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Case Report

Occurrence of colon tumors in a 16-year-old Japanese boy after hematopoietic stem cell transplantation for Diamond Blackfan anemia at age of 4: a case report

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Abstract: Diamond Blackfan anemia (DBA) is a congenital pure red cell aplasia mainly caused by a mutation in ribosomal protein genes. One of the proposed mechanisms for red cell aplasia in DBA is apoptosis caused by constitutive activation of tumor suppressor TP53 protein following defective ribosome biogenesis. Because of this close relationship between ribosome biogenesis and TP53 activation, patients with DBA are considered to be cancer-prone. The association between bone marrow failure and tumor susceptibility in DBA appears paradoxical. Also, the detailed information is lacking on malignancy occurring in patients with DBA. Here, we report a case of a 16-year-old Japanese boy suffering from multiple colon tumors during the follow-up after hematopoietic stem cell transplantation for DBA at the age of 4. Well differentiated tubular adenocarcinoma was detected at the rectum 12 years after the transplantation, followed by multiple tubular adenomas of low to high grade throughout the colon. Endoscopic submucosal dissection was performed for these tumors and the lesions were completely resected. These tumors did not show diffuse and strong TP53 positivity by immunohistochemistry, suggesting that TP53 mutation was not involved in the tumorigenesis as observed in conventional colorectal cancers. Microsatellite instability test and immunohistochemical examination of β-catenin and MLH1 proteins of these tumors showed that WNT signaling or microsatellite instability was less likely to be involved in the present tumors as observed in conventional left-sided or right-sided colon cancers, respectively. To our knowledge, this is the first case report of colon tumors associated with DBA.

Keywords: Colon tumor, Diamond Blackfan anemia, hematopoietic stem cell transplantation

Introduction

Diamond Blackfan anemia (DBA) is a congenital pure red cell aplasia. One of the breakthroughs in investigations for DBA was the discovery that around half of DBA cases are caused by mutations in genes for ribosome protein family, for example, ribosomal protein S19 [1]. This study has led to the conceptual development of ribosomopathy in bone marrow failure [2-4]. Until now, it has been reported that around 48% of DBA cases harbor mutations in genes for ribosomal protein family or GATA1 transcription factor, the latter of which is critical for erythroid lineage development [5-8]. In addition, around 2% of DBA cases were reported to carry mitochondrial gene deletion [6].

Two major hypotheses have been proposed for the mechanisms for red blood cell loss in DBA. The first hypothesis assumes that the loss or haploinsufficiency of the ribosomal protein leads to defective translation of some proteins critical for development of erythroid lineage, including GATA1 transcription factor. Indeed, defects in GATA1 protein expression was reported [9], although some of the defects may be due to mutations in GATA1 protein itself [5, 7, 8]. The other hypothesis presumes that the loss

