 2	PDC Grade 3	Univariate analysis p value	Multivariate analysis p value
Recurrence	+/−	36/156	35/103	n.s	n.s
INF	ab/c	163/29	57/81	<0.0001	<0.0001
Gender	Male/Female	111/81	81/57	n.s	n.s
Age	<60 y/60 y<	53/138	36/98	n.s	n.s
CEA	Normal/Abnormal	103/49	62/46	n.s	n.s
CA19-9	Normal/Abnormal	138/12	88/19	0.0205	n.s
Location	Colon, RS/Rectum	157/35	101/37	n.s	n.s
Macroscopic type	Type 0, 1, 2/type 3, 4, 5	171/16	119/16	n.s	n.s
Circumferential rate	<2/3/subcircumference<	124/55	66/61	0.0027	n.s
Tumor size	<50 mm/50 mm<	127/61	71/60	0.0189	n.s
Histological type	Well/Moderate, poorly	99/92	43/95	0.0002	n.s
Depth of tumor invasion	T2/T3	71/121	22/116	<0.0001	n.s
Lymph node metastasis	+/−	57/133	75/63	<0.0001	n.s
Lympatic invasion	ly0/ly123	42/149	7/130	<0.0001	n.s
Venous invasion	v0/v123	161/30	92/45	0.0003	n.s

INF: invasive growth pattern, PDC: poorly differentiated clusters, RS: rectosigmoid.

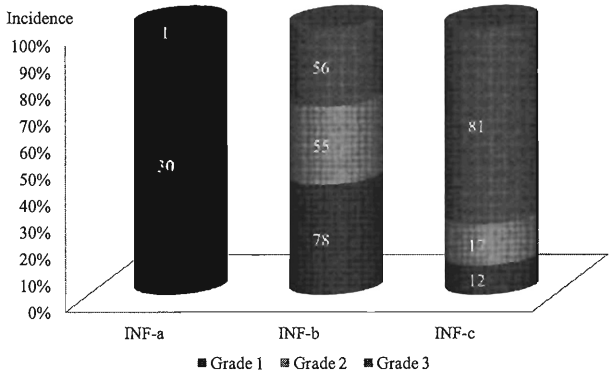

Fig. 3 Association with PDC and INF
In the INF-a, the majority of cases were PDC Grade 1 (30 cases were Grade 1 (96.8%)). In the INF-c, the majority of cases were PDC Grade 3 (81 cases were Grade 3 (73.6%)). However, in the INF-b, 55 cases were Grade 2 (29.1%). PDC Grade 2 did not necessarily coincide with the INF-b.

and 2 had significantly good prognosis than PDC Grade 3 (p = 0.0307) (Fig. 2). In addition, we examined the relationship between PDC appearance and clinicopathological factors. In univariate analysis, significant relations were found between PDC appearance and the invasive growth pattern (INF), CA19-9, circumferential rate of the tumor, tumor size, histological type, depth of tumor invasion, lymph node metastasis, lymphatic invasion, and venous invasion. In addition, a significant relationship was found in INF in logistic regression analysis (p< 0.0001) (Table).

3. Association with PDC and INF

We investigated the association between INF and PDC appearance (p< 0.0001). In the INF-a, the majority of cases were PDC Grade 1 (30 cases were Grade 1 (96.8%)). In the INF-c, the majority of cases were PDC Grade 3 (81 cases were Grade 3 (73.6%)). However, in the INF-b, 78 cases were Grade 1 (41.3%), 55 cases were Grade 2 (29.1%), and 56 cases were Grade 3 (29.7%). Therefore, PDC Grade 2 did not necessarily coincide with the INF-b (Fig. 3).

