Sys Rev Pharm 2024; 15(4): 140-145
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453/DOI: 10.31858/0975-8453.15.4.140-145

Research Article

Clinicopathological Analysis of Poorly Differentiated Adenocarcinoma of Colorectal Cancer

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Article History: *Submitted: 21.03.2024* *Accepted: 05.04.2024* *Published: 12.04.2024*

ABSTRACT

Aim: The purpose of this study was to clarify the malignancy grades and clinic pathological characteristics of poorly differentiated adenocarcinoma of colorectal cancer.

Material and methods: A total of 520 patients diagnosed with differentiated adenocarcinoma in p-Stage I-III who underwent curative resection at our hospital between 2011 and 2018 were included in this study of these, 19 patients were diagnosed with Poorly Differentiated Adenocarcinoma (PDA). All PDA cases were sub-classified into solid and non-solid PDAs based on tumor morphology confirmed by Hematoxylin and Eosin (H&E) stained specimens. In addition, immunostaining was additionally performed for solid PDA, medullary carcinoma, and endocrine cell cancer. The clinic pathological characteristics of each tumor were statistically analyzed.

Results: Compared to Highly Differentiated Adenocarcinomas/Moderately Differentiated Adenocarcinomas (HDA/MDA), PDA was more common in women (p=0.015), larger in size (p=0.033), more frequently located in the right colon (p=0.001), deeper in invasion (p<0.001), venous invasion (p<0.001) and lymph node metastasis (p<0.001). Immunostaining results showed that 5 patients (38.5%) of medullary carcinoma and 5 patients (38.5%) of neuroendocrine neoplasm were included in the solid PDA.

Conclusion: Our study suggests the importance of incorporating immune histological examination for the diagnosis of solid PDA in the future.

Keywords: Colorectal cancer, Clinicopathology, Adenocarcinoma

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INTRODUCTION

Colorectal cancer is the 2nd leading cause of cancer-related death in Japan (National Cancer Registry). The histopathological subtypes of colorectal cancer have different clinic pathological malignancy grades, which significantly affect recurrence and survival (Funahashi K, *et al.*, 1994; Compton CC, *et al.*, 2000). In particular, Poorly Differentiated Adenocarcinoma (PDA) of colorectal cancer is relatively rare, accounting for about 1.7%-7.7% (Nakamura T, *et al.*, 2002; Ueno H, *et al.*, 2021), of all colorectal cancers and therefore has not been sufficiently investigated clinically. In the recent years, several studies have reported that the histological morphology of the tumor invasive front correlates with metastasis, recurrence and oncological outcomes. Specifically, tumor budding can be defined as a single tumor cells or tumor clusters of upto 4 cells at the invasive front, while Poorly Differentiated Clusters (PDC) as clusters of 5 or more tumor cells lacking glandular structure (Basile D, *et al.*, 2022; Bonetti RL, *et al.*, 2016). These Tumor, Node, Metastasis (TMN) stage independent prognostic factors have been shown to be useful in determining the need for additional resection in early-stage colorectal cancer and in deciding the postoperative adjuvant chemotherapy in advanced colorectal cancer (Basile D, *et al.*, 2022; Bonetti RL, *et al.*, 2016; Nakamura T, *et al.*, 2008; Konishi T, *et al.*, 2018). On the other hand, there are cases that are morphologically difficult to differentiate by Hematoxylin and Eosin (H&E) staining alone, although they are diagnosed as the most predominant histological types according to the Japanese classification of colorectal carcinoma (Shida D, *et al.*, 2019; Ishida H, *et al.*, 2016) and the World Health Organization (WHO) tumor classification (WHO, 2019; WHO, 2000). Medullary carcinoma is a relatively new disease concept, described for the 1st time in the 3rd edition of the WHO tumor classification, published in 2000 (WHO, 2000) and has been included in the Japanese classification of colorectal carcinoma since 2013 (Shida D, *et al.*, 2019). In addition, some cases diagnosed with PDA of colorectal cancer may include Neuro Endocrine Neoplasm (NEN) with poor prognosis (Miura S, *et al.*, 2008; Kouchi Y, *et al.*, 2003). It was reported that PDA of colorectal cancer had a poor prognosis compared to HDA and MDA (Nakamura T, *et al.*, 2002; Ueno H, *et al.*, 2021; Miura S, *et al.*, 2008; Osada S, *et al.*, 1997), but when solid type PDA (por1) and non-solid type PDA (por2) were compared. The prognosis of solid PDA was as favorable as that of MDA (Nakamura T, *et al.*, 2002).

