ANTICANCER RESEARCH *41*: 5847-5854 (2021)
doi:10.21873/anticanres.15404

A Case Report of Metachronous Multiple Adenosquamous Carcinoma of the Colon Over-expressing PD-L1 and a Literature Review

SHINICHI AKAHOSHI[1], KENSUKE YAMAMURA[1], YOSHIHIRO KOMOHARA[2], DAIKI YOSHII[2], ERI ODA[1], NOBUTAKA SATO[1], HIDEAKI YUKI[3], KATSUNORI YUKIMURA[4] and TORU BEPPU[1]

[1]*Department of Surgery, Yamaga City Medical Center, Kumamoto, Japan;*
[2]*Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan;*
[3]*Department of Radiology, Yamaga City Medical Center, Kumamoto, Japan;*
[4]*Yukimura Clinic, Kumamoto, Japan*

Abstract. *Background: Colorectal cancer is the third most commonly diagnosed cancer in both men and women, and one of the more widely recognized preventable cancers. Adenosquamous carcinoma (ASC) of the colon/rectum is an uncommon disease that consists of both glandular and squamous components, and the most common site of ACS is the right and transverse colon. Case Report: Here, we present the case of a 78-year-old woman, who complained of abdominal pain. Colonoscopy revealed a circumscribed tumor in the ascending colon, and no specific lesion was detected in the other areas of the colon or rectum. ASC (pT3N0M0) was diagnosed from right hemicolectomy specimens. Three months after the first surgery, the serum levels of tumor markers had gradually increased, and a new tumor was subsequently detected in the sigmoid colon 2 months later. The sigmoid lesion was surgically resected and diagnosed as ASC (pT3N3M0). Strong PD-L1 expression was also found in the squamous component. Conclusion: To our knowledge, this is the first report of a recurrent sigmoid colon ASC that likely originated from the ascending colon, and PD-L1/PD-1 signaling was likely involved in the immune escape mechanism.*

Adenosquamous carcinoma (ASC) of the colon/rectum is an uncommon disease that consists of both glandular and squamous components (1, 2). ASC of the colon accounts for 0.02% to 0.06% of all colorectal malignant tumors. The first case was reported by Herxheimer in 1907 (3). The most common site of ACS is the right and transverse colon (4, 5). Although the clinical manifestations are similar to those of adenocarcinoma, ASC is associated with a poor clinical outcome, with a 5-year overall survival rate of 31%, in comparison to conventional adenocarcinoma (4). ASC has a greater tendency to form both regional and distant metastases, and similar to colorectal adenocarcinoma, the most common metastatic site is the liver, followed by the peritoneum and lung (4). The overall rates of regional metastases and distant metastases were reported to be 46.0% and 42.4%, respectively. In a review of Japanese patients with ASC, Yokoi *et al*. reported that 48% were female, 56% had right-sided colon cancer, 94% were T3 or more, 62% had lymph node metastasis, and 33% were stage IV (6). ASC has been reported to be aggressive, and is diagnosed at advanced stages. Herein, we report a case of recurrent intestinal ASC suspected to be due to implantation metastasis from the ascending colon to the sigmoid colon. In addition, immunohistochemical analysis of the primary and recurrent lesions was performed. We found strong PD-L1 expression in the squamous component, and the level of PD-L1 expression was increased in the recurrent lesion; therefore, immune escape *via* PD-L1/PD-1 signaling may be involved in the progression of ASC.

Correspondence to: Yoshihiro Komohara, MD, Ph.D., Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Honjo 1-1-1, Chuouku, Kumamoto, 860-8556, Japan. Tel: +81 963735095, e-mail: ycomo@kumamoto-u.ac.jp

Key Words: Colon cancer, adenosquamous carcinoma, PD-L1.

Case Report

A 78-year-old Japanese woman was referred to our hospital with a history of abdominal pain. She had a medical history of asthma and ovarian benign tumor. Diagnostic colonoscopy revealed the presence of a large circumferential and obstructive 6-cm mass. No detectable lesion was seen in the sigmoid colon. Biopsy of the mass indicated carcinoma; however, a detailed histological diagnosis was not obtained. The serum

levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were 27.8 ng/ml (normal range=0-5.0 ng/ml) and 78.9 U/ml (normal range=0-37 U/ml), respectively. The squamous cell carcinoma (SCC) antigen level was not measured. Abdominal computed tomography demonstrated thickening of the wall of the ascending colon and no distant metastasis. The patient underwent a right hemicolectomy with D3 lymph node dissection together with partial resection of the duodenum. The pathological diagnosis was ASC without lymph node metastasis (pT3N0M0; Stage IIa). She was followed-up without adjuvant chemotherapy. However, the serum levels of CEA gradually increased 3 months after the first surgery, and a tumor mass was subsequently detected 2 months later in the sigmoid colon by computed tomography and colonoscopy. The CA19-9 levels were also elevated. Biopsy of the tumor revealed a poorly differentiated adenocarcinoma. The values of CEA and CA19-9 before the second operation reached 27.4 ng/ml and 56.4 U/ml, respectively, and the SCC antigen value was 1.2 ng/ml (normal range=0-1.5 U/ml). The patient underwent a sigmoidectomy with D3 lymph node dissection. The pathological diagnosis of the recurrent lesion was ASC (pT3) with lymph node metastasis. She received postoperative adjuvant chemotherapy with a capecitabine plus oxaliplatin regimen for 4 months. No subsequent recurrent lesion has been seen, and all three tumor markers are within normal limits.