4. Disease-free 5-year survival based on PDC Grade according to clinicopathological stage

We investigated the disease-free 5-year survival based on PDC appearance. In the lymph nodes metastasis positive group, there were no significant differences between PDC Grade 1 and 2 and the PDC Grade 3 (p = 0.6145), but in the lymph nodes metastasis negative group, there were significant differences between PDC Grade 1 and 2 and the PDC Grade 3 (p = 0.0089) (Fig. 4). We then divided the lymph node metastasis negative group into Stage I and Stage II, and conducted the same investigation based on PDC appearance. In neither Stage I nor Stage II, there were no significant differences between PDC Grades 1 and 2 and PDC Grade 3 (Stage I; p = 0.2522, Stage II; p = 0.0658) (Fig. 5). In Stage II, there were no significant differences be-

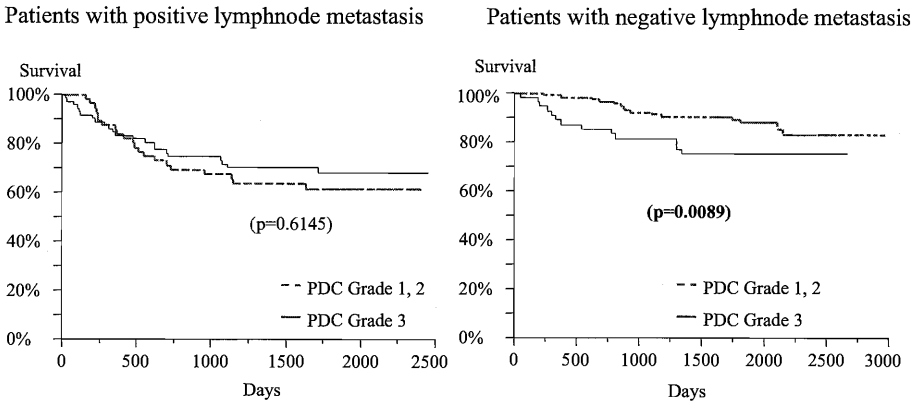

Fig. 4 Disease-free 5-year survival based on PDC Grade according to lymph nodes metastasis

In the lymph nodes metastasis positive group, there was no significant differences for disease free 5-year survival between PDC Grades 1 and 2 and the PDC Grade 3 (p = 0.6145), but in the lymph nodes metastasis negative group, there was significant differences for disease free 5-year survival between PDC Grades 1 and 2 and the PDC Grade 3 (p = 0.0089).

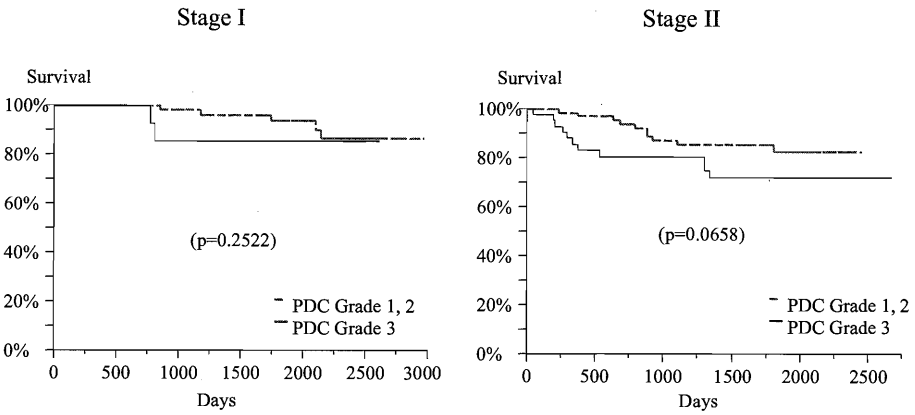

Fig. 5 Disease-free 5-year survival based on PDC Grade according to clinicopathological negative lymph node metastasis

In neither Stage I nor Stage II, there were no significant differences for disease free 5-year survival between PDC Grades 1 and 2 and PDC Grade 3 (Stage I; p = 0.2522, Stage II; p = 0.0658)

tween depth of tumor invasion T3 and T4 in PDC Grade 3 (p = 0.4161), but significant differences between depth of tumor invasion T3 and T4 in PDC Grades 1 and 2 (p = 0.0160) (Fig. 6) were found. In Stage II, PDC Grades 1 and 2 in depth of tumor invasion T3 had a tendency to have good prognosis than any PDC Grade in depth of tumor invasion T4 (p = 0.0058), and PDC Grade 3 in depth of tumor invasion T3 (p = 0.0979) (Fig. 7).