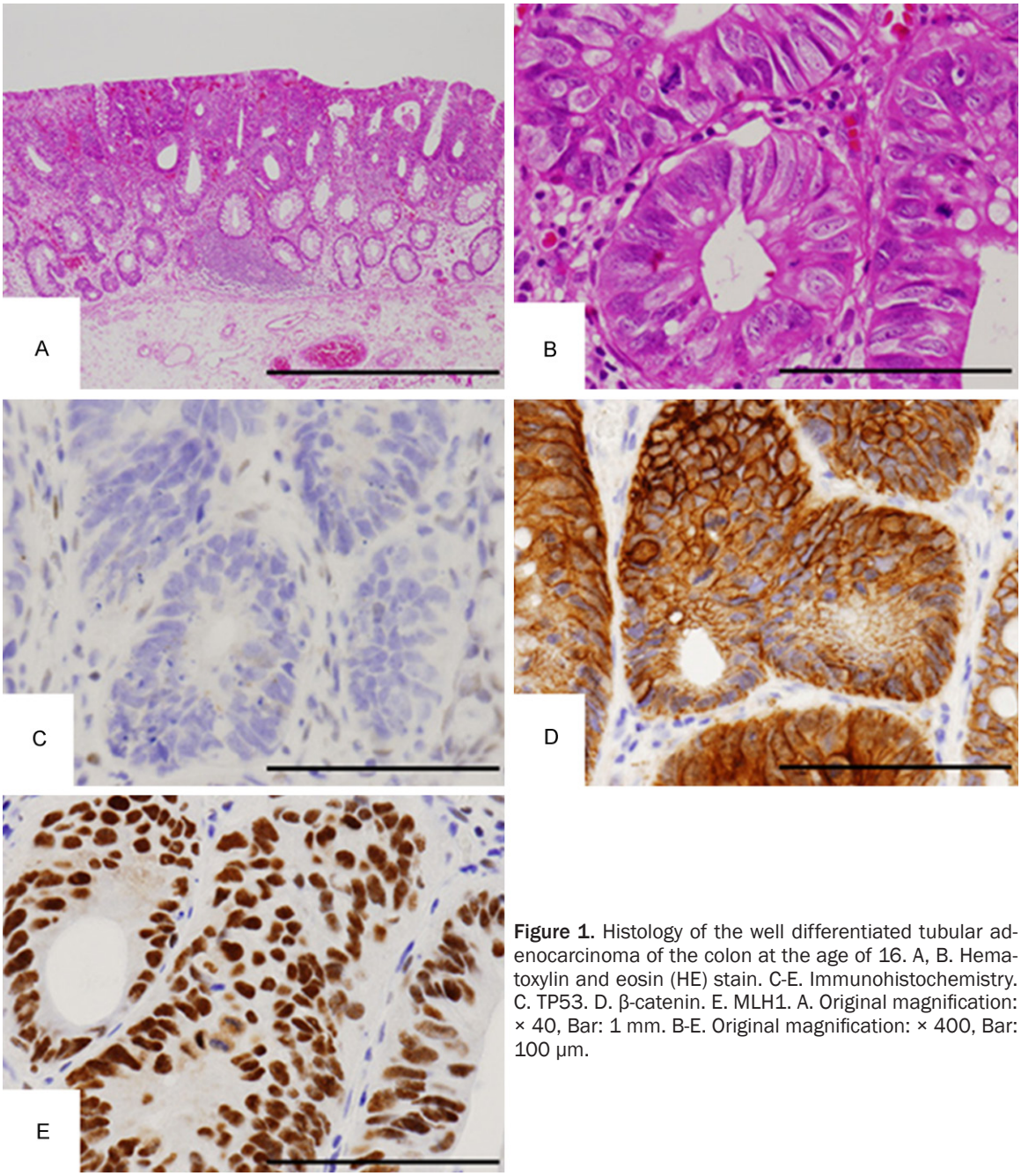

Figure 1. Histology of the well differentiated tubular adenocarcinoma of the colon at the age of 16. A, B. Hematoxylin and eosin (HE) stain. C-E. Immunohistochemistry. C. TP53. D. β-catenin. E. MLH1. A. Original magnification: × 40, Bar: 1 mm. B-E. Original magnification: × 400, Bar: 100 μm.

or haploinsufficiency of the ribosomal protein causes defective ribosome biogenesis, which results in leakage of the ribosomal proteins out of nucleoli. The ribosomal proteins leaked out of nucleolar trap MDM2 proteins, which ubiquitinate and degrade tumor suppressor protein TP53. In summary, defective ribosome biogenesis due to mutations in ribosomal protein genes activates tumor suppressor protein TP53 pathway, yielding apoptosis of red blood cells [2-4]. The latter hypothesis may also be applied for defective hematopoiesis in 5q- syndrome, a subtype of myelodysplastic syndrome: a ribosomal protein gene cluster is localized in chromosome 5q region [2]. These two examples illustrate a close relationship between defective ribosome biogenesis and TP53 activation.