Pathological observation. A circumferential mass lesion with ulceration and a 5-cm diameter was seen, and a whitish solid tumor was observed in the cross-section of the lesion (Figure 1A). The solid tumor had an atypical glandular (adenomatous) structure that indicated grade 1 to 2 (well-to-moderately differentiated) adenocarcinoma, and a solid nest pattern that indicated grade 2 SCC (Figure 1B). The ratio of the adenomatous and squamous components was approximately 1:1, and both components were sequentially converted. Fibromatous stroma and immune cell infiltration were also detectable. As shown in Figure 1C, immunohistochemistry (IHC) of cancer tissue showed that the adenomatous component was positive for CK20, and the squamous component was positive for p40 and CK5/6, which is consistent with ASC. Mutations of the *RAS* gene and microsatellite instability (MSI) were negative, whereas the *BRAF* (V600E) mutation was detected.

The recurrent lesion was a similar all-around mass lesion with ulceration that was 4 cm in diameter, and a whitish solid tumor was observed in the cross-section of the lesion (Figure 2A). It was hypothesized to be a rapid growth tumor. The tumor was composed of grade 1 to 2 ADC and grade 2 SCC, similar to the primary lesion, and was consistent with ASC (Figure 2B). The adenomatous and squamous components comprised 10% and 90% of the tumor, respectively. Two regional lymph node metastases were observed, and the histology of the lymph node metastases was consistent with ASC. The IHC results were the same as those of the primary lesion (Figure 2C). Additional IHC showed positive CEA and CA19-9 staining in both the adenomatous and squamous components (Figure 2C). Both components were also positive for mismatch repair molecules, such as MLH1, MSH2, MSH6, and PMS2 (data not shown). No positive signals of p53 were observed in either of the components, which indicated the so-called "p53-null mutation" (data not shown).

Additional IHC studies were performed, and p40 and CK20 double-positive cells were sporadically observed in the tumor lesion, especially in the transitional zone between the adenomatous and squamous components (Figure 3A). Notably, PD-L1 overexpression was observed in the squamous component, but not in the adenomatous component (Figure 3B). Over 90% of the squamous component was positive for PD-L1. Stromal immune cells, mainly macrophages, were also positive for PD-L1.

Discussion

ASC of the colon is often detected at an advanced clinical stage, when the tumor is already large, and is characterized by a higher incidence of lymph node metastasis when compared to pure adenocarcinoma. The most common site of colorectal ASC is the right and transverse colon, and the liver is the most common site of metastases, followed by the peritoneum and lung (7). The present case showed stage T3 disease without lymph node metastasis in the primary lesion, and recurrence in the sigmoid colon 5 months after the first operation. Even in common colorectal cancer, hematogenous metastasis from the right colon to the sigmoid colon has rarely been reported (8).

The gross appearance and histological growth pattern of the recurrent lesion were similar to those of the primary lesion, indicating that the recurrent lesion originated from the mucosal area. Colorectal cancer spreading by implantation is also rare; however, it has been described in colonoscopic biopsy sites, laparoscopic port sites, and others (9, 10). Since the incidence of ASC is very rare, the de novo carcinogenesis of ASC in other sites has not been clarified. Therefore, implantation metastasis from the ascending colon to the sigmoid colon may have caused the recurrence of ASC in the present case.

Although both the adenomatous and squamous components have the potential for metastasis in ASC of the colon, more aggressive potential was observed in the squamous component (11-13). In the present study, the ratio of the squamous component was significantly higher in the recurrent lesion than in the primary lesion, indicating that SCC is more aggressive than adenocarcinoma. The histogenesis of ASC is not fully understood, despite the fact that previous studies have suggested several hypotheses, *i.e.*,