In this study, we sub-classified poorly differentiated adenocarcinoma of colorectal cancer into solid- and non-solid groups and compared them, but found no statistical significant difference in Overall Survival (OS) between the two groups (*Figure 1*). Thus, in order to differentiate solid PDA from medullary carcinoma and neuroendocrine neoplasm, which are difficult to differentiate by H&E staining alone, we re-examined the clinic pathological characteristics and prognostic factors of PDA using immunohistochemistry.

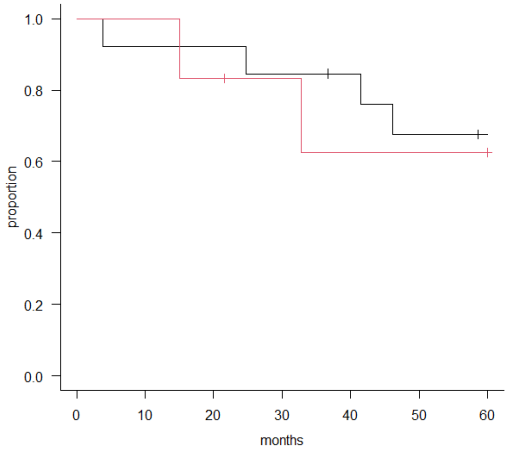

Figure 1: Estimation of OS rate in patients with p-Stage I-III colon cancer by Kaplan-Meier plot
Note: p=0.964, (—): 1 year and (—): 2 years

MATERIALS AND METHODS

Study design and patients cohort

The sample size for this study was determined by the number of patients whose pathology could be re-reviewed at our institution. All the surgeries were performed by a team of physicians with more than 20 years of surgical experience. We examined the factors such as age, gender, family history, maximum tumor diameter, tumor location, depth of invasion, lymphatic invasion, venous invasion, lymph node metastasis, recurrence rate, recurrence pattern and OS. During the study period, we found 24 deaths due to other primary malignancies, 29 deaths due to benign diseases other than cancer deaths including death from old age and 3 deaths due to unknown causes. This study was approved by the Ethics Committee of Dokkyo Medical University Hospital (Approval No: R-52-6J). Further, written informed consent was also obtained from the patient for publication and any accompanying images.

Inclusion criteria: Patients who were diagnosed with differentiated adenocarcinoma and underwent surgery between January, 2011 and December, 2018 and patients with pStage I-III colorectal cancer were included in this study.

Exclusion criteria: Patients those that received preoperative chemotherapy or radiotherapy (n=10), pTis (n=27), transanal endoscopic microsurgery (n=1), non-curative resection (n=5) and synchronous disease stage IV (n=91) were excluded from the study.

Histologic evaluation

For all patients diagnosed with PDA during the study period, whole-mount sections of the tumor including the invasive front were microscopically examined using H&E staining. The pathological evaluation was performed by two expert pathologists with expertise in pathological diagnosis. Immunostaining was performed to differentiate medullary carcinoma from endocrine cell cancer among tumors lacking glandular ductal structure. MutL protein Homolog 1 (MLH1), Deoxyribonucleic Acid (DNA) Mismatch repair protein (MSH2), MSH6 and Postmeiotic Segregation increased 2 (PMS2) were used to test for mismatch repair protein deficiency, while chromogranin A, synaptophysin and Insulinoma-associated 1 (INSM1) were used to detect endocrine cells. Similarly, Mucin-5AC (MUC-5AC) was used to detect the expression of gastric-type mucin. All the immunostaining results were judged by one of the experts.

