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Case Study

Coexistent t(8;21)(q22;q22) Translocation and 5q Deletion in Acute Myeloid Leukemia

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The t(8;21)(q22;q22) translocation is specifically observed in acute myeloid leukemia (AML) M2 subtype, whereas del(5q) is one of the most common cytogenetic aberrations in myelodysplastic syndromes (MDS). Thus, t(8;21)(q22;q22) and del(5q) appear to be mutually exclusive, and the association between them has not been characterized yet. Here, we report an 81-year-old woman with coexistent t(8;21)(q22;q22) and del(5q) at initial diagnosis. The bone marrow was infiltrated with 18.4% myeloblasts, and showed marked myeloid and erythroid dysplasia. Myeloblasts were positive for CD19 and CD56 as well as CD13, CD33, CD34 and HLA-DR. G-banding and spectral karyotyping showed 46,XX,del(5)(q?),t(8;21)(q22;q22)[18]/46,XX[2]. Both del(5)(q?) and t(8;21)(q22;q22) were present in a single clone. Fluorescence *in situ* hybridization (FISH) on metaphase spreads detected a *RUNX1/RUNX1T1* fusion signal on the der(8)t(8;21)(q22;q22), and confirmed deletion of *CSF1R* signaling at 5q33-q34 on the del(5)(q?). Furthermore, FISH on interphase nuclei revealed that the *RUNX1/RUNX1T1* fusion signal and deletion of *CSF1R* signaling were found in 66.0% and 58.0% of interphase cells, respectively, suggesting that del(5)(q?) occurred in cells with *RUNX1/RUNX1T1*. These results indicated a diagnosis of AML with t(8;21)(q22;q22)/*RUNX1/RUNX1T1* rather than MDS, even though the percentage of bone marrow myeloblasts was less than 20%. Based on these findings, together with those of other reported cases, del(5q) seems to be an extremely rare but recurrent secondary aberration in AML with t(8;21)(q22;q22). 〔*J Clin Exp Hematop 55(2) : 181-185, 2015*〕

Keywords: acute myeloid leukemia, chromosome aberrations, coexistence, 5q deletion, *RUNX1/RUNX1T1*

INTRODUCTION

The t(8;21)(q22;q22) translocation involving *RUNX1* at 21q22 and *RUNX1T1* at 8q22 is observed in 10% of cases of acute myeloid leukemia (AML) M2 subtype.[1] This translocation leads to the formation of a *RUNX1/RUNX1T1* fusion gene on der(8)t(8;21)(q22;q22), thus causing leukemic transformation by transcriptional repression of normal *RUNX1* target genes. On the other hand, deletion of the long arm of chromosome 5, del(5q), is one of the most common cytogenetic aberrations in myelodysplastic syndromes (MDS) and accounts for 10% and 40% of *de novo* and therapy-related MDS cases, respectively.[1] Furthermore, according to the World Health Organization classification, del(5q) is defined to be one of the unbalanced abnormalities sufficient for the diagnosis of AML with myelodysplasia-related changes.[1,2] At the diagnosis of AML with myelodysplasia-related changes, recurrent cytogenetic abnormalities, such as t(8;21)(q22;q22), inv(16)(p13.1q22), and t(15;17)(q22;q12), described as "AML with recurrent genetic abnormalities", should be absent. Thus, t(8;21)(q22;q22) and del(5q) seem to be mutually exclusive in patients with AML. Here, we report an unusual case of AML with t(8;21)(q22;q22) and del(5q) at initial diagnosis.

