Development of acute myeloid leukemia and myelodysplastic syndrome with amplification of the MLL gene in two patients treated for multiple cancers

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INTRODUCTION

The MLL gene, currently named KMT2A (lysine [K]-specific methyltransferase 2A), is localized at 11q23, and rearranged with a wide variety of genes resulting from chromosomal translocations in at least 10% of acute leukemia of various types.¹ On the other hand, multiple copies of MLL, i.e. MLL amplification, has been recognized as a recurrent cytogenetic event that occurs in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), therapy-related AML/MDS, and even pro-B cell acute lymphoblastic leukemia (ALL).²⁻³ MLL amplification has been often associated with older age, dysplastic hematopoietic cellular morphology, complex karyotype (three or more numerical/structural cytogenetic abnormalities), del(5q), TP53 mutation, coagulopathy, and very poor clinical outcome.²⁻⁴⁻⁵ Cytogenetically, MLL amplification can be located intrachromosomally...
on homogeneously staining regions or extrachromosomally on double minutes, on extra or derivative chromosome 11, or on ring chromosomes.\textsuperscript{2,4-11}

We previously reported a \textit{de novo} case of AML with amplification of \textit{MLL} identified by cytogenetic methods.\textsuperscript{12} In the present report, we show two additional AML/MDS cases with \textit{MLL} amplification that developed in patients who had been treated for multiple cancers.

**MATERIALS AND METHODS**

\textit{G-banding}

Bone marrow aspirates were incubated in culture medium overnight at 37°C in 5% CO\textsubscript{2}, and then cultured in the presence of 0.1 \(\mu\)g/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.

\textit{FISH}

Four FISH probes: the Vysis LSI \textit{MLL} dual-color, break-apart rearrangement probe, Vysis LSI \textit{EGR1}/\textit{DSS23, D5S721} dual color probe, Vysis \textit{D7S486/CEP7} FISH probe, and Vysis LSI \textit{IGH/BCL2} dual color, dual fusion translocation probe were purchased from Abbott Laboratories, Abbott Park, IL, USA. Denaturing of the chromosome/probe, hybridization, and washing conditions were as recommended by the manufacturer. FISH results were analyzed by 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).

**CASE REPORT**

\textit{Case 1}

A 74-year-old man who had a 9-year-long treatment history for hepatocellular carcinoma was referred to the Hematology Department due to pancytopenia. He had been treated repeatedly with transarterial embolization, transcatheter arterial chemoembolization injecting a mixture of lipiodol and epirubicin, and percutaneous radiofrequency ablation, in addition to interferon. Eight years earlier, he had concurrently received radiation therapy for laryngeal cancer with total dosages of 75 Gy. He had smoked 15 cigarettes a day for 45 years. He was seropositive for hepatitis virus type C.

His hemoglobin level was 8.8 g/dL, white cell count was \(0.9 \times 10^{3}/\mu\)L, and platelet count was \(36 \times 10^{3}/\mu\)L. The white cell differential was 58.0% lymphocytes, 2.0% atypical lymphocytes, 8.0% monocytes, 24.0% segmented neutrophils, 3.0% banded neutrophils, and 5.0% metamyelocytes. The bone marrow showed hypo-or normocellularity composed of 11.6% blasts (Figure 1A). Hypgranular neutrophils and giant granulocytes were observed (Figure 1B), and erythroid cells showed a mild megaloblastoid appearance. Ringed sideroblasts accounted for 20% of erythroblasts.

Two months after presentation, the disease progressed to florid leukemia; the white cell count rose to \(8.1 \times 10^{3}/\mu\)L, consisting of 15.0% lymphocytes, 53.0% monocytes, 0.5% eosinophils, 8.0% segmented neutrophils, 5.0% banded neutrophils, 10.0% metamyelocytes, 0.5% myelocytes, and 8.0% blasts. Neutrophils displaying pseudo-Pelger nuclear abnormality were observed. His lactate dehydrogenase level was 645 IU/L. Blood coagulation test data were: PT, 17.3 sec; APTT, 40.8 sec; fibrinogen, 158 mg/dL; fibrin degradation product, 62 \(\mu\)g/mL; and D-dimer, 33.2 \(\mu\)g/mL. The patient died of leukemia progression 80 days after referral to our department.