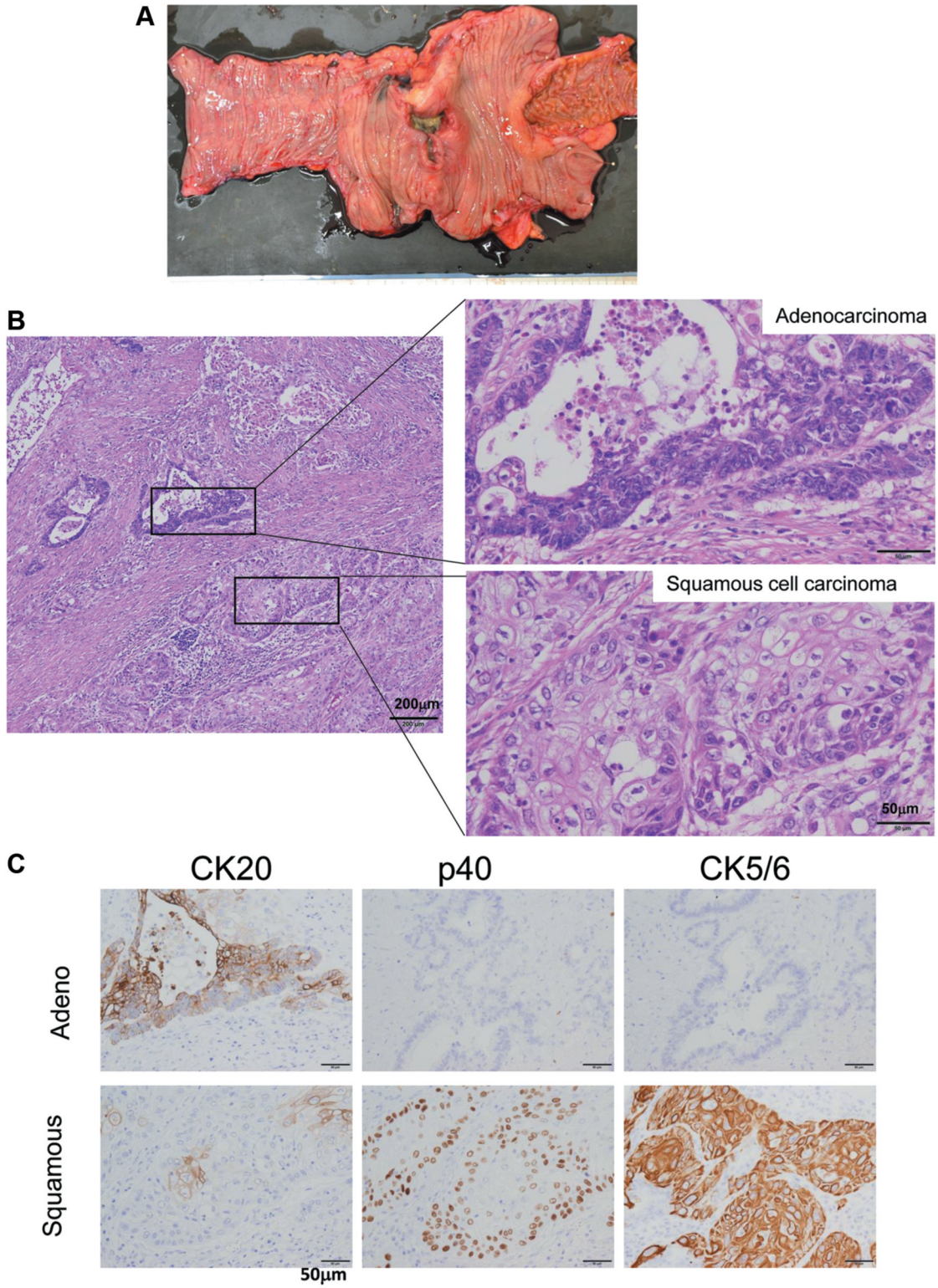

Figure 1. *Pathology of the primary lesion. (A) An ulcer with rolled-up edges and a 5-cm diameter is observed in the resected ascending colon. Cross-section of the carcinoma showing a whitish lesion with extension into the subserosa. (B) Hematoxylin and eosin (H&E) staining indicating that the lesion is composed of an adenomatous component (adenocarcinoma) and a squamous component [squamous cell carcinoma (SCC)]. (C) Immunohistochemical (IHC) staining showing that the adenomatous component is positive for CK20, but negative for p40 and CK5/6, and the squamous component is positive for p40 and CK5/6, but negative for CK20.*