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CASE REPORT

An 81-year-old woman was admitted to our hospital because of fever, progressive anemia, and thrombocytopenia. Three years earlier, she had been diagnosed with mild anemia, but no specific treatment was given. Peripheral blood showed hemoglobin 7.4 g/dL (mean corpuscular volume 100 fL), platelets 66 × 10^9/L, and leukocytes 2.8 × 10^9/L with 2% myelocytes, 1% metamyelocytes, 9% band forms, 54% segmented neutrophils, 7% monocytes, 23% lymphocytes, and 4% myeloblasts. Bone marrow was normocellular with 18.4% myeloblasts, 61.4% mature myeloid cells, 1.0% monocytes,

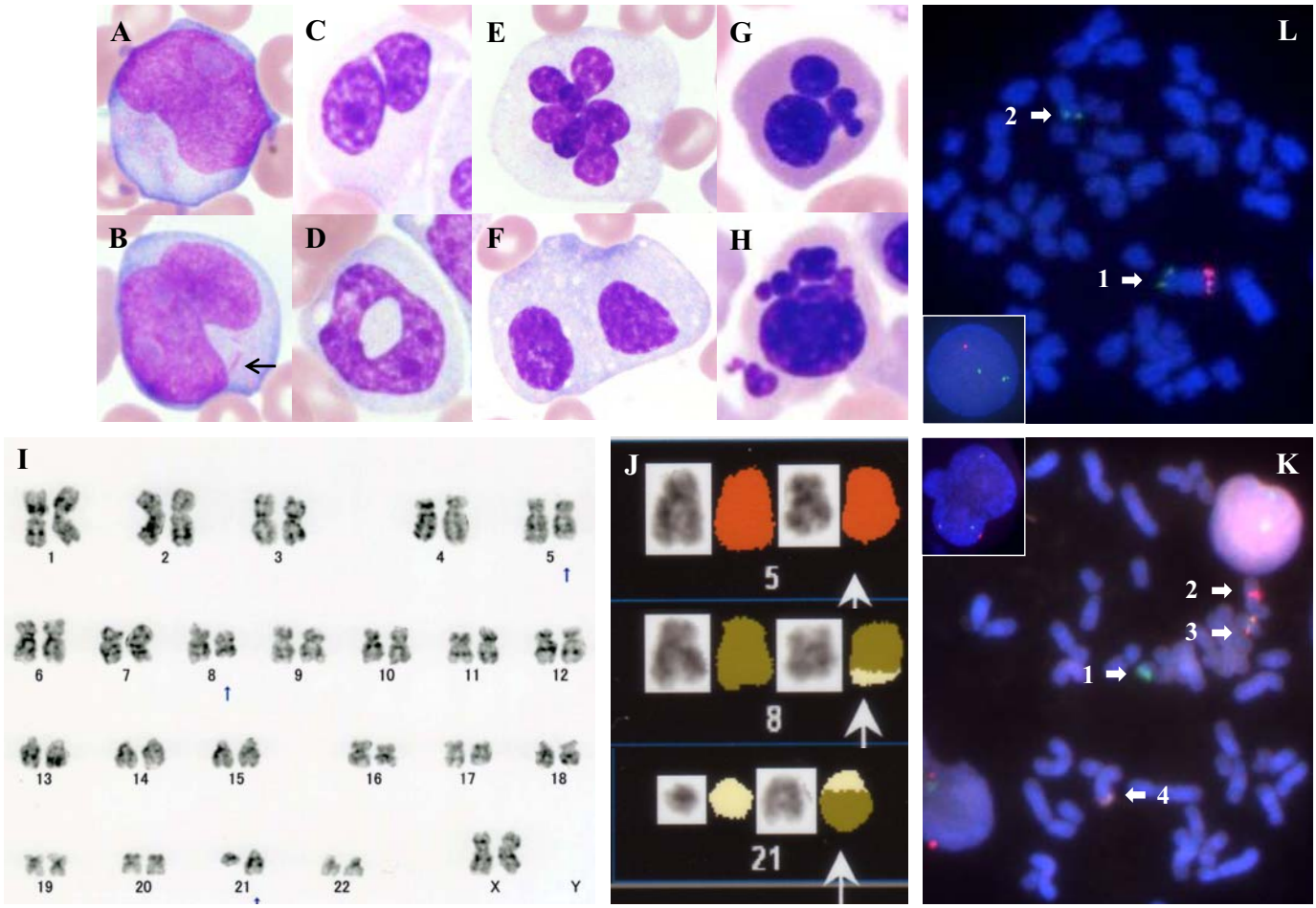

Fig. 1. Bone marrow smear cytology, G-banded karyotype, spectral karyotyping and fluorescence *in situ* hybridization. (***1A-1H***) Bone marrow smears show myeloblasts with convoluted nuclei, fine nuclear chromatin and nucleoli, and azurophilic granules in the basophilic cytoplasm (*1A*); myeloblasts with Auer rods (*black arrow*) (*1B*); hypogranular neutrophils with pseudo-Pelger-Huët anomaly (*1C*); round nuclei (*1D*); abnormal nuclear lobulation (*1E*); separated nuclei (*1F*); and erythroblasts with abnormal nuclear lobulation (*1G*, *1H*). May-Grünwald-Giemsa staining, × 1,000. (***1I***) G-banded karyotype of bone marrow cells at the initial diagnosis: 46,XX,del(5)(q?), t(8;21)(q22;q22). *Arrows* indicate the rearranged chromosomes. (***1J***) Spectral karyotyping of the metaphase spreads after spectrum-based classification (*left side*, reverse DAPI; *right side*, SKY). Only chromosomes 5, 8, and 21 are shown. Two derivative chromosomes, der (8)t(8;21)(q22;q22) and der(21)t(8;21)(q22;q22), and del(5)(q?) have been confirmed. *Arrows* indicate the rearranged chromosomes. (***1K***) Fluorescence *in situ* hybridization (FISH) analyses with Vysis LSI AML1/ETO Dual Color, Dual Fusion Translocation Probe (Abbott Molecular, Abbott