Discussion

Several studies have reported that dedifferentation at the front of carcinoma is an important factor to determine the prognosis in colorectal cancer[1)2)]. However, it is difficult to make a clear distinction between moderately differentiated adenocarcinoma and well differentiated adenocarcinoma. Furthermore, there is no consensus determining the histological type based on reference to the most histologically active portion or the dominant area of tissue[9)10)]. On the other hand, PDC is only defined by the expression of clusters regardless of the histological type. PDC Grade showed a tendency to increase as the clinicopathological stage became more advanced. PDC Grade 3 showed significantly poor

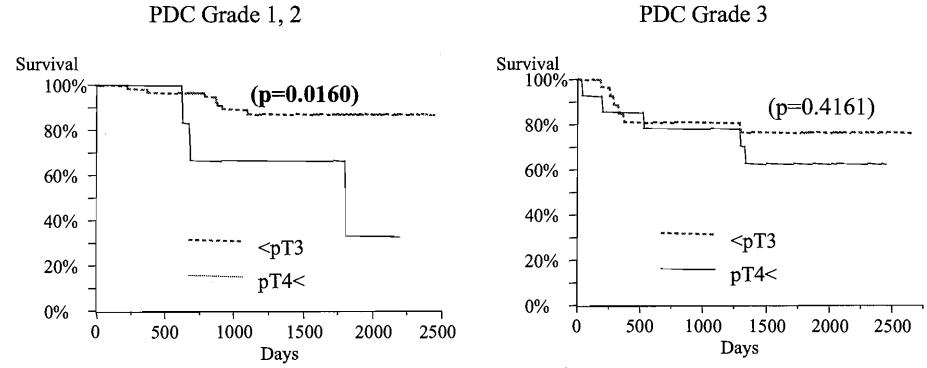

Fig. 6 Disease-free 5-year survival based on depth of tumor invasion according to PDC Grade in Stage II
In Stage II, there were no significant differences for disease free 5-year survival between depth of tumor invasion T3 and T4 in PDC Grade 3 (p = 0.4161), but there was significant differences for disease free 5-year survival between depth of tumor invasion T3 and T4 in PDC Grades 1 and 2 (p = 0.0160).

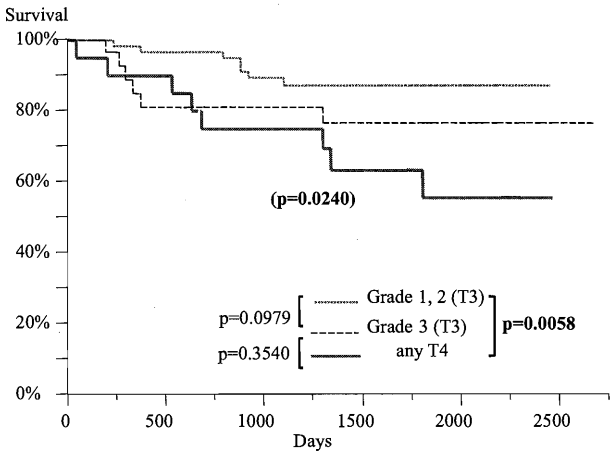

Fig. 7 Relationship between Disease-free 5-year survival and combination of PDC Grade and depth of tumor invasion in Stage II
PDC Grades 1 and 2 in depth of tumor invasion T3 had a tendency to good prognosis than any PDC appearance in depth of tumor invasion T4 (p = 0.0058), and PDC Grade 3 in depth of tumor invasion T3 (p = 0.0979).

prognosis than PDC Grades 1 and 2. The cancer with high frequency of PDC expression in the margin of invasion front of the colorectal cancer may have potential tumor invasion. INF shows the format of invasive cancer growth, which is described in the Japanese Classification of Colorectal Carcinoma Second English Edition[11]. In this study, INF had a strong correlation with PDC. INF is classified into 3 cancer nest developmental types under a low-power field, and is determined subjectively. However, regarding PDC, classification is determined more objectively than INF, based on the number of clusters at the invasive front of tumor.