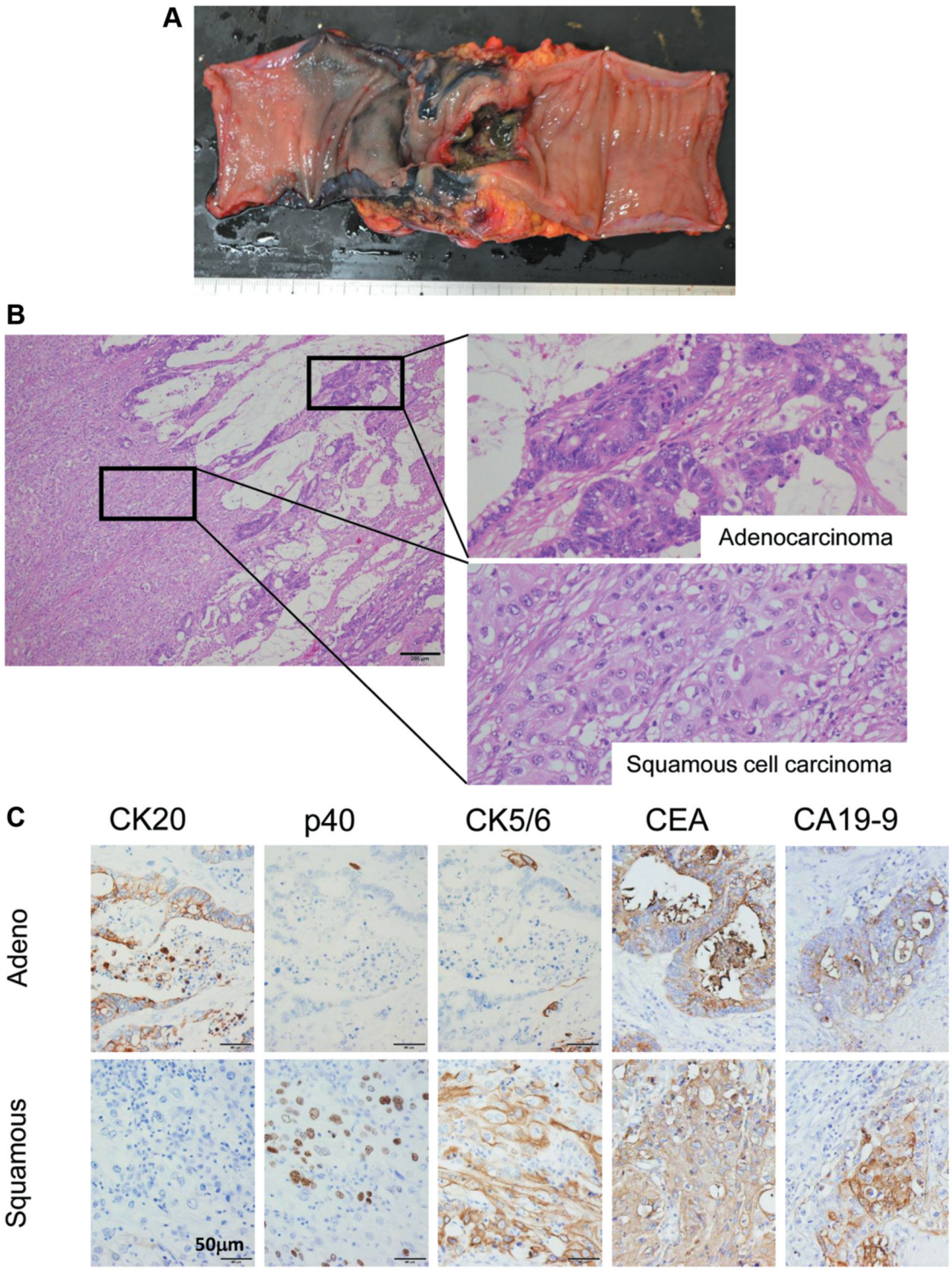

Figure 2. *Pathology of the recurrent lesion. (A) An ulcer with slightly rolled-up edges and a 4-cm diameter is observed in the resected sigmoid colon. Cross-section of the carcinoma showing a whitish lesion with extension into the subserosa. Black ink was injected into the mucosa near the tumor before surgical resection. (B) H&E section indicating that the lesion is composed of an adenomatous component (adenocarcinoma) and a squamous component (SCC), similar to the primary lesion. (C) The adenomatous component is positive for CK20, but negative for p40 and CK5/6, and the squamous component is positive for p40 and CK5/6, but negative for CK20. Strong CEA expression is observed in both the adenomatous and squamous components. CA19-9 expression is seen in both the adenomatous and squamous components; however, the expression levels were weaker in the adenomatous component than in the squamous component.*