\textit{Case 2}

The second case was a 68-year-old man who had been treated for bladder, laryngeal, and esophageal cancers for the preceding 12 years. He had undergone partial cystectomy followed by oral doxifluridine, partial resec-
Therapy-related AML/MDS with MLL amplification

A 67-year-old man presented with a 6-month history of hoarseness. A laryngoscopic examination revealed a nodular lesion of the lower larynx followed by radiation therapy (50 Gy), and repeated endoscopic esophageal mucosal resection. Six months before presentation, he had received additional surgery for recurrence of laryngeal cancer and chemotherapy consisting of cisplatin and docetaxel and oral TS-1. He had smoked 40 cigarettes a day for 30 years.

The patient was admitted to the Hematology Department due to leukocytosis. On admission, percutaneous endoscopic gastrostomy tube and tracheostomy tubes were placed. His performance status was 3. His hemoglobin level was 7.1 g/dL, white cell count was 63.1 × 10⁹/µL, and platelet count was 34 × 10⁹/µL. The white cell differential was 9.0% lymphocytes, 19.5% monocytes, 0.5% eosinophils, 10.0% segmented neutrophils, 9.5% banded neutrophils, 19.5% metamyelocytes, 4.0% myelocytes, and 28.0% blasts. His lactate dehydrogenase level was 1,557 IU/L and C-reactive protein was 26.8 mg/dL. Blood coagulation test data were: PT, 18.8 sec; APTT, 40.7 sec; and D-dimer, 46.9 µg/mL. The bone marrow showed 90% cellularity and included 72.9% blasts exhibiting 75% positivity for peroxidase staining (Figure 1C). Granulocytes and megakaryocytes showed the morphological features of myelodysplastic syndrome (Figure 1D, E).

The patient was complicated by aspiration pneumonia and induction chemotherapy was considered to be inadequate. He died of respiratory failure on the 6th day of hospitalization.

Figure 1. Appearance of the bone marrow (Wright staining; original magnification, ×100 objective). (A and B) Case 1. Blasts and dysplastic granulocytes are shown. (C to E) Case 2. Blasts have abundant cytoplasm containing azurophilic granules. Two hypogranular neutrophils in D and a dysplastic megakaryocyte in E are shown.
CYTOGENETIC STUDIES

The G-banded karyotype of case 1 showed heterogeneity among the metaphase cells analyzed; nevertheless, del(7)(q31), −13, two types of ring chromosomes (r1 and r2), and one double minute were observed in at least two metaphase cells (Figure 2A). The karyotype according to the ISCN was: 42~47,XY,del(7)(q31),−13,+r1,+r2,1dmin[cp5]/46,XY[8]. We hybridized the chromosome preparation with the dual-color MLL probe. As shown in Figure 3A and B, normal chromosome 11 homologues were labeled with the yellow (fusion) signals (i.e. consisting of the green 5′ MLL and red 3′ MLL signal) at 11q23, while one ring chromosome (r2) and the double minute were brightly labeled with the probe. Accordingly, 78% of interphase nuclei counted carried 8 to 10 copies of the MLL signal (Figure 3C). FISH of metaphase cells using the D7S486/CEP7 probe showed that the del(7) chromosome was labeled with the green centromere signal, but lacked the red signal at 7q31 [ish del(7)(CEP7+,D7S486−,IGH+) (Figure 3D)]. The der(11) chromosome carried two tandemly aligned MLL signals (Figure 3E), and the telomeric end was labeled with the D5S23, D5S721 green probe at 5p15.2 [ish der(11)(MLL×2, [D5S23, D5S721]+)] (Figure 3G), indicating that the short arm of chromosome 5 was translocated to the der(11), while a segment of the long arm of chromosome 5 corresponding to the EGR1 locus on 5q31 was deleted. Finally, around 8 MLL signals were clustered on the r(11) chromosome [ish r(11)(MLL×8)] (Figure 3E). Taken together, the karyotype is described as: 44−46,XY,add(2)(q37),−5,der(7)t(7;14)(q36;q11),der(11)t(5;11)(p11;q25)?dup(11)(q13q23),−14,add(15)(q26),−19,+r,+1~2mar[cp9]. Accordingly, interphase nuclei showed the signal pattern indicative of del(5q) (Figure 3G), del(7q), and amplification of the MLL gene copy (Figure 3F).