In view of this, it is not unexpected but appears paradoxical that DBA is a risk factor for cancer susceptibility and the precise mechanism for this susceptibility has remained unclear. A

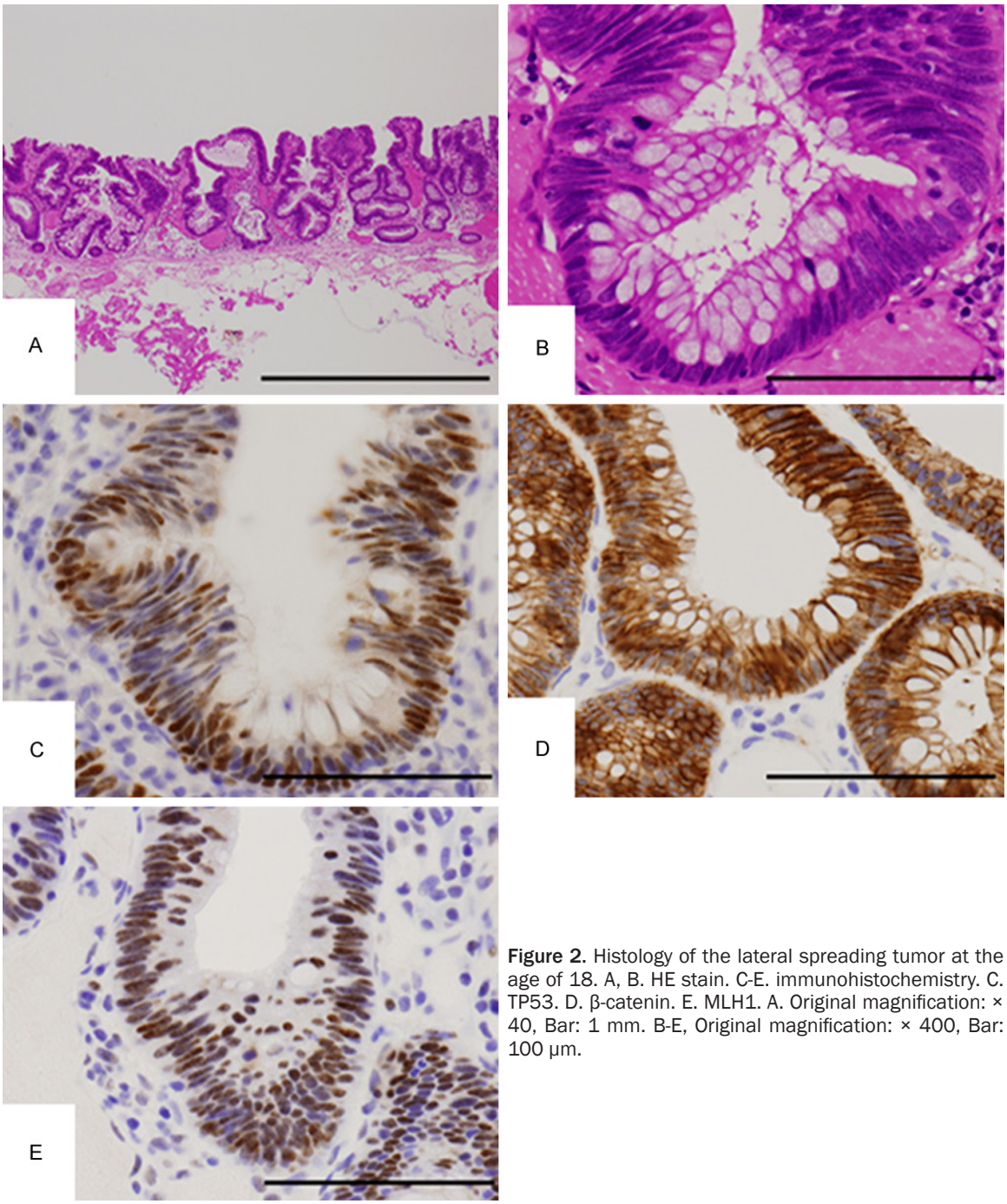

Figure 2. Histology of the lateral spreading tumor at the age of 18. A, B. HE stain. C-E. immunohistochemistry. C. TP53. D. β-catenin. E. MLH1. A. Original magnification: × 40, Bar: 1 mm. B-E, Original magnification: × 400, Bar: 100 μm.

cohort study reported that patients with DBA are cancer-prone; they are associated with increased incidence of solid tumor such as colon cancer and osteosarcoma, besides hematologic malignancy such as acute myeloid leukemia and myelodysplastic syndrome [10]. However, the detailed information on their precursor lesions and prognosis is lacking on malignancy occurring in patients with DBA.