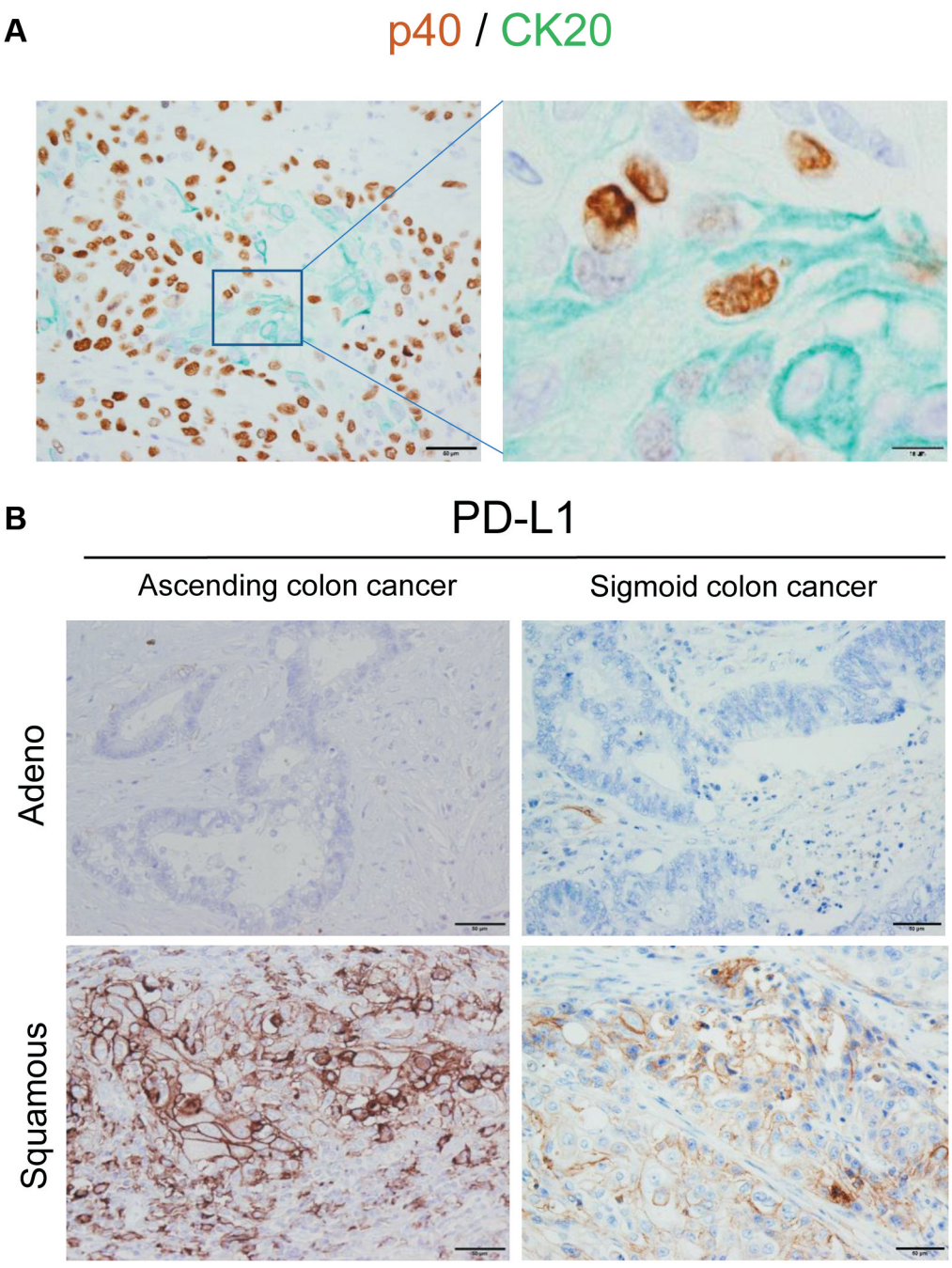

Figure 3. *Additional immunohistochemistry (IHC) stainings. (A) Double-IHC for p40 (brown nuclear staining pattern) and CK20 (green membrane staining pattern). (B) PD-L1 expression is observed in the squamous component, but not in the adenomatous component in both the primary and recurrent lesions.*

the presence of embryologic nests of ectodermal cells, squamous metaplasia of the intestinal mucosa, and the presence of pluripotent stem cells of endodermal origin that are capable of multidirectional differentiation (14, 15). In a previous gene sequence analysis of ASC of the pancreas, *TP53* mutations and 3p deletions were frequently observed (16). This study also found that the adenomatous and squamous components shared crucial mutation events and similar genomic variations, suggesting that the two cellular components may develop from the same progenitor cancer

Table I. *Literature review of PD-L1 expression in adenosquamous carcinoma.*

Reference number	Number of cases	Organ	Gender (male/female)	ICI therapy	Comments on PD-L1
25	6	Lung	6/0	No	PD-L1-positive cancer cells were seen in all cases. Homogenous expression of PD-L1 was seen in 1 case, whereas heterogenous expression of PD-L1 was seen in 5 cases. PD-L1 expression was restricted to ADC in 2 cases and to SCC in 3 cases.
26	28	Lung	23/5	No	PD-L1-positive cancer cells were seen in 17 cases. PD-L1 expression was detected in ADC in 6 cases and in SCC in 11 cases.
27	72	Lung	38/34	No	PD-L1-positive cancer cells were seen in 35 cases. Homogenous expression of PD-L1 was seen in 5 cases. PD-L1 expression was detected in ADC in 15 cases and in SCC in 25 cases.
28	51	Lung	33/18	No	PD-L1-positive cancer cells were seen in 20 of 51 cases. When the two components were examined separately in 36 cases, PD-L1 was detected in ADC in 4 cases and in SCC in 14 cases.
29	5	Lung	N.D.	Yes	No detailed pathological observation of PD-L1 expression was described in the paper. Three cases and 2 cases showed a PR and PD, respectively, in response to ICI therapy, and the frequency of achieving a PR was higher when compared to cases with other common histologies of cancer.
30	1	Lung	0/1	Yes	PD-L1 expression was seen in 1% to 10% of ADC and in >90% of SCC. A PR was achieved with ICI therapy.
31	7	Uterine cervix	0/7	No	PD-L1-positive cancer cells were seen in 3 cases. PD-L1 expression was restricted to SCC in all cases.
32	56	Pancreas	35/21	No	PD-L1-positive cancer cells were seen in 6 cases. PD-L1 expression was restricted to SCC in all cases.
33	6	Pancreas	4/2	No	PD-L1-positive cancer cells were seen in 5 cases. PD-L1 expression was restricted to SCC in all cases.

ICI: Immune checkpoint inhibitors targeting PD1/PD-L1 signaling; ADC: adenocarcinoma component; SCC: squamous cell carcinoma component; PR: partial response; PD: progressive disease; N.D.: no description.

cells. In the present case, p40 (SCC marker) and CK20 (adenocarcinoma marker) double-positive cells were sporadically observed in the tumor tissues, supporting the hypothesis that the two components are derived from the same pluripotent stem cells or progenitor cancer cells.

In the present study, IHC for p40 was performed to confirm the pathological diagnosis. p40 is one of the p63 isoforms (ΔNp63) that has been identified in recent years to be specifically expressed in SCC (17). The sensitivity and specificity of p40 for SCC has been reported to be 90% to 100% in lung cancer and head and neck cancer. Since the squamous component of the present ASC case was also positive for p40, IHC for p40 might be useful for the diagnosis of ASC in the intestine.