Park, IL, USA) on metaphase spreads. *Arrows* indicate 1) *RUNX1* signal (*green*) on a normal chromosome 21, 2) *RUNX1T1* signal (*red*) on a normal chromosome 8, 3) *RUNX1T1/RUNX1* signal (*red*/*green*, *yellow*) on the der(21)t(8;21)(q22;q22), and 4) *RUNX1/RUNX1T1* fusion signal (*red*/*green*, *yellow*) on the der(8)t(8;21)(q22;q22). FISH on interphase nuclei confirmed one *green*, one *red*, and two *yellow* signals (*inset*). (***1L***) FISH with Vysis LSI CSF1R SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes (Abbott Molecular) on metaphase spreads. *Arrows* indicate 1) D5S23, D5S721 signal (*green*) at 5p15.2 and *CSF1R* signal (*red*) at 5q33-34 on a normal chromosome 5, and 2) D5S23, D5S721 signal (*green*) on the del(5)(q?). FISH on interphase nuclei confirmed one *red* and two *green* signals (*inset*).

5.2% lymphocytes, and 12.2% erythroblasts. Myeloblasts had few azurophilic granules and Auer rods in the basophilic cytoplasm (Fig. 1A & 1B). Marked myeloid dysplasia with hypogranulation, pseudo-Pelger-Huët anomaly, and separated nuclei, and erythroblasts with abnormal nuclear lobulation were also detected (Fig. 1C-1H). The number of megakaryocytes was low, and no dysplastic changes were apparent. Myeloblasts were positive for myeloperoxidase, and immunophenotypically positive (> 20%) for CD13 (57.7%), CD19 (30.0%), CD33 (21.2%), CD34 (96.2%), CD56 (97.7%), and HLA-DR (93.7%). Initially, because of the low percentage of bone marrow blasts and marked bilineage dysplasia, we diagnosed the patient with MDS, refractory anemia with excess blasts-2, according to the World Health Organization

classification.[1] Furthermore, a computed tomography scan of the chest revealed multiple small nodules in both lungs. Because of her advanced age and suspected miliary tuberculosis, she did not receive chemotherapy and returned to the hospital closest to her home for comfort care.

