CASE REPORT

Outcome of colorectal cancer in Diamond–Blackfan syndrome with a ribosomal protein S19 mutation

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Abstract

Diamond–Blackfan anemia is an autosomal dominant syndrome, characterized by anemia and a predisposition for malignancies. Ribosomal proteins are responsible for this syndrome, and the incidence of colorectal cancer in patients with this syndrome is higher than the general population. This patient's Diamond–Blackfan anemia was caused by a novel ribosomal protein S19 gene mutation, and he received chemotherapy for colorectal cancer caused by it. In his cancer, ribosomal proteins S19 and TP53 were overexpressed. He received 5FU and cetuximab; however, his anemia made chemotherapy difficult, and he did not survive long. Patients with Diamond–Blackfan anemia should be screened earlier and more often for colorectal cancer than usual.

Abbreviations

CRC	Colorectal cancer
DBA	Diamond–Blackfan anemia
O/E ratio	Observed/expected ratio
RPS19	Ribosomal protein S19
CT	Computed tomography
PS	Performance status
Cmab	Cetuximab
FL	5-Fluorouracil and calcium levofolinate hydrate
PFS	Progression-free survival
RPL	Ribosomal protein L
D130fs	Aspartic acid at codon 130 with subsequent frame-shifting

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Introduction

Diamond–Blackfan anemia (DBA) is characterized by red cell aplasia and congenital anomalies [1]. The majority of DBA cases result from autosomal dominant, loss of function mutations in approximately 20 genes encoding subunits associated with ribosomal proteins [1]. Approximately 25% of DBA cases are associated with ribosomal protein S19 (RPS19) gene mutations [2]. RPS19 is a 16-kDa protein required for the maturation of 18S rRNA and plays a role in the biogenesis of the 40S small ribosomal subunit [3]. Mice bearing the germ line mutations in this gene display anemia [4]. Decreased levels of RPS19 reduce rRNA synthesis in both erythroid and non-erythroid cell lines [5] and have been implicated in defective erythropoiesis. *RPS19* cDNA was first isolated from colon tissue, and it is expressed to a greater degree in colorectal cancer (CRC) tissue than the surrounding normal colon tissue [6]. An increased risk for CRC has recently been described in patients with DBA [7]. The observed/expected (O/E) ratio for CRC was 45, while the O/E ratio for all site malignancies was 4.8. In this report,

we describe the characteristics of advanced CRC in a DBA patient and his course of treatment in order to highlight the difficulties.

Case report

The patient was a 41-year-old male. He had suffered from red cell aplasia from birth and was diagnosed with DBA. There was no family history of DBA. He had been treated with steroids as an infant resulting in bilateral cataracts. Since then, his DBA had been treated with periodic red cell transfusions. His previous physician finally noted jaundice at age 40, and endoscopic retrograde cholangiopancreatography noted obstruction of the extrahepatic biliary duct, so a stent was introduced to improve the obstructive jaundice. Computed tomography (CT) as part of his evaluation found a tumor in the sigmoid colon, which biopsy revealed to be a well-differentiated adenocarcinoma. Sigmoidectomy was performed to prevent obstruction, but the tumor had invaded the abdominal wall. Furthermore, metastasis to multiple lymph nodes surrounding the extrahepatic biliary duct was apparent at surgery. This case was diagnosed as stage IVb by the Union for International Cancer Control (UICC) TNM staging system: T4b, N2b, M1a, after which he was transferred to our hospital for chemotherapy. At his