We investigated the possibility of PDC which could classify prognosis colorectal cancer. In neither Stage I nor Stage III, there was no significant prognostic difference between PDC Grades 1 and 2 and PDC Grade 3. Depth invasion of Stage I depends on the muscularis propria. For cancer with high frequency of PDC appearance, radical surgery of the intestinal serosa, which is the last barrier to tumor invasion, obtained similar outcome. However, Stage III was the initial stage of lymph node metastases for other sites. We believe that in Stage III, the prognosis is governed not by the PDC situation of the primary tumor but by situations site of metastasis. In order to verify whether this is correct, we need to examine the occurrence of PDC in lymph node metastasis. In addition, there were no significant differences in the outcome between PDC Grades 1 and 2 and PDC Grade 3 in Stage II. However, significant differences could be found in the case of depth invasion for Stage II, between T3 and T4 among PDC Grades 1 and 2. We believe that both PDC Grades 1 and 2 and depth of tumor invasion T3 cases in Stage II could determined as the low risk recurrence group of Stage II.

The main problem which must be solved in the future is how to determine PDC, as this is deter-

mined without specifying the inside or outside of vascular invasion, making it difficult to distinguish between PDC and marked highly invasion of the vessels (ly3: severe lymphatic invasion and v3: severe venous invasion). In addition, PDC forms massive cluster compared with tumor budding, but is sometimes difficult to distinguish from fibroblasts, histiocytes and vascular endothelial cells. To resolve this problem, double immunostaining with D2-40 and CD-31 based on cytokeratin is necessary to clarify the differences between PDC and vascular invasion[12].

According to the national registry of domestic colorectal cancer, the cumulative 5-year survival rate of patients with curative resection is 94.3% in Stage 0, 90.6% in Stage I, 81.2% in Stage II, 71.4% in Stage IIIa, and 56.0% in Stage IIIb[13]. In Stage III, it is known that the surgery with add on adjuvant chemotherapy group has a better prognosis than the surgery alone group[14)~16)]. However, no consensus has been obtained at present on the use of adjuvant chemotherapy for Stage II. It is very important to select a group with a high and low risk of metastasis in Stage II. Evaluation of PDC is thought to be useful when selecting the low risk group with metastatis.

Conclusion

The PDC grade tended to increase with stage progression. PDC Grade 1 and 2 had a significantly better prognosis than PDC Grade 3. A significant relationship between PDC with several clinicopathological factors could be seen, such as the relationship between PDC and INF in multivariate analysis. In Stage II, PDC Grade 1 and 2 in depth of tumor invasion T3 had a tendency to have good prognosis compared with others.

The authors indicated no conflicts of interest.