G-banding analysis of bone marrow cells at diagnosis showed 46,XX,del(5)(q?),t(8;21)(q22;q22)[18]/46,XX[2] (Fig. 1I). Both del(5q) and t(8;21)(q22;q22) were present in a single clone. Spectral karyotyping (SKY) confirmed del(5)(q?) and two derivative chromosomes, der(8)t(8;21)(q22;q22) and der(21)t(8;21)(q22;q22) (Fig. 1J). Specifically, del(5)(q?) was due to the deletion alone and not to an unbalanced translocation with another chromosome. However, precise breakpoints of del(5)(q?) could not be identified by G-banding and SKY because of inadequate quality of metaphase spreads. Fluorescence *in situ* hybridization (FISH) on metaphase spreads detected a *RUNX1/RUNX1T1* fusion signal on the der(8)t(8;21)(q22;q22) in 15 of 20 metaphase spreads (Fig. 1K). In addition, FISH revealed deletion of *CSF1R* signaling at 5q33-q34 on the del(5)(q?) in 14 of 20 metaphase spreads (Fig. 1L). FISH on interphase nuclei demonstrated that the *RUNX1/RUNX1T1* fusion signal and deletion of *CSF1R* signaling were found in 66 of 100 cells and 58 of 100 cells, respectively (Fig. 1K & 1L, *inset*). The t(8;21)(q22;q22) translocation is defined to be one of the recurrent cytogenetic abnormalities specifically observed in AML. Morphological features such as granular blasts, Auer rods, myeloid dysplasia, and co-expression of CD19 seem to be typical findings of AML with t(8;21). Thus, we revised the final diagnosis to AML with t(8;21)(q22;q22)/*RUNX1/RUNX1T1*, even though the percentage of bone marrow myeloblasts was less than 20%.[1]

DISCUSSION

Here we presented the coexistence of t(8;21)(q22;q22) and del(5q) at the initial diagnosis of AML with *RUNX1/RUNX1T1*. The t(8;21)(q22;q22) is a primary recurrent genetic event, with the exception of Philadelphia (Ph) chromosome-positive chronic myeloid leukemia (CML).[3] On the other hand, del(5q) could be an additional abnormality as part of a complex karyotype as well as a single aberration leading to 5q- syndrome.[4] In the present case, FISH revealed that *RUNX1/RUNX1T1* rearrangement was detected more frequently than *CSF1R* deletion in bone marrow interphase cells, suggesting that del(5q) occurred in cells with t(8;21)(q22;q22). Thus, we concluded that t(8;21)(q22;q22) and del(5q) were primary and secondary abnormalities, respectively, although both cytogenetic aberrations coexisted in all abnormal metaphase spreads analyzed by G-banding and SKY. Furthermore, the diagnosis was AML with t(8;21)(q22;q22) rather than MDS, even though the percentage of bone marrow myeloblasts was less than 20%. FISH on neutrophils in the peripheral blood may be useful for the differential diagnosis between MDS and *de novo* AML, but unfortunately, we could not perform this procedure.

Additional cytogenetic and molecular genetic abnormalities are frequently observed in AML with t(8;21)(q22;q22).[5] Over 70% of patients have cytogenetic aberrations, such as loss of sex chromosomes, del(9)(q22), and trisomy 8; mutations of *NRAS* and *KIT* are also common. However, to our knowledge, the association between t(8;21)(q22;q22) and del(5q) has not been characterized yet. We searched the Mitelman database and found eight cases harboring coexistent t(8;21)(q22;q22) and del(5q) among 1,589 cases of AML with t(8;21)(q22;q22) (Table 1).[6-14] As observed in the present case, both del(5q) and t(8;21)(q22;q22) were present in a single clone in these reported cases. Three cases (No. 1, 2 & 6) had del(5q) in their subclones, whereas other cases had coexistent del(5q) in the stem line. Only the present case showed del(5q) as a sole additional abnormality in the stem line. Therefore, del(5q) seems to be an extremely rare (8/

Table 1. Reported cases of hematological malignancies with coexistent t(8;21)(q22;q22) and del(5q)