Staging and surveillance protocol

Preoperative diagnosis was performed using contrast-enhanced computed tomography scans of the chest, abdomen and pelvis. Further, colonoscopy, magnetic resonance imaging and positron emission tomography techniques were also followed. According to the colorectal cancer treatment guidelines (Ishiguro M, *et al.*, 2013), postoperative adjuvant chemotherapy was administered to patients diagnosed with high-risk p-Stage II/III colorectal cancer based on the histological evaluation of surgical specimens. Postoperative surveillance was conducted for all colorectal cancer patients.

Statistical analysis

The primary endpoint of the study was OS. Patients were followed up for 5 years after surgery or until death and OS was estimated using Kaplan-Meier plot. Patients who were alive without recurrence at the final follow-up were censored. Each pathological parameter was evaluated for its association with OS using the log-rank test. Hazard Ratio (HR) and 95% Confidence Interval (CI) were calculated using the Cox proportional hazards model. Continuous variables were analyzed using either the Mann-Whitney U test or the Kruskal-Wallis test. Categorical variables were analyzed using either Fisher's exact test or the Chi-square (χ^2) test. Easy R (EZR) version 1.61 was used for all statistical analysis and all tests were two-tailed with $p<0.05$ indicating a statistically significant difference.

RESULTS

The median observation period was 64 (2-147) months while the median age was 70 (25-93) years with 326 men (62.7%) and 194 women (37.3%). Among all the patients 342 had colon cancer (65.8%) and 178 had rectal cancer (34.2%). Of these, 312 were MDA (60.0%), 189 were HDA (36.3%) and 19 were PDA (3.7%). There was 1 patient (0.2%) of familial adenomatous polyposis and 2 patients (0.4%) with ulcerative colitis. The pathological stage distribution for each adenocarcinoma included, 189 HDAs who had 85 patients with p-Stage I (16.3%), 62 patients with p-Stage II (11.9%), 42 patients having p-Stage III (8.1%); 312 MDAs had 63 patients with p-Stage I (12.1%), 132 patients of p-Stage II (25.4%) and 117 patients of p-Stage III (22.5%). Similarly, 19 patients had no case of p-Stage I (0%), 6 patients with p-Stage II (1.2%) and 13 patients with p-Stage III (2.5%). The rate of PDA found in advanced cancer was significantly higher ($p<0.001$). Postoperative adjuvant chemotherapy was administered to p-Stage II (76 (38.5%)) and p-Stage III patients (116 (67.4%)). When compared to HDA and MDA, PDA showed higher incidence in women ($p=0.015$) with larger tumor size ($p=0.033$), higher incidence in the right colon ($p=0.001$), deeper invasion ($p<0.001$), higher positive lymphatic vessel invasion ($p<0.001$), higher lymph node metastases ($p<0.001$) and higher proportion of patients receiving postoperative adjuvant chemotherapy ($p<0.001$) (*Table 1*).

Table 1: Base-line characteristics of the patients

Pathological characteristics	tub1 (n=189)	tub2 (n=312)	por (n=19)	p
Gender (male:female)	123:66	197:115	6:13	0.015
Age (mean ± SD*)	68.7 ± 10.9	69.9 ± 10.6	72.4 ± 15.5	0.119
Tumor size (mean ± SD*)	42.6 ± 26.2	45.2 ± 20.7	51.9 ± 23.2	0.033
Location of cancer				
Cecum	12 (6.3%)	17 (5.4%)	4 (21.1%)	0.413
Ascending colon	35 (18.5%)	49 (15.7%)	7 (36.8%)	
Transverse colon	16 (8.5%)	37 (11.9%)	3 (15.8%)	
Descending colon	12 (6.3%)	14 (4.5%)	0	
Sigmoid colon	54 (28.6%)	80 (25.6%)	2 (10.5%)	
Rectum	60 (31.7%)	115 (36.9%)	3 (15.8%)	
Right side colon	63 (33.3%)	103 (33.0%)	14 (73.7%)	0.001
Left side colon	126 (66.6%)	209 (67.0%)	5 (26.3%)	
Depth of invasion				
Shallower than MP**	98 (51.9%)	76 (24.4%)	3 (15.8%)	<0.001
Deeper than MP**	91 (48.1%)	236 (75.6%)	16 (84.2%)	