first visit, we found the patient to be emaciated and pale, with an Eastern Cooperative Oncology Group performance status (PS) of 3. He was emergently admitted to the hospital, where CT examination revealed a left lung nodule, para-aortic, peri-celiac arterial, and left subclavian lymph node swelling, a mild pleural effusion, moderate peritoneal ascites, and a nodule in the greater omentum (Fig. 1a–e). An asymptomatic blood clot found in his left pulmonary artery was treated with edoxaban. Laboratory data were as follows: WBC: $4300/\mu L$, RBC: $233 \times 10^4/\mu L$, hemoglobin: 7.6 g/dL, HCT: 22.4%, Plt: $21.7 \times 10^4/\mu L$, AST: 40 U/L, ALT: 42 U/L, total bilirubin: 0.4 mg/dL, creatinine: 0.6 mg/dL, CEA: 24.7 ng/mL, CA19-9: 31.5 U/mL. Endoscopic examination detected extra-luminal stenosis of the duodenal bulb, possibly due to abdominal dissemination, and local recurrence at the anastomosis site in the colon (Fig. 1f, 1g). He accepted supportive care alone due to his poor PS. Intravenous hyperalimentation for three weeks and periodic red blood cell transfusion gradually improved his PS, at which time chemotherapy was considered. As his colon cancer had no detectable mutations in the *RAS/BRAF* genes, cetuximab (Cmab), an anti-epidermal growth factor receptor antibody, was initiated together with a continuous infusion of 5-fluorouracil (5FU) and calcium levofolinate hydrate (FL). He was treated with 60% of the standard dose of 5FU due to his frail physical status. He did not achieve partial response of
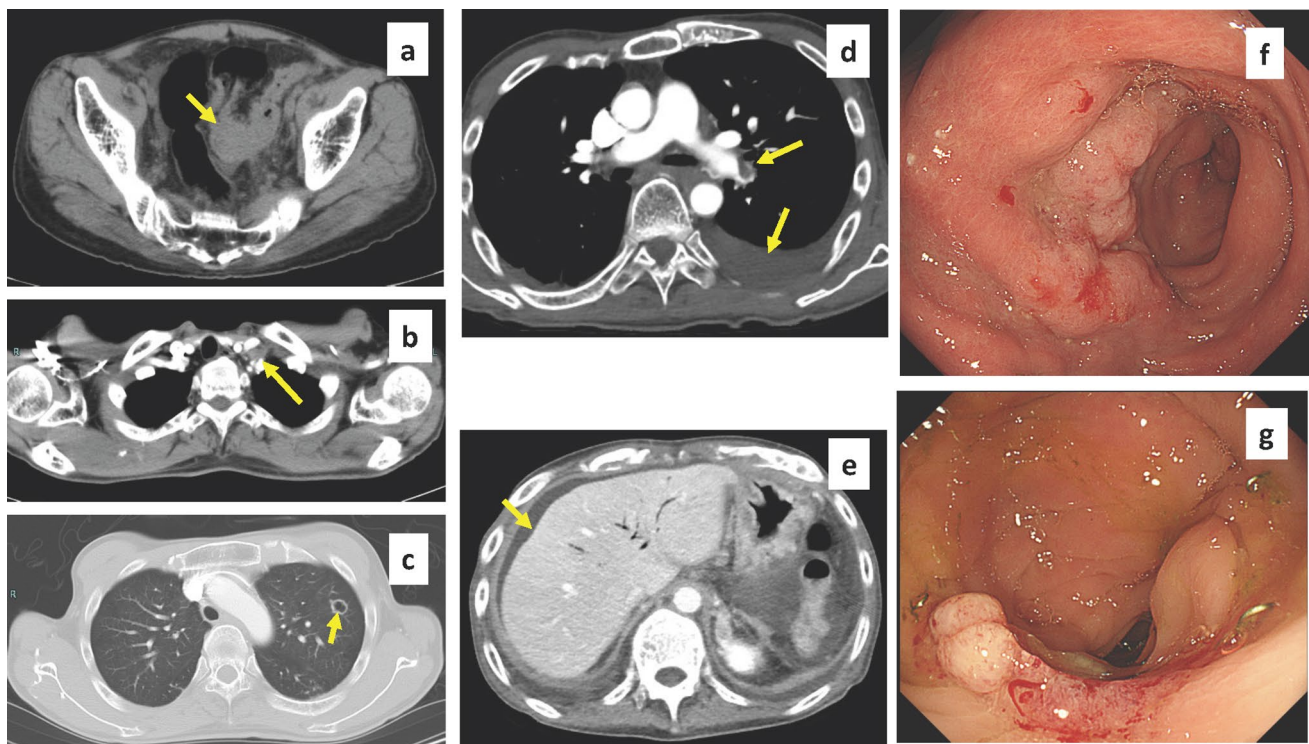

Fig. 1 Clinical features. **a** Sigmoid colon tumor, (**b**) left subclavian lymph node swelling, (**c**) left lung tumor, (**d**) pleural effusion, (**e**) ascites, (f) duodenal invasion, (**g**) recurrence at the anastomosis site

Response Evaluation Criteria in Solid Tumors criteria, but all lesions were controlled with 20 cycles of Cmab plus FL for 11 months. His progression-free survival (PFS) was estimated to be 290 days. During this period, no adverse events other than grade 3 anemia, grade 2 hypomagnesemia, and skin rash occurred. Following 3 months of in-hospital chemotherapy, he was able to be managed as an outpatient. Following disease progression, oxaliplatin was added to FL, but it could not control his condition. Second-line chemotherapy was commenced under his consent. After one week, there were no abnormalities including laboratory data. However, on the 10th day of chemotherapy, the patient died suddenly. Autopsy imaging indicated rapid progression of pleural effusion. He died 328 days after the start of chemotherapy.

