Tandem triplication of the long arm of chromosome 1, trp(1)(q21q32), in two cases with myelodysplastic syndromes

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We report here two cases with myelodysplastic syndromes (MDS) carrying tandem triplication of the long arm of chromosome 1. The first case involved a 66-year-old man who presented with thrombocytopenia and monocytosis. Although hematopoietic dysplasia was minimal, cytogenetic analysis of the bone marrow revealed trp(1)(q21q32) in all metaphases analyzed, and the karyotype was determined as 46,XY,trp(1)(q21q32),add(3)(p25)[20]. The second case was a 58-year-old woman who initially presented with bilateral pulmonary infiltration indicative of alveolar proteinosis. Blood tests showed pancytopenia with few blasts, and the bone marrow picture fulfilled the criteria of refractory cytopenia with multilineage dysplasia. The karyotype was 46,XX,trp(1)(q21q32)[20]. Fluorescence in situ hybridization using a probe for the pre-B-cell leukemia homeobox 1 gene localized on 1q23 showed 3 fluorescence signals tandemly aligned on the trp(1)(q21q32) chromosome. Because trp(1)(q21q32) was the sole chromosomal abnormality (case 2) or was accompanied by only a single additional abnormality (case 1), trp(1)(q21q32) may be the primary cytogenetic change in a small fraction of MDS cases.

Keywords: tandem triplication of 1q, trp(1)(q21q32), myelodysplastic syndromes, FISH

INTRODUCTION

Gains of the long arm of chromosome 1 (1q) are found in diverse hematological malignancies of both myeloid and lymphoid origin. Structural abnormalities leading to 1q gain include unbalanced translocation [t(1q)], tandem duplication [dup(1q)], isochromosome, and jumping translocation. In lymphoid tumors, t(1q)/dup(1q) is most frequently identified in Burkitt lymphoma or high-grade B-cell lymphoma in association with the primary MYC translocations, and is associated with a poor prognosis. On the other hand, in myeloid tumors, whole-arm unbalanced translocation der(1;7)(q10;p10), resulting in total trisomy for 1q, is observed in myelodysplastic syndromes (MDS) and acute myeloid leukemia with preceding MDS. Nevertheless, as der(1;7) (q10;p10) also results in monosomy for 7q, this abnormality may represent an equivalent to del(7q), which is one of the most common cytogenetic abnormalities in MDS.

Tandem duplication [dup(1q)] and tandem triplication of 1q [trp(1q)] have been described in sporadic case reports of MDS or related diseases, or have been listed as single cases in large series of MDS cases. We report here two cases with MDS that carried trp(1q) as the sole
cytogenetic abnormality or in association with another single abnormality. To confirm trp(1q), we performed fluorescence in situ hybridization (FISH) using a probe localized within the triplicated segment.

MATERIALS AND METHODS

G-banding

Bone marrow aspirates were incubated in culture medium overnight at 37°C in 5% CO₂ and then cultured in the presence of 0.1 μg/mL colcemid for 2 hr. After harvesting, the cells were treated with hypotonic solution and fixed with methanol:acetic acid (3:1). Chromosomes were banded by trypsin-Giemsa staining, and the results of chromosome analysis were described according to the ISCN 2013.¹⁶

FISH

The Vysis LSI TCF3/PBX1 Dual Color, Dual Fusion Translocation Probe was purchased from Abbott Laboratories (Abbott Park, IL, USA). The probe was hybridized with cytogenetic preparations or bone marrow smear slides. Denaturing of the chromosome/probe, hybridization, and washing conditions were as recommended by the manufacturer. FISH results were analyzed using a fluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped with DAPI, fluorescein isothiocyanate (FITC), and tetramethylrhodamine B isothiocyanate (TRITC) fluorescence filters, as well as a DAPI/FITC/TRITC triple band-pass filter (Nikon Corporation).