Another interesting result of IHC in this case was that PD-L1 expression was restricted to the squamous component and was not observed in the adenomatous component. PD-L1 expression in colorectal adenocarcinoma has been reported to be around 30% (18). In lung cancer, PD-L1-positive cancer cells were observed in 72% of SCC cases and 27% of adenocarcinoma cases (19, 20). Thus, squamous cancer cells potentially express more PD-L1 than adenocarcinoma cells. In addition, immune checkpoint inhibitors (ICIs) have shown efficacy in patients with MSI-high/mismatch repair-deficient (MSI-H/dMMR) cancers. A recent report described the successful treatment of MSI-H colorectal cancer by anti-PD-1 antibody (21). It has also been reported that a case with metastasized colon ASC, in which the squamous component expressed PD-L1, was successfully treated by anti-PD-1 antibody therapy (22). High levels of PD-L1 expression were also observed in the squamous component in both the primary and recurrent lesions in the present case. A high PD-L1 expression level on tumor cells predicts a beneficial anti-tumor effect of anti-PD-1/PD-L1 therapy in non-small cell lung cancer, head and neck cancer, and melanoma (23, 24).

Although few reports on PD-L1 expression in colorectal cancer have been published, there have been several reports on PD-L1 expression in ASC of the lung, uterine cervix, and pancreas (Table I). From four studies describing PD-L1 in lung ASC, 68 of 157 cases (43.3%) were positive for PD-L1, and PD-L1 was preferentially expressed in SCC (25-28). Two of the four studies described the anti-cancer effect of ICIs in lung ASC; they reported that 4 and 2 of 6 cases showed a partial response and progressive disease, respectively, and that the efficacy of ICI therapy appeared to be higher in ASC than in other cancers with common histological subtypes (29, 30). In a report of ASC of the uterine cervix, 3 of 7 cases were positive for PD-L1, and PD-L1 expression was restricted to SCC (31).

Two studies reported PD-L1 expression in pancreatic ASC. Lee *et al*. showed that 6 of 56 cases were positive for PD-L1, and that the PD-L1-positive ratio was similar to that of ADC, which is a common histological subtype of pancreatic cancer (32). Tanigawa *et al*. reported that 5 of 6 cases of pancreatic ASC were positive for PD-L1, and that PD-L1 expression was restricted to SCC (33). Several different monoclonal antibodies against PD-L1 are commercially available, but their sensitivity and specificity have been reported to vary (34); as such, the use of different antibody clones might cause discrepancies between the results of different studies.

Taken together, SCC in ASC preferentially expressed PD-L1 in several organs. Although patients with ASC generally show a worse clinical course than those with the usual adenocarcinoma in several organs, ICI therapy targeting PD-1/PD-L1 might be a promising approach for patients with recurrent or metastatic ASC.

Conflicts of Interest

The Authors declare no conflicts of interest associated with this manuscript.

Authors' Contributions

SA and KY gathered the patient`s data and wrote the manuscript. SA, KY, EO, NS, HY, KY, and TB participated in the surgery. YK and DY were responsible for pathological diagnosis of this case. SA, KY, YK, DY, EO, NS, HY, KY, and TB discussed the data and helped write the manuscript. All Authors approved the final manuscript.

Acknowledgements

The Authors would like to thank Mr. Takenobu Nakagawa for a technical assistance. The Ethics Committees of Kumamoto University approved the content of this manuscript (#2224), and written informed consent was obtained from the patient before surgery. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

References

1 Petrelli NJ, Valle AA, Weber TK and Rodriguez-Bigas M: Adenosquamous carcinoma of the colon and rectum. Dis Colon Rectum *39(11)*: 1265-1268, 1996. PMID: 8918436. DOI: 10.1007/BF02055120

2 Cagir B, Nagy MW, Topham A, Rakinic J and Fry RD: Adenosquamous carcinoma of the colon, rectum, and anus: epidemiology, distribution, and survival characteristics. Dis Colon Rectum *42(2)*: 258-263, 1999. PMID: 10211505. DOI: 10.1007/BF02237138

3 Comer TP, Beahrs OH and Dockerty MB: Primary squamous cell carcinoma and adenocanthoma of the colon. Cancer *28(5)*: 1111-1117, 1971. PMID: 5125659. DOI: 10.1002/1097-0142(1971)28:5<1111::aid-cncr2820280504>3.0.co;2-v

4 Masoomi H, Ziogas A, Lin BS, Barleben A, Mills S, Stamos MJ and Zell JA: Population-based evaluation of adenosquamous carcinoma of the colon and rectum. Dis Colon Rectum *55(5)*: 509-514, 2012. PMID: 22513428. DOI: 10.1097/DCR.0b013e3182420953

5 Choi JW and Park HU: Adenosquamous carcinoma of the ascending colon: a case report and review of the literature. Ann Coloproctol *29(2)*: 83-86, 2013. PMID: 23700577. DOI: 10.3393/ac.2013.29.2.83

6 Yokoi K, Tanaka N, Furukawa K, Seya T, Ohaki Y and Tajiri T: Case of adenosquamous carcinoma of the ascending colon. J Nippon Med Sch *75(4)*: 242-246, 2008. PMID: 18781050. DOI: 10.1272/jnms.75.242