Genetic testing was conducted for DBA-related genes including *RPS19*, *RPS27*, *RPS7*, *RPS14*, *RPS10*, *RPS24*, *RPS26*, *RPS17*, *Ribosomal Protein L (RPL)11*, *RPL5*, *RPL26*, *RPL27*, and *RPL35A* as well as *TP53* and *GATA1* prior to his death. Among them, only *RPS19 c.384_385del* resulting in p.D130fs was detected as a germ line mutation. He had no family history of DBA and was likely the result

of a spontaneous mutation. He was not married and had no children. Bone marrow biopsy indicated pure red cell aplasia (Fig. 2a–e). Immunohistochemistry revealed RPS19 expression as well as TP53 in the erythroblasts (Fig. 2f–h). However, compared to the normal surrounding tissue, RPS19 and TP53 were overexpressed in the colorectal cancer cells (Fig. 3).

Discussion

DBA, an autosomal dominant syndrome with an incidence of 5–10 per million live births, predisposes carriers to many types of malignancies [8]. We report a case of DBA with an *RPS19 c.384_385del* (p.D130fs) mutation in his germ line which has never been reported previously. Campagnoli et al. reported four *RPS19 c.383_384delAA* mutations resulting in p.D130Sfs*23 among DBA patients [9], and our mutation is almost identical to theirs at the amino acid level. Gregory et al. reported on the structure and function of RPS19, and that aspartic acid at codon 130
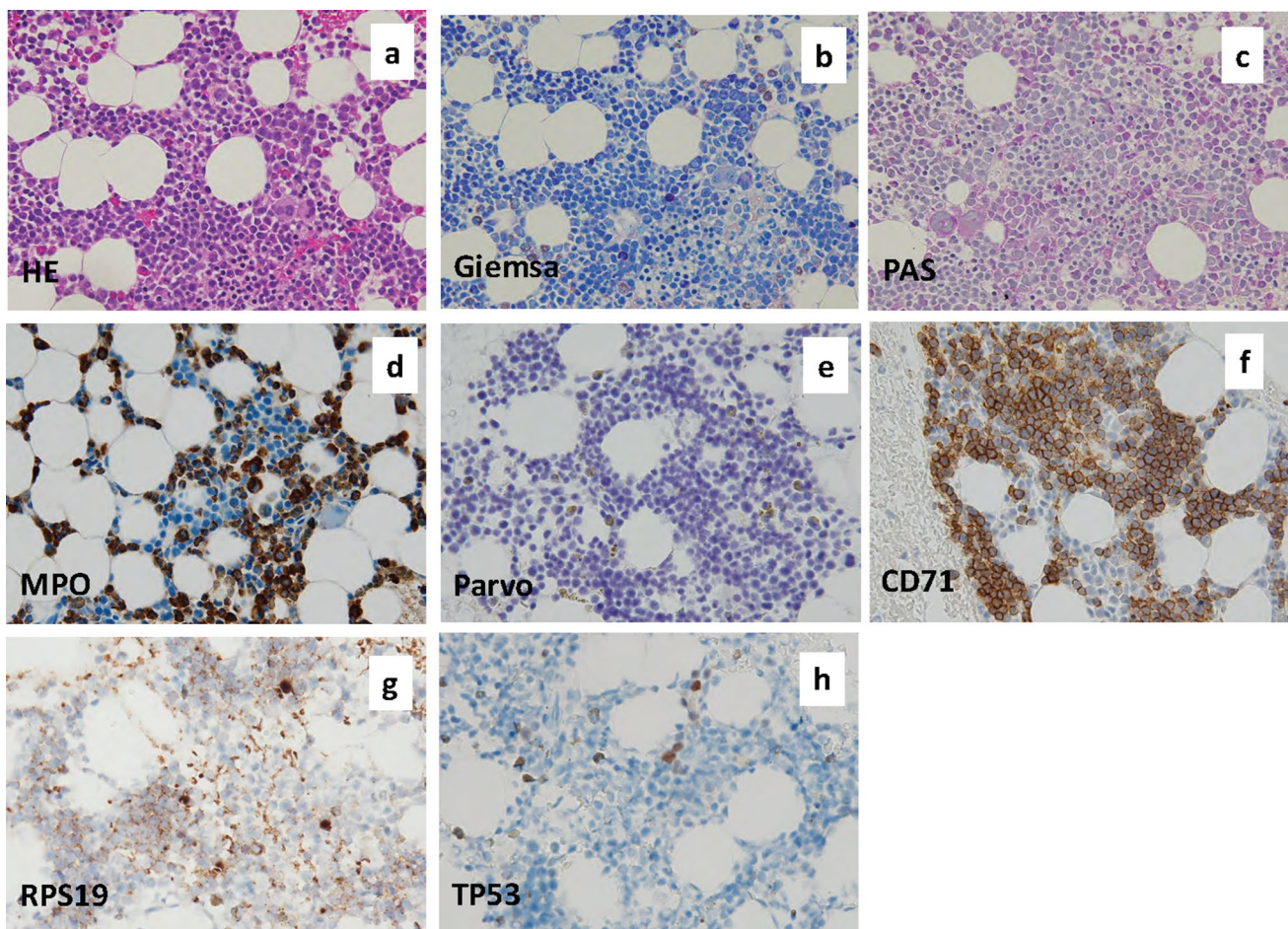