RESULTS

Case 1

The first case involved a 66-year-old man who was under diagnostic workup of lung cancer. His hemoglobin level was 13.3 g/dL, white cell count 7.94 × 10³/µL, and platelet count 45 × 10³/µL. The white cell differential was 21.0% lymphocytes, 18.0% monocytes (absolute monocyte count, 1.43 × 10³/µL) including immature forms, 44.0% segmented neutrophils, 15.0% banded neutrophils (absolute neutrophil count, 4.68 × 10³/µL), and 2.0% metamyelocytes. Giant platelets were also observed. The bone marrow showed hypocellularity and the differential count revealed 24.3% monocytes and 0.4% blasts. There were a few hypogranular neutrophils occasionally associated with Döhle bodies and giant granulocytes, and erythroid cells showed a mild megakaryoblastoid appearance (Figure 1). Megakaryocytes were too few to evaluate their morphology. G-banding analyses of the bone marrow aspirates revealed the tandem triplication trp(1)(q21q32) and additional materials of the short arm of chromosome 3 in all metaphases analyzed. The karyotype was 46,XY,trp(1)(q21q32),add(3)(p25)[20] (Figure 2A, Figure 3).

As a bronchoscopy revealed a polypoid tumor obliterating the right B2 bronchus and the biopsy showed squamous cell carcinoma, right upper lobectomy was considered to be the first priority. Platelet concentrates were transfused immediately before the surgery. Unfortunately, the patient developed cardiac complication after the surgery and finally died of pneumonia.

Case 2

The second case involved a 58-year-old woman who presented with long-lasting productive coughing. Chest X-ray showed bilateral pulmonary infiltrates and computed tomography revealed patchy ground-glass opacification with thickened interlobular septa, predominantly in the lower lungs. Bronchoalveolar lavage yielded milky fluids and cytological examination revealed amorphous materials and macrophages with foamy cytoplasm. The levels of KL-6 (normal, <500 U/mL) and surfactant protein-D (<110 ng/ml) were 3,313 U/mL and 294.1 ng/mL, respectively. The patient’s hemoglobin level was 6.4 g/dL, white cell count 0.97 × 10³/µL, and platelet count 110 × 10³/µL. Neutrophils displayed hypogranular
cytoplasm and pseudo-Pelger nuclear abnormality. The bone marrow showed hypercellularity with 3.0% blasts. There were a series of dysplastic hematopoietic precursors, including erythroblasts with nuclear irregularity, giant granulocytes, and mononucleate/multinucleate megakaryocytes and micromegakaryocytes (Figure 1). The serum level of iron was 19 µg/dL, total iron binding capacity 393 µg/dL, and ferritin 43 ng/mL; iron

Case 1

![Image](image1.png)

Case 2

![Image](image2.png)

Figure 1. Appearance of the bone marrow (Wright staining)

**Case 1.** Hypogranular neutrophils, a Döhle body in the cytoplasm (arrow), mature- and immature-form monocytes, and two blasts (asterisk) are shown. Erythroblasts show a mild megaloblastoid appearance (original magnification, ×1,000).

**Case 2.** Neutrophils with hypogranular cytoplasm and pseudo-Pelger nuclear abnormality (top, ×1,000), binucleate/multinucleate megakaryocytes (bottom left, ×400), and dysplastic erythroblasts (bottom right, ×1,000) are shown.
deficiency was apparent by bone marrow examination. Cytogenetic analysis revealed trp(1)(q21q32) as the sole chromosomal abnormality in all metaphases analyzed; the karyotype was 46,XX,trp(1)(q21q32)[20] (Figure 2B, Figure 3).

The patient has been transfusion-dependent and required 2 units of red cell concentrates every 4 weeks to keep the hemoglobin level above 7.0 g/dL. Nevertheless, other hematologic parameters as well as the lung involvement have been stable. She is currently scheduled to undergo allogeneic hematopoietic stem cell transplantation.

**FISH**

To confirm trp(1)(q21q32), we performed FISH using a probe corresponding to a locus within the triplicated segment q21-q32. The FISH probe consisted of Vysis LSI TCF3 (transcription factor 3; also known as E2A) on chromosome 19p13.3 labeled by SpectrumGreen (green signal) and Vysis LSI PBX1 (pre-B-cell leukemia...
homeobox 1) on 1q23 labeled by SpectrumOrange (red signal). Cytogenetic preparations of the two cases were hybridized with the probe and showed 3 red signals tandemly aligned on the trp(1)(q21q32) chromosome and a single red signal localized on normal chromosome 1q (Figure 4). Accordingly, hybridization of the bone marrow smear slide showed that 70% (case 1) and 91% (case 2) of interphase nuclei had 4 red and 2 green hybridization signals (Figure 4).