Case 2 also showed karyotype heterogeneity. Clonally occurring numerical and structural abnormalities were add(2), −5, der(7), der(11), −14, add(15), and a ring chromosome which appeared to be derived from chromosome 11 [r(11)] (Figure 2B). The der(7) chromosome was labeled by the green CEP7 probe at the centromere and the IGH probe at a telomeric end (not shown), but lacked the red D7S486 signal at 7q31 [ish der(7)(CEP7+,D7S486−,IGH+) (Figure 3H)]. The der(11) chromosome carried two tandemly aligned MLL signals (Figure 3E), and the telomeric end was labeled with the D5S23, D5S721 green probe at 5p15.2 [ish der(11)(MLL×2, [D5S23, D5S721]+)] (Figure 3G), indicating that the short arm of chromosome 5 was translocated to the der(11), while a segment of the long arm of chromosome 5 corresponding to the EGR1 locus on 5q31 was deleted. Finally, around 8 MLL signals were clustered on the r(11) chromosome [ish r(11)(MLL×8)] (Figure 3E). Taken together, the karyotype is described as: 44−46,XY,add(2)(q37),−5,der(7)t(7;14)(q36;q11),der(11)t(5;11)(p11;q25)?dup(11)(q13q23),−14,add(15)(q26),−19,+r,+1~2mar[cp9]. Accordingly, interphase nuclei showed the signal pattern indicative of del(5q) (Figure 3G), del(7q), and amplification of the MLL gene copy (Figure 3F).

Figure 2. G-banded karyotype obtained from the bone marrow. (A) Case 1. Arrows indicate the del(7)(q31), −13, and −21. Inset: Partial karyotype of chromosome 7 showing del(7)(q31) (arrow). (B) Case 2. Arrows indicate the −5, add(7), der(11), and −14. Insets: Partial karyotypes of chromosome 2 and 15 showing add(2) and add(5) (arrows), respectively. The karyotypes according to the ISCN are described in the text.
Figure 3. FISH. (A to D) Case 1. The MLL hybridization signals are localized at two chromosome 11 homologues (arrow heads) and the r2 and dmin in A (arrows), while the metaphase shown in B lacks the dmin. Three interphase nuclei in C carry 8 to 10 MLL signals, while a diploid nucleus is labeled with 2 signals (asterisk). D shows the del(7)(q31) that lacks the red D7S486 signal at 7q31. (E to H) Case 2. The MLL hybridization signals are localized at the normal chromosome 11 (arrow head), der(11), and ring (arrows). Interphase nuclei shown in E and F are labeled with multiple hybridization signals. G and H show the der(11) labeled with the green D5S23, D5S721 probe at 5p15.2 and der(7) lacking the red D7S486 signal at 7q31. Asterisk indicates a nucleus showing the one red and two green signal pattern indicative of del(5q).
DISCUSSION

The clinical features of the two present cases corresponded well with those of MLL-amplification-associated AML/MDS; namely, dysplastic hematopoietic cellular morphology, complex karyotype, laboratory abnormalities indicative of disseminated intravascular coagulation, and very short survival.\(^2\) According to the 2008 World Health Organization (WHO) classification, the diseases of the two cases are categorized into refractory anemia with excess of blasts-2 (RAEB-2) and AML with myelodysplasia-related changes (AML-MRC), respectively.\(^{13,14}\) del(7q) observed in both cases or loss of whole chromosome 7 is associated with up to 70% of patients with therapy-related AML/MDS,\(^{15}\) even though it is not yet known what genes located on 7q are involved in the leukemogenesis. Thus, it is likely that long-lasting cytotoxic treatment for multiple cancers was responsible for the development of both diseases. Alternatively, these two patients may have carried genetic susceptibility predisposing them to cancer development.

On the other hand, in the 2008 WHO classification, cases of AML, MDS, and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) that arise after chemotherapy and/or radiation therapy for prior neoplastic or non-neoplastic disorders are categorized together into therapy-related myeloid neoplasms (TR-MNs).\(^{15}\) In general, 10% to 20% of AML, MDS, and MDS/MPN cases are estimated to be therapy-related.\(^{15,16}\) A search of the literature identified a total of 151 cases of AML/MDS with MLL amplification (excluding duplication cases) and 34 (23%) of these cases had had primary disorders and/or received cytotoxic treatment consisting of chemotherapy alone, radiation alone, or both.\(^{2,11,17-24}\) However, the term of TR-MNs, in definition, includes any myeloid neoplasm that develops in a patient with a history of chemotherapy/radiotherapy, and cumulative exposure to therapies and other confounding variables are not considered.\(^{16}\) Table 1 compares the features of 3 cases of AML/MDS with MLL amplification treated in our institution (i.e. one de novo and two therapy-related cases),\(^{12}\) showing that the three cases shared karyotype complexity with del(5q)/del(7q), coagulopathy, and very short survival. Thus, it remains to be determined whether there is an essential difference between TR-MNs and their de novo counterparts in clinical features, cytogenet-