In this paper, we reported a case of a 16-year-old Japanese boy suffering from multiple colon tumors 12 years after hematopoietic stem cell transplantation for DBA. The colon tumors included well differentiated tubular adenocarcinoma and tubular adenomas of low to high grade. To our knowledge, this is the first case report on the colon tumors associated with DBA.

Case report

A 16-year-old Japanese boy was admitted to our hospital because of aggravating diarrhea which had been persisting after cholecystectomy for cholelithiasis at the age of 13. At first, irritable bowel syndrome was suspected. Colonoscopy was performed and a 5 mm-sized sessile polyp was found at the rectum 10 cm from the anus. Histological examination of the biopsy of the tumor made us highly suspicious of well differentiated tubular adenocarcinoma. Endoscopic submucosal dissection of the tumor was successfully performed. The final diagnosis was well differentiated tubular adenocarcinoma *in situ* without submucosal invasion, associated with adenomatous component (**Figure 1A** and **1B**). The lesion was completely resected.

The patient suffered from severe anemia starting at birth, which turned out to be pure red cell aplasia due to DBA, diagnosed by the age of around 1 month. His family history was not remarkable except for lung cancer of his paternal grandfather. The anemia was resistant to steroid pulse therapy and totally 65 times of transfusion resulted in iron overload. At the age of 4, the patient underwent hematopoietic stem cell transplantation for DBA with conditioning regimen using cyclophosphamide (at 60 mg/kg/day for 2 days) and total body irradiation (at 3 Gy/day for 4 days). He overcame acute graft-versus-host disease (GVHD), which caused diarrhea of over 1000 ml/day, with the aid of calcineurin inhibitor administration and steroid pulse therapy. Other complications after the transplantation included convulsion, which appeared one and a half month later, and glucose intolerance. During the follow-up after the transplantation, overt hematological abnormalities were not noticed.

At the age of 17, the follow-up colonoscopy revealed eight colon polyps up to 8 mm in size at the transverse colon, sigmoid colon, and rectum. The biopsy showed that four out of the eight polyps were tubular adenoma of low grade (data not shown). In the rest of the polyps, atypical glands were sparsely observed (data not shown). The subsequent colonoscopy at the age of 18 showed again eight colon polyps up to 10 mm in size. In addition, a laterally spreading tumor (LST) of 9 × 12 mm in size was observed at the descending colon. All of these lesions were endoscopically resected. Histological examination of the resected specimen showed that all of the colon polyps were tubular adenoma of low grade except the LST of the descending colon, which was tubular adenoma with moderate to severe atypia (**Figure 2A**, **2B**).

The colon tumors occurring at such a young age of this patient made us suspicious of a hidden familial background for cancer susceptibility, including familial adenomatous polyposis and Lynch syndrome. These possibilities were examined for the adenocacinoma at the age of 16 and the LST at the age of 18. The adenocarcinoma cells were immunohistochemically negative for TP53 (**Figure 1C**). The LST cells were only partially and weakly positive for TP53 in a mosaic pattern, as observed typically for tubular adenoma (**Figure 2C**). These results of TP53 immunohistochemistry suggest that TP53 mutation was not involved in the tumorigenesis as observed in conventional colorectal cancers. In both tumors, the immunohistochemical analysis of β-catenin and MLH1 protein expression showed that the former was localized at the membrane (**Figures 1D** and **2D**) and the latter was expressed in the nucleus (**Figures 1E** and **2E**), suggesting that WNT signaling or microsatellite instability (MSI) related to DNA mismatch repair was less likely to be involved as observed in conventional left-sided or right-sided colon cancers, respectively. In fact, MSI test using 5 microsatellite markers (BAT26, BAT25, D2S123, D5S346 and D17S250) according to the revised Bethesda panel [11, 12] revealed that this case was MSI-low (data not shown). These results were inconsistent with the possibility of familial adenomatous polyposis or Lynch syndrome. The patient is now kept under close observation.