References

1) **Ueno H, Mochizuki H, Hashiguchi Y et al**: Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology **127**: 385–394, 2004
2) **Tanaka S, Haruma K, Oh-e H et al**: Conditions of curability after endoscopic resection for colorectal carcinoma with submucosally massive invasion. Oncol Rep **7**: 783–788, 2000
3) **Ueno H, Murphy J, Jass J et al**: Tumor "budding" as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology **40**: 127–132, 2002
4) **Kawauchi H, Koike M**: A Preliminary Report of the Research Project of the "Tumor budding/Sprouting" by Japanese Society for Carcinoma of the Colon and Rectum. *In* Daicho shikkan NOW 2007, pp87–92, Nihon Medical Center (2007)
5) http://jsccr.umin.jp/75/file/teigi4.pdf
6) **Ueno H, Hashiguti Y, Kajiwara Y et al**: Proposed objective criteria for "grade 3" in early invasive colorectal cancer. Am J Clin Pathol **134**: 312–322, 2010
7) **Ueno H, Mochizuki H, Hashiguchi Y et al**: Histological grading of colorectal cancer. A simple and objective method. Ann Surg **247**: 811–818, 2008
8) Japanese Society for Cancer of the Colon and Rectum edt: Japanese Classification of Colorectal Carcinoma Second English Edition, Kanehara, Tokyo (2009)
9) **Riddell RH, Petras RE, Williams GT et al**: Colorectal carcinoma. *In* Atlas of tumor pathology-Tumors of the intestines (Rosai J, Sobin L eds), pp133–188, Armed Forces Institute of Pathology, Washington (2003)
10) **Compton CC**: Colorectal carcinoma: diagnostic, prognostic, and molecular features. Mod Pathol **16**: 376–388, 2003
11) Japanese Society for Cancer of the Colon and Rectum edt: 6 Outcome survey. *In* Japanese Classification of Colorectal Carcinoma Second English Edition, p27, Kanehara, Tokyo (2009)
12) Japanese Society for Cancer of Colon and Rectum edt: Clinical questions 2. *In* JSCCR Guidelines 2010 for the Treatment of Colorectal Cancer, p42, Kanehara Shuppan (2010)
13) Japanese Society for Cancer of Colon and Rectum edt: deta. *In* JSCCR Guidelines 2010 for the Treatment of Colorectal Cancer, p59. Kanehara Shuppan (2010)
14) **Kato T, Ohashi Y, Nakazato H et al**: Efficacy of oral UFT as adjuvant chemotherapy to curative resection of colorectal cancer: multicenter prospective randomized trial. Langenbeck Arch Surg **386**: 575–581, 2002
15) **Akasu T, Moriya Y, Ohashi Y et al**: Adjuvant chemotherapy with uracil-tegafur for pathological stage III rectal cancer after mesorectal excision with selective lateral pelviclymphadenectomy: a multicenter randomized controlled trial. Jpn J Clin Oncol **36**: 237–244, 2006
16) **Sakamoto J, Hamada C, Yoshida S et al**: An individual patient deta meta-analysis of adjuvant therapy with uracil-tegafur (UFT) in patients with curatively resected rectal cancer. Br J Cancer **96**: 1170–1177, 2007

大腸癌における低分化胞巣の出現と臨床病理学的因子の関連についての検討

[1]東京女子医科大学第二外科学教室
[2]同　総合研究所
[3]同　第一病理学教室

橋本（ハシモト）　拓造（タクゾウ）[1]・板橋（イタバシ）　道朗（ミチオ）[1]・小川（オガワ）　真平（シンペイ）[1]
廣澤知一郎（ヒロサワトモイチロウ）[1]・番場（バンバ）　嘉子（ヨシコ）[1]・加治（カジ）　早苗（サナエ）[1]
清水（シミズ）　悟（サトル）[2]・柴田（シバタ）　亮行（ノリユキ）[3]・亀岡（カメオカ）　信悟（シンゴ）[1]

Cur A 大腸癌 330 例を対象に低分化胞巣（poorly differentiated clusters：PDC）の発現を調べた．PDC は間質浸潤を呈する癌胞巣中，5 個以上の細胞から構成される腺腔形成の乏しい癌胞巣である．PDC の数をカウントし Grade 1（4 個以下），Grade 2（5〜9 個），Grade 3（10 個以上）と定義した．病期の進行とともに PDC Grade は上昇した．Grade 2 までの群と Grade 3 の群の間には累積 5 年無再発生存率に有意差を認めた．また PDC は IFN と有意な相関関係を認めた．リンパ節転移の有無別で検討を行ったところ，転移陽性例で有意差は認められなかったが，転移陰性症例において Grade 2 までの群と Grade 3 の群の間には累積 5 年無再発生存率に有意差を認めた．さらに Stage II の症例において PDC Grade 2 までででかつ深達度 T3 の症例は深達度 T4 の症例や PDC Grade 3 かつ深達度 T3 の症例に比べて予後は良好である傾向にあった．