Esophageal Squamous Cell Carcinoma Developed 11 Years After Allogeneic Bone Marrow Transplantation for Acute Lymphatic Leukemia

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Younger patients (aged <30 years) presenting with esophageal cancer are rare. Bone marrow transplantation offers a curative therapy in patients with malignant and nonmalignant lymphohematopoietic diseases and other disorders. However, one important late complication in transplantation survivors is the development of secondary malignancies including solid tumors. Although some solid cancers have been demonstrated to occur after bone marrow transplantation, only a few cases of esophageal squamous cell carcinoma have thus far been reported. We herein describe the case of a 27-year-old male with esophageal squamous cell carcinoma, who was diagnosed with T-cell-type acute lymphatic leukemia at the age of 12 and relapsed 5 years later. He achieved a second complete remission and underwent bone marrow transplantation at the age of 17. A genetic analysis revealed germ-line lineage-derived chimeric cellular populations of the donor and patient on both the esophageal squamous cell carcinoma and non tumorous portions of the patient’s esophageal mucosa with a preponderance of the patient’s germ-line lineage-derived cells, suggesting that repopulated donor-derived hemopoietic stem cells in the esophageal epithelia only partially contributed to the carcinogenesis of esophageal squamous cell carcinoma several years after bone marrow transplantation. Multiple events occurring during the course of treatment for primary hematological disorder may play an important role in the development of esophageal squamous cell carcinoma.

Key words: ESCC – bone marrow transplantation – short tandem repeat analysis

INTRODUCTION

Esophageal cancer (EC), histologically including both esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), continues to be a fatal disease throughout the world. ESCC is the most common esophageal malignancy internationally, thus accounting for more than 90% of cases of EC (1). In both low- and high-incidence areas of the world, EC is rare (0.5–6.3%) in individuals younger than age 30 (2).

Allogeneic hematopoietic stem cell transplantation from bone marrow, peripheral blood or cord blood offers curative...
therapy for malignant and nonmalignant lymphohematopoietic diseases and other disorders. Improvements in outcomes after transplantation have led to continuous increases in long-term survival among recipients. The development of secondary neoplasms is one of the serious late complications that can occur after transplantation and is associated with considerable morbidity and mortality (3,4). There is evidence that epithelial repopulation by bone marrow-derived stem cells can induce cancer in host tissues if provided with any stimulation in most epithelial malignancies (5–8). Although previous studies using large cohorts of transplant recipients have demonstrated that a variety of solid tumors can develop secondarily to bone marrow transplantation (BMT), only a few cases of ESCC occurring after allogeneic hematopoietic stem cell transplantation have been reported in these cohorts (4,9–13) (Table 1) and clinical case reports (14,15).

In this report, we describe the case history of a young adult male diagnosed with ESCC at the age of 27 who had been previously diagnosed with T-cell-type acute lymphoblastic leukemia (T-ALL) and eventually achieved a complete remission at age 17 by means of allogeneic BMT followed by prolonged chronic graft-versus-host disease (GVHD). We also attempt to address the question of the origin (donor or recipient) of the ESCC that developed after allogeneic BMT using a short tandem repeat (STR) analysis.

### CASE REPORT

**PATIENT**

A 12-year-old male patient was diagnosed with T-ALL. He achieved a complete response after undergoing combination chemotherapy according to the protocol of Tokyo Children’s Cancer Study Group (TCCSG) Studies L92–13 (16).

**Table 1.** Incidence of secondary solid tumors including ESCC after bone marrow transplantation (BMT)

<table>
<thead>
<tr>
<th>BMT recipients</th>
<th>No. of cases developed secondary tumors</th>
<th>ESCC (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>No. of institutes</td>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>19229</td>
<td>235 (IBMTR)</td>
<td>80 (0.4%)</td>
<td>Curtis et al. (25)</td>
</tr>
<tr>
<td>1036</td>
<td>45 (EBMT)</td>
<td>53 (5.1%)</td>
<td>Kolb et al. (9)</td>
</tr>
<tr>
<td>2129</td>
<td>1 (USA)</td>
<td>29 (1.4%)</td>
<td>Bhatia et al. (4)</td>
</tr>
<tr>
<td>605</td>
<td>1 (China)</td>
<td>9 (1.5%)</td>
<td>Au et al. (10)</td>
</tr>
<tr>
<td>1451</td>
<td>16 (NBMTG)</td>
<td>19 (1.3%)</td>
<td>Shimada et al. (11)</td>
</tr>
<tr>
<td>170</td>
<td>1 (Taiwan)</td>
<td>8 (4.7%)</td>
<td>Chen et al. (12)</td>
</tr>
</tbody>
</table>

BMT, bone marrow transplantation; ESCC, esophageal squamous cell carcinoma; IBMTR, International Bone Marrow Transplantation Registry; EBMT, European Bone Marrow Transplantation Registry; NBMTG, Nagoya Blood and Marrow Transplantation Group.