Lymphatic invasion				
Negative	129 (68.3%)	125 (40.0%)	4 (21.1%)	<0.001
Positive	60 (31.7%)	187 (60.0%)	15 (78.9%)	
Venous invasion				
Negative	99 (52.4%)	95 (30.4%)	5 (26.3%)	<0.001
Positive	90 (47.6%)	217 (69.6%)	14 (73.7%)	
Lymph node metastasis				
Negative	146 (77.2%)	195 (62.5%)	6 (31.6%)	<0.001
Positive	43 (22.8%)	117 (37.5%)	13 (68.4%)	
Adjuvant chemotherapy				
Negative	137 (72.5%)	174 (55.8%)	9 (47.4%)	<0.001
Positive	52 (27.5%)	138 (44.2%)	10 (52.6%)	
Note: (*): Standard Deviation (SD) and (**): Muscularis Propria (MP)				

Microscopic examination of H&E stained specimens from all 19 patients diagnosed with PDA revealed 13 patients of solid PDA and 6 patients of non-solid PDA. We found no statistically significant differences in clinical pathological characteristics between the solid- and non-solid PDA groups (*Table 2*) or in their OS (*Figure 1*). In all the patients with HDA, MDA, and PDA in p-Stage I-III, patients having PDA had the poorest OS (p=0.013) (*Figure 2*).

Table 2: Comparison of clinical pathological characteristics between solid- and non-solid PDA groups

Pathological characteristics	Por1 (n=13)	Por2 (n=6)	p
Gender (male:female)	5:8	1:5	0.675
Age (mean ± SD*)	75.9 ± 9.9	64.8 ± 23	0.403
Tumor size (mean ± SD*)	50.7 ± 25.4	54.5 ± 19.5	0.539
Location of cancer			
Cecum	3 (23.1%)	1 (16.7%)	0.315
Ascending colon	3 (23.1%)	4 (66.7%)	
Transverse colon	3 (23.1%)	0	
Descending colon	0	0	
Sigmoid colon	1 (7.7%)	1 (16.7%)	
Rectum	3 (23.1%)	0	
Right side colon	9 (69.2%)	5 (83.3%)	1
Left side colon	4 (30.8%)	1 (16.7%)	
Depth of invasion			
Shallower than MP**	3 (23.1%)	0	0.517
Deeper than MP**	10 (76.9%)	6 (100%)	
Lymphatic invasion			
Negative	4 (30.8%)	1 (16.7%)	1
Positive	9 (69.2%)	5 (83.3%)	
Venous invasion			
Negative	4 (30.8%)	1 (16.7%)	1
Positive	9 (69.2%)	5 (83.3%)	
Lymph node metastasis			
Negative	4 (30.8%)	2 (33.3%)	1
Positive	9 (69.2%)	4 (66.7%)	
Adjuvant chemotherapy			
Negative	7 (53.8%)	2 (33.3%)	0.628
Positive	6 (46.2%)	4 (66.7%)	
Note: (*): Standard Deviation (SD) and (**): Muscularis Propria (MP)			