Fig. 2 Bone marrow immunohistochemistry. **a** HE, (**b**) Giemsa, (**c**) PAS, (**d**) myeloperoxidase, (**e**) parvovirus, (**f**) CD71, (**g**) RPS19, (**h**) TP53
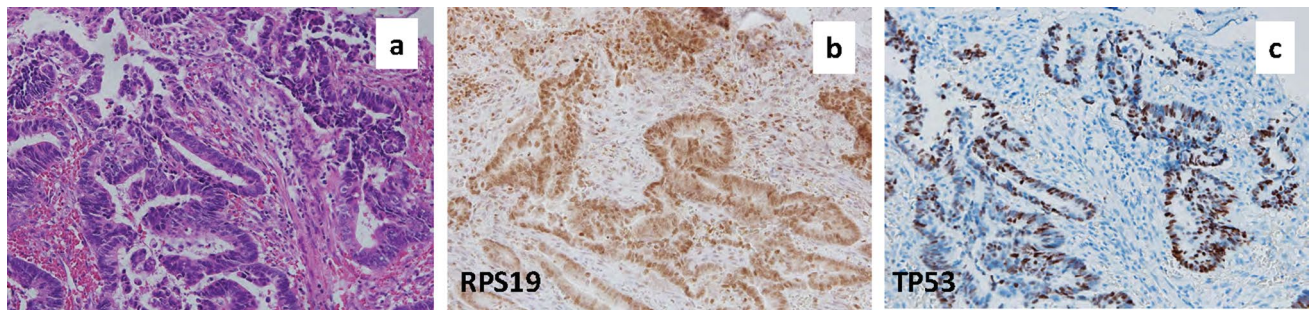
Fig. 3 Colon cancer immunohistochemistry. **a** HE, (**b**) RPS19, (**c**) TP53

is located in the alpha-helix 6 of RPS19 [10]. The next amino acid, leucine at codon 131, faces the hydrophobic core and is significantly involved in HSP19 function. We believe that *RPS19 c.384_385del* (p.D130fs) causes a loss of function and is responsible for DBA. Campagnoli et al. did not report on carcinogenesis in individuals bearing p.D130Sfs*23. These RPS19 mutations could interfere with ribosomal biogenesis. An *RPS19* knockout mutation in mice is lethal, and a heterozygous *RPS19* mutation leads to haplo-insufficiency of RPS19 with severe anemia [11]. The exact mechanism causing anemia is still unknown. However, the wild-type *RPS19* allele might compensate by expression in normal tissue. A knockout mutation of *RPS19* in zebrafish leads to erythroid defects, with an over-expression of TP53 [12]. The specific relationship between down-regulation of RPS19 and overexpression of TP53 is still not clear. However, it is believed that the extra-ribosomal function of RPS19 might contribute to tumor progression, invasion, and metastasis [13]. In our case, a novel *RPS19* mutation, *RPS19 c.384_385del* (p.D130fs), might have been responsible for DBA, and the high level of both RPS19 and TP53 expressions was observed in the colon cancer cells of this patient.

It has been indicated that DBA-related ribosomal proteins such as RPL11 can suppress TP53 degradation by MDM2, resulting in TP53 overexpression [14]. RPS19 is overexpressed in colon tissue, where it was first discovered. Haplo-insufficiency of RPS19 in colon cells of DBA patients can be compensated for and may be upregulated during colo-rectal carcinogenesis. Similarly, RPS19 overexpression may activate TP53 expression in CRC.

Elevated levels of RPS19 expression are associated with a poorer prognosis for colon cancer, while lower levels are associated with a better prognosis [6]. TP53 overexpression is also associated with an increased risk of death, but it does not relate to chemotherapeutic effect [15]. The progression-free survival (PFS) of the first line drugs, FL plus Cmab, was 9.6 months in this case which is comparable to the outcome of FOLFOX4 plus Cmab in the pivotal TAILOR trial (median survival of 9.2 months) [16].

This is the first report to our knowledge of chemotherapy for colon cancer in a DBA patient. Chemotherapy itself was very difficult in the presence of his significant anemia. Chemotherapy for patients with DBA should be considered on an individual basis. In his case, the frequency of red cell transfusion did not change. The patient was considered able to tolerate FL despite his anemia. Recent data indicate a one-year survival rate of 75% in stage IV, left-sided colon cancer [17]. This patient survived for 328 days from the start of chemotherapy which is less than the median survival time. For these reasons, and the frequency of occurrence, we conclude that DBA patients should be surveyed much more carefully for CRC.

Compliance with ethical standards

References

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