As a relapse in the bone marrow was confirmed at the age of 17, the patient was initially treated with ALL-oriented combination chemotherapy modified from the protocol of St. Jude Total Therapy XI (17) as induction chemotherapy; however, he showed only a partial response. Since the patient obtained a complete response to a second round of an acute myeloid leukemia-oriented regimen modified from the TCCSG M91–13 protocol (18), continuous intensification chemotherapy was administered to prepare for transplantation of allogeneic hematopoietic stem cells from bone marrow. The transplantation conditioning regimen included cyclophosphamide, etoposide and total body irradiation (TBI) (12 Gy in six fractions over 3 days), and GVHD prophylaxis included short-term methotrexate and cyclosporine A. On Day 0, bone marrow from a compatible blood type and human leukocyte antigen identical sibling donor was infused. Bone marrow engraftment was eventually confirmed on Day 28. On Day 14, erythema palmaris diagnosed as acute GVHD (grade 1) was observed, which spontaneously regressed without treatment. On Day 34, intraoral vitiligo appeared, and the patient was diagnosed with extensive chronic GVHD according to the results of a biopsy of the intraoral tissue. In addition, the patient experienced a restrictive pulmonary disorder with cytomegalovirus antigenemia and pneumocystis carinii pneumonia starting on Days 67 and 68, respectively. Therapy for chronic GVHD and the pulmonary diseases included steroid pulse therapy, sulfamethoxazole, trimethoprim and dihydroxyphenylglycine. Finally, the patient received immunosuppressive agents for 3 months.

The patient developed dysphagia at the age of 27, and an evaluation revealed EC. An upper gastrointestinal series (G-Is) showed an ulcerative lesion 3 cm in diameter in the lower thoracic esophagus (Fig. 1A). Esophago-gastro-duodenoscopy (EGD) revealed an irregularly elevated lesion with ulceration in the right wall 33–36 cm from the incisors (Fig. 1B). An endoscopic biopsy specimen yielded a diagnosis of moderately differentiated ESCC. Computed tomography (CT) showed no mediastinal or abdominal lymph node swelling or distant metastasis. Blood chemistry revealed only slight depression in the hematopoietic function (white blood cell count 2.1 × 10³/ml, red blood cell count 242 × 10³/ml, hemoglobin level 8.9 g/dl and platelet count 12.0 × 10³/ml); however, a bone marrow aspiration indicated normal findings. The clinical diagnosis was lower thoracic ESCC T2N0M0 stage IB (UICC-TMN, 7th edition). After the blood count was elevated to within normal ranges with an oral iron supplement and vitamin B12 preparation, thoracoscopic esophagectomy with lymphadenectomy was performed. The resected specimen was an apparently circumscribed ulcerative lesion similar to a caldera measuring 31 × 24 mm (Fig. 1C) that showed moderately differentiated SCC invading the adventitia (Fig. 1D) on histopathologic examination.

The patient had an uncomplicated postoperative course for 18 months until the pancytopenia reoccurred. He was diagnosed with acute myelocytic leukemia following a bone
marrow biopsy, and an STR analysis demonstrated that the leukemic cells exhibited the recipient’s germ-line lineage-derived pattern. Although he received chemotherapy, the patient died 4 months after recurrence.

MOLECULAR TEST

In order to determine whether donor-derived cells were present in the ESCC tumor, we compared the genetic pattern of DNA obtained from the patient’s peripheral blood, which was derived from the donor, with that of formalin-fixed paraffin-embedded primary ESCC tumor samples and corresponding noncancerous esophageal mucosa using macrodissection. STR analysis of eight STR loci (CSF1PO, D13S317, D16S539, D5S818, D7S820, TH01, TPOX and vWA) and the amelogenin locus was performed using powerPlex1.2 system (Promega, Madison, WI) and Applied Biosystems 3130 × 1 Genetic Analyzers with GeneMapper ver. 3.7 (Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions.

Figure 1. (A) Upper gastrointestinal series (G-Is) revealed an ulcerative lesion 3 cm in diameter at the lower thoracic esophagus (left, side view; right, front view). (B) Esophago-gastro-duodenoscopy revealed an apparently circumscribed ulcerative lesion at 33–36 cm from incisors. (C) The resected specimen showed an apparently circumscribed ulcerative lesion like a caldera measuring 31 × 24 mm. Bar, 10 mm. (D) Histopathological examination showed that the tumor was a moderately differentiated squamous cell carcinoma invading adventitia (hematoxylin and eosin staining). Bars, 1 mm (upper) and 10 μm (lower).
Based on the results of STR analysis, two distinct genetic patterns were observed in both the patient’s nontumorous esophageal mucosa and the ESCC tumor. In addition, a donor-derived pattern was clearly observed in the patient’s peripheral blood and germ-line lineage-derived pattern (Fig. 2, Table 2). These two patterns formed a chimeric population consisting predominantly of the patient’s germ-line lineage and to a lesser extent, as estimated by the fluorescence intensity of each peak on electropherogram, the donor graft. This chimeric pattern with a predominance of the recipient germ-line lineage in the esophagus, especially in the tumorous region, indicated that the ESCC primarily originated from recipient germ-line lineage-derived cells, although it is possible that repopulation of the transplanted bone marrow-derived cells of the donor as well as host epithelial stem cells in the patient’s esophageal epithelia might have occurred after BMT.