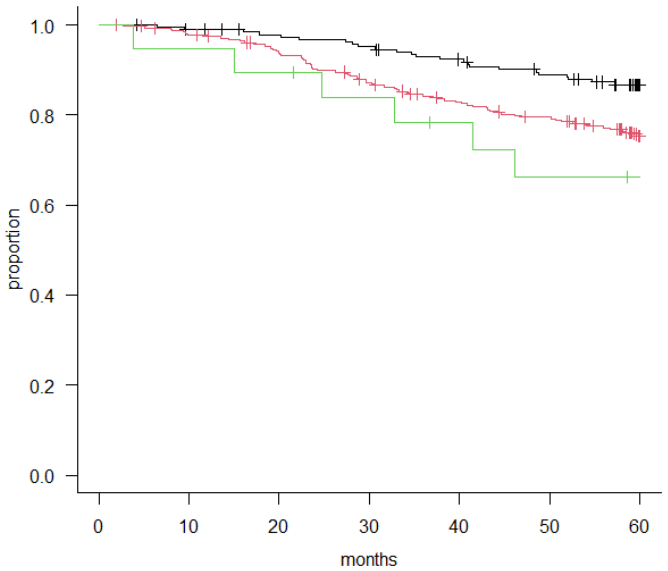

Figure 2: Estimation of OS rate in patients with PDA
Note: p=0.013, (—): 1 year; (—): 2 years and (—): 3 years

Cox's regression analysis was performed to examine HDA, MDA and PDA in p-Stage I-III and based on the analysis, depth of tumor depth, lymphatic vessel invasion and postoperative adjuvant chemotherapy were identified as independent prognostic factors. We found the invasion depth (HR:

2.82, 95% CI: 1.72-4.65 and p<0.001), lymph node metastasis (HR: 1.72, 95% CI: 1.17-2.52 and p=0.005) and postoperative adjuvant chemotherapy (HR: 0.58, 95% CI: 0.39-0.85 and p=0.005).

Among the 13 patients diagnosed as solid PDA, immunostaining results showed that 5 patients (38.5%) were medullary carcinoma and 5 patients (38.5%) were endocrine tumor. All the endocrine tumors were Mixed Neuroendocrine-non-neuroendocrine Neoplasm (MiNEN) and 2 out of 5 endocrine tumors were identified as Microsatellite Instability (MSI)-high due to deficiency of MLH1 and PMS2. Solid PDA showed poor expression of gastric-type mucin and no weakening in expression of mismatch repair gene products was observed. The clinical pathological features of medullary carcinoma were that it tended to be more common in older women, all were located in the right colon and the maximum tumor size was generally larger. Furthermore, compared to solid PDA and MiNEN, medullary carcinoma showed significantly lower lymph node metastasis (p=0.028) in lower proportion of patients who received postoperative adjuvant chemotherapy (p=0.039). It was clarified that medullary carcinoma and MiNEN were more common in the right colon, while solid PDA was more common in the left colon (p=0.003).

DISCUSSION

In this study, we aimed to compare PDA with HDA and MDA of colorectal cancer and to clarify the malignancy grades and clinical-pathological characteristics. All cases of PDA were detected at an advanced stage, i.e., p-Stage II or higher which had deep invasion with significantly higher rates of vascular invasion and lymph node metastasis. Previous studies have shown that the rate of lymph node metastasis in PDA (44%-79%) is higher than that in other differentiated adenocarcinomas (28%-51%) (Nakamura T, *et al.*, 2002; Ueno H, *et al.*, 2021; Miura S, *et al.*, 2008; Sato H, *et al.*, 1998; Ueno H, *et al.*, 2014) and the same is true in the present study. These results may indicate the high degree of malignancy in PDA. However, it has been pointed out that there are tumors with relatively good prognosis even among PDA with poor prognosis and various sub-classifications have been proposed (Nakamura T, *et al.*, 2002; Miura S, *et al.*, 2008; Osada S, *et al.*, 1997). Solid PDA is considered to have a better prognosis than non-solid PDA (Nakamura T, *et al.*, 2002; Goi T, *et al.*, 2004; Masuda H, *et al.*, 2005). In this study, there was no statistically significant difference in the prognosis between solid- and non-solid PDAs. Upon re-examination of H&E stained specimens of the solid PDA cases, it was found that other carcinomas such as medullary carcinoma and endocrine cell carcinoma, which are morphologically similar to solid PDA, might be included in the solid PDA cases.