7 Frizelle FA, Hobday KS, Batts KP and Nelson H: Adenosquamous and squamous carcinoma of the colon and upper rectum: a clinical and histopathologic study. Dis Colon Rectum *44(3)*: 341-346, 2001. PMID: 11289278. DOI: 10.1007/BF02234730

8 Desch CE, Benson AB 3rd, Somerfield MR, Flynn PJ, Krause C, Loprinzi CL, Minsky BD, Pfister DG, Virgo KS, Petrelli NJ and American Society of Clinical Oncology: Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. J Clin Oncol *23(33)*: 8512-8519, 2005. PMID: 16260687. DOI: 10.1200/JCO.2005.04.0063

9 Gomes RM, Kumar RK, Desouza A and Saklani A: Implantation metastasis from adenocarcinoma of the sigmoid colon into a perianal fistula: a case report. Ann Gastroenterol *27(3)*: 276-279, 2014. PMID: 24975988.

10 Tan WJ, Ng NZ, Chen YD, Chee YHM, Foo FJ, Tang CL and Chew MH: Synchronous polypectomy during endoscopic diagnosis of colorectal cancer - is the risk of tumour implantation at the polypectomy site significant? BMC Gastroenterol *18(1)*: 133, 2018. PMID: 30157767. DOI: 10.1186/s12876-018-0861-4

11 Cerezo L, Alvarez M, Edwards O and Price G: Adenosquamous carcinoma of the colon. Dis Colon Rectum *28(8)*: 597-603, 1985. PMID: 3893953. DOI: 10.1007/BF02554156

12 Kontozoglou TE and Moyana TN: Adenosquamous carcinoma of the colon – an immunocytochemical and ultrastructural study. Report of two cases and review of the literature. Dis Colon Rectum *32(8)*: 716-721, 1989. PMID: 2666053. DOI: 10.1007/BF02555782

13 Yokose T, Yamamoto S, Nagase T, Kanai T, Takano K, Fujii T, Tsutsui M, Nakagawa M, Ochiai H and Kameyama K: Long-term survival with extended lateral lymphadenectomy for lateral lymph node recurrence after laparoscopic abdominoperineal resection for rectal adenosquamous carcinoma: a case report. Surg Case Rep *4(1)*: 32, 2018. PMID: 29633041. DOI: 10.1186/s40792-018-0440-5

14 Comer TP, Beahrs OH and Dockerty MB: Primary squamous cell carcinoma and adenocanthoma of the colon. Cancer *28(5)*: 1111-1117, 1971. PMID: 5125659. DOI: 10.1002/1097-0142(1971)28:5<1111::aid-cncr2820280504>3.0.co;2-v

15 Steele VE and Nettesheim P: Unstable cellular differentiation in adenosquamous cell carcinoma. J Natl Cancer Inst *67(1)*: 149-154, 1981. PMID: 6942185.

16 Fang Y, Su Z, Xie J, Xue R, Ma Q, Li Y, Zhao Y, Song Z, Lu X, Li H, Peng C, Bai F and Shen B: Genomic signatures of pancreatic adenosquamous carcinoma (PASC). J Pathol *243(2)*: 155-159, 2017. PMID: 28722109. DOI: 10.1002/path.4943

17 Nobre AR, Albergaria A and Schmitt F: p40: a p63 isoform useful for lung cancer diagnosis - a review of the physiological and pathological role of p63. Acta Cytol *57(1)*: 1-8, 2013. PMID: 23221041. DOI: 10.1159/000345245

18 Liang M, Li J, Wang D, Li S, Sun Y, Sun T, Zhang J, Chen X, Li Q and Sun S: T-cell infiltration and expressions of T lymphocyte co-inhibitory B7-H1 and B7-H4 molecules among colorectal cancer patients in northeast China's Heilongjiang province. Tumour Biol *35(1)*: 55-60, 2014. PMID: 23873101. DOI: 10.1007/s13277-013-1006-6

19 Lee SE, Kim YJ, Sung M, Lee MS, Han J, Kim HK and Choi YL: Association with PD-L1 expression and clinicopathological features in 1000 lung cancers: a large single-institution study of surgically resected lung cancers with a high prevalence of EGFR mutation. Int J Mol Sci *20(19)*: 4794, 2019. PMID: 31561631. DOI: 10.3390/ijms20194794

20 Shinchi Y, Komohara Y, Yonemitsu K, Sato K, Ohnishi K, Saito Y, Fujiwara Y, Mori T, Shiraishi K, Ikeda K and Suzuki M: Accurate expression of PD-L1/L2 in lung adenocarcinoma cells: A retrospective study by double immunohistochemistry. Cancer Sci *110(9)*: 2711-2721, 2019. PMID: 31294893. DOI: 10.1111/cas.14128

21 Hirsch D, Gaiser T, Merx K, Weingaertner S, Forster M, Hendricks A, Woenckhaus M, Schubert T, Hofheinz RD and Gencer D: Clinical responses to PD-1 inhibition and their molecular characterization in six patients with mismatch repair-deficient metastatic cancer of the digestive system. J Cancer Res Clin Oncol *147(1)*: 263-273, 2021. PMID: 32776177. DOI: 10.1007/s00432-020-03335-2