Discussion

In this paper, we reported a case of a 16-year-old Japanese boy suffering from multiple colon tumors 12 years after hematopoietic stem cell transplantation for DBA. The colon tumors included well differentiated tubular adenocarcinoma and tubular adenomas of low to high grade. Endoscopic submucosal dissection was successfully performed and the lesions were completely resected.

Reflecting presumed tumor-susceptibility of DBA, there were some reports on DBA-asso-

ciated tumors [13-17]. According to one cohort study, the observed-to-expected ratio for all cancers combined in DBA patients was 5.4 ($P < 0.05$) [10]. The observed-to-expected ratio was 36 for colon cancer, while the ratios were 287 for myelodysplastic syndrome, 28 for acute myeloid leukemia, and 33 for osteogenic sarcoma [10]. However, there were no case reports on the colon tumors associated with DBA.

Tumor susceptibility of this patient is considered to be related to DBA and its pathogenesis in itself. DBA is a congenital pure red cell aplasia mainly caused by a mutation in ribosomal protein genes. Two plausible hypotheses for DBA-associated tumors may be proposed. The first hypothesis is related to dysregulated protein translation caused by ribosomal dysgenesis in DBA [18, 19]. This may be illustrated by anti-tumor effects of mammalian target of rapamycin inhibitors and proteosomal inhibitors. The second hypothesis is related to apoptosis caused by constitutive activation of TP53 subsequent to trapping of TP53 inhibitor MDM2 by ribosomal proteins leaked out of nucleoli following ribosome dysgenesis [2-4]. It seems to be counterintuitive that in patients with DBA, cell-autonomous activation of apoptosis-inducing TP53 makes the cell more susceptible to tumorigenesis. It may be due to hormesis effect of TP53, in that low level of cell-autonomous activation of TP53 induces adaptive advantage for survival of the tumorous clones [20]. TP53 activation may work as a selective pressure for the survival and enrichment of the tumor cells with growth advantage. Indeed, it was reported that myelodysplastic syndrome might arise in the bone marrow of patients with aplastic anemia [21]. The association of osteosarcoma and cell-autonomous TP53 mutation was well established, as illustrated by the case of Li-Fraumeni syndrome [22]. Osteosarcoma in DBA patients may be related to cell-autonomous activation of TP53. In our case, both of the colon tumors of the patient did not show diffuse and strong positivity for TP53 by immunohistochemistry, suggesting that they were free from TP53 mutation. It remains to be determined whether cell-autonomous TP53 activation in the tumor cells in our case is involved in the tumorigenesis. In both of the tumors in the present case, the immunohistochemical analysis of β-catenin and MLH1 protein expression showed that the former was localized at the membrane and the latter was expressed in the nucleus. Together with MSI test (data not shown), these data suggest that WNT signaling or MSI was less likely to be involved as observed in conventional left-sided or right-sided colon cancers, respectively.

The factor involved in cancer susceptibility of this patient may be status post hematopoietic stem cell transplantation. It is generally known that the experience of hematopoietic stem cell transplantation is a risk factor for subsequent occurrence of malignancy [23]. In fact, there is a case report of the occurrence of osteosarcoma after hematopoietic stem cell transplantation for DBA [17]. It was considered that radiation and/or antitumor drugs used in the conditioning regimen may be associated with secondary malignancies following stem cell transplantation [23]. In our case, conditioning regimen included cyclophosphamide and total body irradiation.

This patient experienced acute GVHD and persistent diarrhea at least for last three years, which provided for the patient the opportunity for colonoscopy, leading to the discovery of the colon tumors. The diarrhea might be partly due to chronic GVHD, and the chronic GVHD might induce atypical glands that were sparsely observed in the colon (data not shown). It is tempting to speculate that these atypical glands were precursor lesions to adenoma; however, it is generally assumed that graft-versus-tumor effect is associated with the presence of GVHD, which may preclude the occurrence of the tumors.

Currently, the detailed information on the precursor lesions and prognosis is lacking on malignancy occurring in patients with DBA. Accumulation and examination of larger number of cases such as ours in future will be necessary to define the spectrum and prognosis of tumors associated with DBA. In the case of this patient, close follow-up will be required.

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Disclosure of conflict of interest

None.

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