PDA is an adenocarcinoma with poor lumen formation or being positive for intracellular mucins despite negative ductal formation (Arai T, *et al.*, 2013; Shida D, *et al.*, 2019) (*Figure 3*). Medullary carcinoma was considered as a subtype of PDA and was treated as the same tumor as PDA before 2013 in Japan. Pathologically, medullary carcinoma resembles solid Pancreatic Ductal Adenocarcinoma (PDAC) in its lack of a glandular ductal structure and its trabecular and medullary growth patterns. However, it is distinguished by the frequent presence of inflammatory cell infiltration, predominantly by neutrophils and lymphocytes within and at the periphery of the tumor. Immunostaining findings characteristically show deficiency in MLH1 and Caudal-related homeobox transcription factor 2 (CDX-2) (Masuda H, *et al.*, 2005). Neuroendocrine neoplasm, particularly large cell type endocrine cell cancer, is morphologically similar to solid PDA due to its solid, pavement stone-like growth pattern of cancer cells and lack of lumen formation and therefore, immunohistological staining is required for differentiation (Ueno H, *et al.*, 2021) (*Figure 4*). In our study, immunostaining results revealed that 10 out of 13 (78%) patients of solid PDA included medullary carcinoma and endocrine tumor, suggesting that the true incidence of solid PDA may be even lower than previously reported (Nakamura T, *et al.*, 2002; Ueno H, *et al.*, 2021).

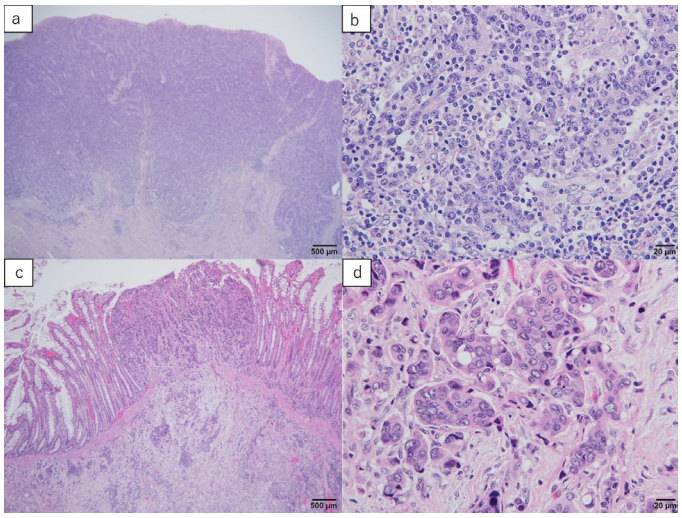

Figure 3: H&E of cancer cells with solid, pavement stone-like growth patterns without lumen formation exhibit expansive growth, (a): Por1 (20X); (b): Por1 (400X); H&E staining of cancer cells with palisading, nested patterns exhibiting infiltrative growth, (c): Por2 (20X) and (d): Por2 (400X)

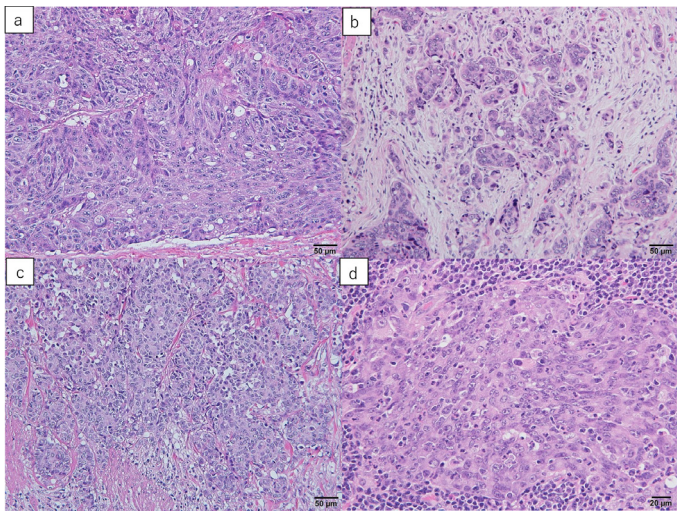

Figure 4: H&E staining comparison, (a): Por1 (200X); (b): Por2 (200X); (c): MiNEN (200X) and (d): Medullary carcinoma (400X)