22 Evert K, Stiegler C, Schäfer C, Palme K, Horndasch E, Reitinger S, Rau BM, Dietmaier W and Evert M: [Successful pembrolizumab therapy in metastasized adenosquamous carcinoma of the colon]. Pathologe *40(5)*: 540-545, 2019. PMID: 30350176. DOI: 10.1007/s00292-018-0546-3

23 Tomela K, Pietrzak B, Schmidt M and Mackiewicz A: The tumor and host immune signature, and the gut microbiota as predictive biomarkers for immune checkpoint inhibitor response in melanoma patients. Life (Basel) *10(10)*: 219, 2020. PMID: 32992737. DOI: 10.3390/life10100219

24 Mino-Kenudson M: Programmed cell death ligand-1 (PD-L1) expression by immunohistochemistry: could it be predictive and/or prognostic in non-small cell lung cancer? Cancer Biol Med *13(2)*: 157-170, 2016. PMID: 27458525. DOI: 10.20892/j.issn.2095-3941.2016.0009

25 Zito Marino F, Rossi G, Montella M, Botti G, De Cecio R, Morabito A, La Manna C, Ronchi A, Micheli M, Salatiello G, Micheli P, Rocco D, Accardo M and Franco R: Heterogeneity of PD-L1 expression in lung mixed adenocarcinomas and adenosquamous carcinomas. Am J Surg Pathol *44(3)*: 378-386, 2020. PMID: 31688140. DOI: 10.1097/PAS.0000000000001400

26 Hlaing AM, Furusato B, Udo E, Kitamura Y, Souda M, Masutani M and Fukuoka J: Expression of phosphatase and tensin homolog and programmed cell death ligand 1 in adenosquamous carcinoma of the lung. Biochem Biophys Res Commun *503(4)*: 2764-2769, 2018. PMID: 30100056. DOI: 10.1016/j.bbrc.2018.08.037

27 Liu Y, Dong Z, Jiang T, Hou L, Wu F, Gao G, He Y, Zhao J, Li X, Zhao C, Zhang W, Tian Q, Pan Y, Wang Y, Yang S, Wu C, Ren S, Zhou C, Zhang J and Hirsch FR: Heterogeneity of PD-L1 expression among the different histological components and metastatic lymph nodes in patients with resected lung adenosquamous carcinoma. Clin Lung Cancer *19(4)*: e421-e430, 2018. PMID: 29609906. DOI: 10.1016/j.cllc.2018.02.008

28 Shi X, Wu S, Sun J, Liu Y, Zeng X and Liang Z: PD-L1 expression in lung adenosquamous carcinomas compared with the more common variants of non-small cell lung cancer. Sci Rep *7*: 46209, 2017. PMID: 28387300. DOI: 10.1038/srep46209

29 Manglaviti S, Brambilla M, Signorelli D, Ferrara R, Lo Russo G, Proto C, Galli G, De Toma A, Occhipinti M, Viscardi G, Beninato T, Zattarin E, Bini M, Lobefaro R, Massa G, Bottiglieri A, Apollonio G, Sottotetti E, Di Mauro RM, Trevisan B, Ganzinelli M, Fabbri A, de Braud FGM, Garassino MC and Prelaj A: Immune-checkpoint inhibitors in advanced non-small cell lung cancer with uncommon histology. Clin Lung Cancer, 2021. PMID: 34334296. DOI: 10.1016/j.cllc.2021.06.013

30 Griswold CR, Kerrigan K and Patel SB: Combination of local ablative therapy and continuation of immune checkpoint inhibitor (ICI) therapy provides durable treatment response past oligometastatic progression in NSCLC: A case report. Case Rep Oncol *12(3)*: 866-871, 2019. PMID: 31824281. DOI: 10.1159/000504473

31 Reddy OL, Shintaku PI and Moatamed NA: Programmed death-ligand 1 (PD-L1) is expressed in a significant number of the uterine cervical carcinomas. Diagn Pathol *12(1)*: 45, 2017. PMID: 28623908. DOI: 10.1186/s13000-017-0631-6

32 Lee SM and Sung CO: PD-L1 expression and surgical outcomes of adenosquamous carcinoma of the pancreas in a single-centre study of 56 lesions. Pancreatology *21(5)*: 920-927, 2021. PMID: 33773917. DOI: 10.1016/j.pan.2021.03.004

33 Tanigawa M, Naito Y, Akiba J, Kawahara A, Okabe Y, Ishida Y, Ishikawa H, Hisaka T, Fujita F, Yasunaga M, Shigaki T, Sudo T, Mihara Y, Nakayama M, Kondo R, Kusano H, Shimamatsu K, Okuda K, Akagi Y and Yano H: PD-L1 expression in pancreatic adenosquamous carcinoma: PD-L1 expression is limited to the squamous component. Pathol Res Pract *214(12)*: 2069-2074, 2018. PMID: 30477643. DOI: 10.1016/j.prp.2018.10.006

34 Zajac M, Scott M, Ratcliffe M, Scorer P, Barker C, Al-Masri H, Rebelatto MC and Walker J: Concordance among four commercially available, validated programmed cell death ligand-1 assays in urothelial carcinoma. Diagn Pathol *14(1)*: 99, 2019. PMID: 31477145. DOI: 10.1186/s13000-019-0873-6

Received July 16, 2021
Revised September 14, 2021
Accepted September 15, 2021