**Figure 4.** FISH of the bone marrow cells using the Vysis LSI TCF3/PBX1 Dual Color, Dual Fusion Translocation Probe. The probe was hybridized with the chromosome preparation or bone marrow smear slides. Red signals representing the PBX1 on 1q23 and green signals representing the TCF3 on 19p13.3 are indicated by arrowheads of each color. **Case 1.** A metaphase spread was banded by trypsin-Giemsa (A), labeled by the PBX1 probe (B, TRITC fluorescence filter), and labeled by the TCF3 probe (C, FITC fluorescence filter). In B, 3 red signals are tandemly aligned on the trp(1)(q21q32) chromosome. In D, two nuclei carrying 4 red and 2 green signals are shown, and a diploid cell carrying 2 red and 2 green signals is indicated by an asterisk. **Case 2.** A metaphase spread was stained by DAPI (A, DAPI fluorescence filter), labeled by the PBX1 probe (B), and labeled by the TCF3 probe (C). Interphase nuclei carrying 4 red and 2 green signals are shown in D.
DISCUSSION

Although case 1 lacked unequivocal hematopoietic dysplasia, the presence of clonal cytogenetic abnormality in addition to unexplained thrombocytopenia and peripheral and marrow monocytosis suggested that this case may fall into the MDS unclassifiable category of the WHO classification. On the other hand, case 2 fulfilled the diagnostic criteria of refractory cytopenia with multilineage dysplasia (RCMD), even though iron deficiency exacerbated the anemia. It appeared that the patient’s lung disease represented pulmonary proteinosis, which develops secondarily in association with underlying MDS. According to the MDS International Prognostic Scoring System (IPSS) and Revised IPSS (IPSS-R) stratification, case 1 was scored as IPSS 0.5 (INT-1) and IPSS-R 3 (low risk), and case 2 was scored as IPSS 1.0 (INT-1) and IPSS-R 5 (high risk).

Patients with MDS may have single or multiple chromosomal changes at the time of diagnosis. The most common chromosomal abnormalities observed in MDS are del(5q), -7/del(7q), +8, del(20q), and −Y. Other abnormalities listed in the IPSS-R include inv(3)/t(3q)/del(3q), del(11q), del(12p), i(17q), +19, and +21, and these abnormalities are placed into prognostic subgroups. Although abnormalities of 1q are not listed in the IPSS-R scheme, 1q abnormalities are commonly observed in MDS either as a sole chromosome abnormality or in association with other abnormalities. These include unbalanced translocation between 1q and variable partner chromosomes, for example, der(1;7)(q10;p10), or tandem duplication of a 1q segment, resulting in total or partial trisomy 1q. This cytogenetic observation was confirmed by the genome-wide SNP array-based study of a large number of MDS cases, showing gain of genomic material of 1q with or without loss of that of 7q in a significant fraction of cases.

Tandem triplication, trp(1q), which is considered to be an expanded variant of dup(1q), has often been described in myeloid diseases (Table 1). In MDS, although the presence of triplicated segments varied among cases, trp(1q) was observed as the sole chromosomal abnormality (as in case 2 of the present report) or was accompanied by only a limited number of additional abnormalities (as in case 1), suggesting that trp(1q) is the primary cytogenetic change in these cases, and may play a pathogenetic role in a minority of MDS cases.

Table 1. Myeloid diseases associated with triplication of 1q reported in the literature