| Table 1. Three cases of AML/MDS associated with MLL amplification treated in our institution |
|---------------------------------|-----------------|-----------------|
| **Age/sex**                     | Okumura et al.\(^1\) | Case 1          | Case 2          |
| **Disease**                     | AML-MRC          | RAEB-2          | AML-MRC         |
| **Dysplastic morphology**       | +                | +               | +               |
| **Prior neoplasm**              | −                | + (Hepatocellular carcinoma, laryngeal cancer) | + (Bladder cancer, laryngeal cancer, esophageal cancer) |
| **History of chemo-radiotherapy** | −               | +               | +               |
| **Coagulopathy**                | +                | +               | +               |
| **Karyotype heterogeneity**     | −                | +               | +               |
| **Clonal abnormalities**        | del(5q), der(11), −18, del(20q) | del(7q), −13, two rings, double minute | add(2), −5, der(7), der(11), −14, add(15), ring |
| **del(5q) by FISH**             | +                | −               | +               |
| **del(7q) by FISH**             | −                | +               | +               |
| **MLL amplification**           | ?hsr at der(11)  | ring, dmin     | ring, duplication at der(11) |
| **Survival**                    | 3 days           | 80 days         | 6 days          |

\(^1\)Reference #12.

AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; RAEB-2, refractory anemia with excess of blasts-2.
Amplification of chromosome band 11q23, including the unrearranged MLL gene, is a recurrent abnormality in therapy-related MDS and AML, and is closely related to mutation of the TP53 gene and to previous therapy with alkylating agents. Genes Chromosomes Cancer 2001;31:33-41.


多重癌の治療歴があり，MLL 遺伝子増幅を認めた急性骨髄性白血病・骨髄異形成症候群の 2 症例

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MLL 遺伝子増幅は，急性骨髄性白血病 (AML) や骨髄異形成症候群 (MDS) に認められる recurrent な染色体異常である。今回我々は，多重癌に対する治療歴があり，MLL 遺伝子増幅を認めた AML/MDS の 2 症例を報告する。症例 1 は，74 歳の男性で，汎血球減少ため当院血液内科を紹介受診した。9 年間にわたる肝癌治療歴と喉頭癌に対する放射線治療歴があった。骨髄は MDS の RAEB-2 に該当したが，短期間で進行し第 80 病日に死亡した。骨髄の染色体分析では，del(7)(q31)，−13，2 種類の ring，1 個の double minute (dmin) を認めた。症例 2 は 68 歳の男性で，白血球增多のため血液内科を紹介受診した。肺腺癌，喉頭癌，食道癌に対して 12 年間治療を受けていた。骨髄検査では芽球 72.9%，多彩な細胞異型を認め，AML with myelodysplasia-related changes に該当した。第 3 病日に死亡した。染色体分析では，add(2)，−5，der(7)，der(11)，−14，add(15) と第 11 染色体に由来する ring [r(11)] を認めた。Vysis LSI MLL dual-color, break-apart rearrangement probe を用いた FISH を行ったところ，症例 1 では 1 個の ring と dmin 上で MLL シグナルが増幅，症例 2 では r(11) 上で MLL シグナルが 2 個に増加し，r(11) 上で MLL シグナルが増幅していた。一方，症例 1 では del(7q) が，症例 2 では del(5q) と del(7q) がそれぞれのプローブを用いた FISH で確認された。これらの 2 症例の臨床病態は，過去に報告されている MLL 増幅 AML/MDS の病態（高齢発症，複雑核型，凝固異常，予後不良など）に合致した。2 症例とも過去に受けた多重癌に対する化学放射線療法が AML/MDS の発症に関与したと考えられた。

キーワード：治療関連急性骨髄性白血病・骨髄異形成症候群，MLL 遺伝子増幅，FISH，多重癌