DISCUSSION

EC including ESCC is rare in individuals aged younger than 30 years in both low- and high-incidence areas (2). The development of second malignancies, including ESCC, following BMT is recognized as one of the most serious complications in transplant recipients, and BMT performed at the age of 17 may have contributed to the pathogenesis of ESCC as a probable risk factor in the present case. Although the mechanisms underlying the development of second malignancies are unclear, previous studies have shown TBI (19,20) and chronic GVHD (9) to be risk factors for secondary malignancies. It has also been shown that persistent chronic GVHD and prolonged use of immunosuppressive therapy place patients at an especially high risk for SCC of the oral cavity and skin (21). Our patient also had these risk factors, which may have contributed to the development of ESCC, although SCC of the esophagus (ESCC) is unlikely to be a second malignancy (Table 1). In addition, it is known that secondary cancer can develop in organs affected by GVHD (22). This phenomenon might have occurred in this case and can be an adverse effect of GVHD.

There is evidence that epithelial repopulation by donor-derived bone marrow stem cells occurs after BMT and that bone marrow-derived stem cells can induce cancer in host tissues given adequate stimulation such as chronic inflammation (5–8,21). Most studies conducted to date concerning the role of donor-derived bone marrow stem cells in transplant recipients have relied on the identification of sex chromosomal anomalies in sex-mismatched transplants and failed to reach consistent conclusions for the origins of the tumors (10,23).

In the present case, we found a chimeric, genetically mixed population consisting predominantly of the patient’s germ-line lineage in the patient’s normal and neoplastic epithelia (Fig. 2, Table 2). These two patterns formed a chimeric population consisting predominantly of the patient’s germ-line lineage and to a lesser extent, as estimated by the fluorescence intensity of each peak on electropherogram, the donor graft. This chimeric pattern with a predominance of the recipient germ-line lineage in the esophagus, especially in the tumorous region, indicated that the ESCC primarily originated from recipient germ-line lineage-derived cells, although it is possible that repopulation of the transplanted bone marrow-derived cells of the donor as well as host epithelial stem cells in the patient’s esophageal epithelia might have occurred after BMT.

Table 2. Results of STR analysis

<table>
<thead>
<tr>
<th>STR locus</th>
<th>Peripheral blooda</th>
<th>Nontumorous esophageal mucosaa,b</th>
<th>ESCC tumora</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF1PO</td>
<td>12</td>
<td>11, 12, 13</td>
<td>11, 12, 13</td>
</tr>
<tr>
<td>D13S317</td>
<td>8, 10</td>
<td>8, 10</td>
<td>8, 10</td>
</tr>
<tr>
<td>D16S539</td>
<td>9</td>
<td>9, 10, 11</td>
<td>9, 10, 11</td>
</tr>
<tr>
<td>D5S818</td>
<td>8, 9</td>
<td>8, 9, 11, 13</td>
<td>8, 9, 11, 13</td>
</tr>
<tr>
<td>D7S820</td>
<td>8, 11</td>
<td>8, (11), 12</td>
<td>8, (11), 12</td>
</tr>
<tr>
<td>TH01</td>
<td>7, 9</td>
<td>(7), 9</td>
<td>(7), 9</td>
</tr>
<tr>
<td>TPOX</td>
<td>8, 11</td>
<td>8, 9, 11</td>
<td>8, (9), 11</td>
</tr>
<tr>
<td>vWA</td>
<td>15, 17</td>
<td>15, 17</td>
<td>(15), 17, 18</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>X, Y</td>
<td>X, Y</td>
<td>X, Y</td>
</tr>
</tbody>
</table>

Note: the number in each cell represents the identified variant alleles for each locus, and refers to complete 4 bp repeat units for each allele.

aPredominant variant alleles estimated by the intensity of each peak on the electropherogram were in boldface type, whereas those showing relatively very small peak were in parentheses.

bUnderline, possible variant allele of the patient’s germ-line lineage.

Figure 2. Representative results of short tandem repeat (STR) analysis of genomic DNA in two STR loci (left, TPOX; right, D5S818).
noncancerous esophageal mucosa were obtained through macrodissection, and almost identical results in each tissue were confirmed by carefully repeating the sample preparations and analyses.

Taken together, the results suggested that the normal epithelial and cancer cells in the esophageal mucosa were not primarily derived from donor-derived pluripotent stem cells in our patient. Therefore, it is possible that risk factors present both before and after BMT may have contributed to the carcinogenesis of ESCC in this case. Further research is needed to illuminate poorly understood risk factors associated with carcinogenesis of the esophagus in younger people.

Conflict of interest statement
None declared.

References