Case No.	Age/ Sex	Diagnosis	Karyotypes	References
1	80/F	AML M2	46,XX,t(8;21)(q22;q22)/46,sl,**del(5)(q1?1q1?3)**	Bernstein R, *et al*., 1982 [7]
2	13/F	AML M2	46,XX,add(4)(q?),t(8;21)(q22;q22)/46,XX,**del(5)(q?)**,t(8;21)	Prigogina EL, *et al*., 1986 [8]
3	62/M	AML	45,XY,**del(5)(q21q23)**,t(8;21)(q22;q22),-9,del(11)(q23),add(12)(q23),-20	GFCH 1990 [9]
4	40/M	AML M2	46,XY,der(1)t(1;8)(p32;q2?3),**del(5)(q13)**,der(8)t(8;21)(q22;q22),der(21)t(8;21)t(1;8)	Calabrese G, *et al*., 1996 [10]
5	31/M	LCH → AML M2	45,X,-Y,**del(5)(q?)**,t(8;21)(q22;q22)	Aslan V, *et al*., 2002 [11]
6	17/M	AML M2	45,X,-Y,t(8;21)(q22;q22)/45,sl,add(3)(q?26),**del(5)(q?33)**/46,XY	Viehmann S, *et al*., 2003 [12]
7	25/F	AML M2	46,XX,r(1)(p36p11),**del(5)(q22q34)**,der(8)t(8;21)(q22;q22),der(21)t(8;21)t(8;18)(q23;?)t(1;18)(q?;?)	Xu W, *et al*., 2010 [13]
8	22/M	AML M2	45,X,-Y,**del(5)(q21)**,t(8;21)(q22;q22),i(9)(q10)	Gmidène A, *et al*., 2012 [14]
9	81/F	AML M2	46,XX,**del(5)(q?)**,t(8;21)(q22;q22)[18]/46,XX[2]	present case

F, female; M, male; AML, acute myeloid leukemia; LCH, Langerhans cell histiocytosis; GFCH, Groupe Français de Cytogénétique Hématologique. The deletion 5q is indicated in bold letters.

1,589, 0.50%) but recurrent secondary aberration in AML with t(8;21)(q22;q22). One case (No. 5) showed similar hematological findings to the present case, such as low leukocyte count (1.9×10^9/L), intracytoplasmic granules and Auer rods of blasts, and positivity for myeloperoxidase, CD13, CD19, CD33, and HLA-DR; however, the bone marrow showed 80% of blast infiltrates, and myeloid dysplasia was not apparent.[11] Unfortunately, common clinical and genetic features could not be detected because only limited data were available in the other reported cases.

With regard to the association between del(5q) and other recurrent genetic abnormalities, del(5q) was detected in 3 out of 899 cases of AML M3 with t(15;17)(q22;q12-21) (0.33%), and in 3 out of 888 cases of AML with inv(16)(p13q22) (0.34%).[6] Coexistence of del(5q) with recurrent genetic abnormalities appears to be a very rare genetic event. In addition, del(5q) is often observed in Ph-positive CML as an unrelated cytogenetic abnormality after successful treatment with imatinib.[15] On the other hand, Maekawa *et al*. reported a rare case of Ph-positive CML with del(5q) as a clonal evolution.[16] Interestingly, this case progressed to erythroblastic crisis. The present case also presented erythroid dysplasia, which is not a typical finding of AML with t(8;21). However, the association between an additional del(5q) and dyserythropoiesis is uncertain.

Deletion of 5q as a secondary change was also found in AML with another *RUNX1*-related translocation. The t(7;21)(p22;q22) translocation, which results in the fusion of *RUNX1* at 21q22 and *USP42* at 7p22, is an uncommon but specific cytogenetic abnormality in AML: only 10 cases have been reported.[17,18] Interestingly, 8 of 10 cases showed loss of 5q material as an additional abnormality at the initial diagnosis or at relapse. Leukemic cells in all 8 cases showed aberrant CD56 expression but lacked CD19 expression. Thus, the association between t(7;21) and del(5q) may be nonrandom, but the clinical significance of this connection remains to be elucidated.[18] Although in the present case leukemic cells also expressed high CD56 and low CD19 levels, at the moment, it is unknown whether similar clinical findings are observed in AML with t(8;21) and del(5q). Future observations will clarify the possible association between *RUNX1* rearrangement and del(5q) in AML.

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