Knox RD, *et al.*, 2015, reported that medullary carcinoma accounted for 91 patients (2.8%) out of 3295 surgical resection patients between 1998 and 2012 and a different meta-analysis also reported a prevalence of 2.7% (Pyo JS, *et al.*, 2016). It is estimated that medullary carcinoma accounts for about 2%-3% of all colorectal cancers. Since about 2/3rd of those diagnosed with PDA in the elderly >65 years of age are considered to have medullary carcinoma where the possibility of medullary carcinoma should be the 1st consideration for PDA occurring in the right colon in women >80 years of age (Arai T, *et al.*, 2004). Medullary carcinoma in our study was more common in the elderly women in the right colon, although the prevalence was less than previous reports, i.e., 5 patients out of 520 (0.96%). It has been reported that many cases of PDA show MSI (Okuno M, *et al.*, 1989; Kawabata Y, *et al.*, 1999), which may be attributed to the inclusion of medullary carcinoma in these studies. Furthermore, among advanced or recurrent colorectal cancers, MSI-high tumors are known to be resistant to 5-Fluorouracil (5-FU) based chemotherapy (Ribic CM, *et al.*, 2003; Kim GP, *et al.*, 2007; Barratt PL, *et al.*, 2002) and no consensus has been reached on the

effectiveness of chemotherapy based on oxaliplatin and irinotecan. As the efficacy of anti-Programmed Cell Death Ligand 1 (PD-L1) antibody drugs for MSI-high tumors has been reported for which appropriate diagnosis and postoperative treatment strategies are necessary (Le DT, *et al.*, 2015).

While neuroendocrine neoplasms displaying histologically neuroendocrine patterns are classified as highly differentiated in the WHO classification (WHO, 2019), they were further categorized into Neuroendocrine Tumors (NETs) Grade 1 (G1)-G3 in this study according to the mitotic figures based on proliferative capacity and Kiel-67 (Ki-67) index. Poorly differentiated tumors with a Ki-67 index >20% were categorized as Neuroendocrine Carcinomas (NECs) and tumors with ≥ 30% of both non-neuroendocrine and neuroendocrine components were categorized as MiNEN. Notably, all the patients in our study were classified as MiNEN. MiNEN is considered to be synonymous with the disease previously referred to as Mixed Adeno-Neuroendocrine Carcinoma (MANEC) (WHO, 2019) and most MiNENs are deemed to be high-grade tumors equivalent to NET G3. However, some MiNEN cases have a mixture of NET G2 and adenocarcinoma that have relatively good prognoses (Kasahara K, *et al.*, 2020). Ki-67 index testing was not performed in this study, but patients with relatively good prognosis were included; further investigation is under consideration. Since no treatment strategy has been established for primary colorectal NEC, chemotherapy with a combination of platinum-based drugs and etoposide or irinotecan, similar to that used for small cell lung cancer is recommended (Japan neuroendocrine tumor society, 2019). As the treatment strategies for PDA and endocrine tumors are quite different, differential diagnosis is necessary as in the case with medullary carcinoma mentioned earlier.

CONCLUSION

The above findings indicate that PDA is a highly malignant tumor, often diagnosed at an advanced stage which is associated with poor prognosis. There are few reports on the pathological features of solid PDA, so contrary to previous reports the tumor size may be smaller and may occur predominantly in the left colon. Furthermore, solid PDA is morphologically similar to medullary carcinoma and neuroendocrine neoplasm in H&E stained specimens alone, making differentiation difficult. Therefore, immunohistological examination should be performed in all cases whenever possible. Since PDA is very rare, further examination and accumulation of cases will be necessary in the future.

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