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Disease</th>
<th>1q triplication</th>
<th>Additional abnormality</th>
<th>Author (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/male</td>
<td>ET</td>
<td>trp(1)(q21q32)</td>
<td>None</td>
<td>Knuutila (1983)</td>
</tr>
<tr>
<td>2</td>
<td>52/male</td>
<td>MDS</td>
<td>trp(1)(q21q32)</td>
<td>+8</td>
<td>Papenhausen (1984)</td>
</tr>
<tr>
<td>3</td>
<td>21/male</td>
<td>RAEB</td>
<td>trp(1)(q32q44)</td>
<td>+mar</td>
<td>Berger (1993)</td>
</tr>
<tr>
<td>5</td>
<td>43/male</td>
<td>RAEB-T</td>
<td>trp(1)(q21q41)</td>
<td>None</td>
<td>Tien (1994)</td>
</tr>
<tr>
<td>6</td>
<td>38/male</td>
<td>FA, MDS-RA</td>
<td>trp(1)(q12-21q32-q32)</td>
<td>add(11)(p15), add(21)(q22)</td>
<td>Ferro (2001)</td>
</tr>
<tr>
<td>7</td>
<td>28/male</td>
<td>CML</td>
<td>trp(1)(q24q31)</td>
<td>t(9;22)(q34;q11.2)</td>
<td>Park (2009)</td>
</tr>
<tr>
<td>8</td>
<td>57/female</td>
<td>MF</td>
<td>trp(1)(q21q32)</td>
<td>None</td>
<td>Park (2009)</td>
</tr>
<tr>
<td>9</td>
<td>64/male</td>
<td>MDS-RCMD</td>
<td>trp(1)(q21q32)</td>
<td>None</td>
<td>Ha (2011)</td>
</tr>
<tr>
<td>10</td>
<td>66/male</td>
<td>MDS-U</td>
<td>trp(1)(q21q32)</td>
<td>add(3)(p25)</td>
<td>Present report</td>
</tr>
<tr>
<td>11</td>
<td>58/female</td>
<td>MDS-RCMD</td>
<td>trp(1)(q21q32)</td>
<td>None</td>
<td>Present report</td>
</tr>
</tbody>
</table>

ET, essential thrombocytosis; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess of blasts; MDS-RA, MDS, refractory anemia; RAEB-T, RAEB in transformation; FA, Fanconi anemia; CML, chronic myeloid leukemia; MF, myelofibrosis; MDS-RCMD, MDS, refractory cytopenia with multilineage dysplasia; MDS-U, MDS, unclassified.
trp(1) theoretically leads to partial tetrasomy of 1q, it is possible that this abnormality exerts a gene dosage effect localized within this region, thereby causing higher levels of gene product involved in the pathogenesis of MDS. At present, more case studies are required to determine the prognostic implications of trp(1q) in MDS.

REFERENCES
1番染色体長腕の tandem triplication [trp(1)(q21q32)] を認めた骨髄異形成症候群の 2 例

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1番染色体長腕 (1q) の tandem triplication [trp(1)(q21q32)] を認めた骨髄異形成症候群 (MDS) の 2 例を報告する。

症例 1: 60 代男性。肺癌の術前検査で血小板減少と単球増多が認められた。骨髄は低形成で、芽球 0.4%、単球 24.3%、顆粒球系と赤芽球系に軽度の異形成を認めた。染色体分析では、分析した全ての分裂中期核に trp(1)(q21q32) を認め、3 番染色体短腕の構造異常を伴っていた。核型は、46,XY,trp(1)(q21q32),add(3)(p25)[20] であった。

症例 2: 50 代女性。咳嗽が続くため受診、画像検査と気管支肺洗浄検査で肺胞蛋白症が示唆された。血液検査では汎血球減少と少数の芽球が認められた。骨髄は過形成で、芽球 3.0%、3 系統に異形成を認め、MDS の refractory cytopenia with multilineage dysplasia に該当した。核型は、46,XX,trp(1)(q21q32)[20] であった。

FISH 解析：Vysis TCF3/PBX1 dual fusion probe を用いた FISH を行ったところ、trp(1)(q21q32) 染色体上で 1q23 に位置する PBX1 シグナルが 3 個連続していた。間期核は、赤 (PBX1) シグナル 4 個、緑 (TCF3) シグナル 2 個のパターンを示した。

考案：1q の gain は造血器腫瘍にしばしば認められる二次的な異常であるが、今回の 2 症例では、単独異常（症例 2）または 1 個の特異性のない異常を伴うのみ（症例 1）であったことから、trp(1)(q21q32) は MDS の primary cytogenetic change の一つと考えられた。

キーワード：1 番染色体長腕の tandem triplication，trp(1)(q21q32)，骨髄異形成症候群，FISH