A der(13)t(7;13)(p13;q14) with monoallelic loss of RB1 and D13S319 in myelodysplastic syndrome

Yamamoto, Katsuya ; Ito, Mitsuhiro ; Minagawa, Kentaro ; Urahama, Norinaga ; Sada, Akiko ; Okamura, Atsuo ; Matsui, Toshimitsu

A novel translocation der(13)t(7;13)(p13;q14) involving 13q14 with monoallelic loss of *RB1* and *D13S319* in myelodysplastic syndrome

Katsuya Yamamoto, Mitsuhiro Ito, Kentaro Minagawa, Norinaga Urahama, Akiko Sada, Atsuo Okamura and Toshimitsu Matsui

Hematology/Oncology, Department of Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Corresponding author: Toshimitsu Matsui
Address: Hematology/Oncology, Department of Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
TEL: +81-78-382-5885
FAX: +81-78-382-5899
E-mail: matsui@med.kobe-u.ac.jp

Abstract

Deletions or translocations of chromosome band 13q14, the locus of the retinoblastoma (*RB1*) gene, have been observed in a variety of hematological malignancies including myelodysplastic syndrome (MDS). We describe here a novel unbalanced translocation der(13)t(7;13)(p13;q14) involving 13q14 in a patient with MDS. A 66-year-old woman was admitted for precise examination of progressive anemia. She was diagnosed as MDS, refractory anemia with excess of blasts (RAEB-I) because of 7.4% blasts and trilineage dysplasia in the bone marrow cells. The blasts were positive for CD4, CD10, CD16 and CD41a as well as CD13, CD14 and CD33. Chromosome analysis showed complex karyotypes as follows; 46,XX,del(6)(q?),-7,der(13)t(7;13)(p13;q14),+mar1. Spectral karyotyping revealed that these cytogenetic aberrations were derived from complex translocations involving chromosomes 6, 7 and 13. As a result, the revised karyotype was 46,XX,der(6)t(6;7)(q11;?),der(7)del(7)(p13?)t(6;7)(q?;q11)t(6;13)(q?;q?),der(13)t(7;13)(p13;q14). Fluorescence *in situ* hybridization (FISH) analyses demonstrated that one allele of the *RB1* gene and the microsatellite locus *D13S319,* located at 13q14 and telomeric to the *RB1* gene, was deleted. Considering other reported cases, our results indicate that submicroscopic deletions accompanying 13q14 translocations are recurrent cytogenetic aberrations in MDS. The *RB1* gene and/or another tumor suppressor gene in the vicinity of *D13S319* may be involved in the pathogenesis of MDS with 13q14 translocations by monoallelic deletion.

1. Introduction

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by ineffective hematopoiesis that leads to bone marrow failure, leukemic transformation, and clonal cytogenetic aberrations [1]. Total or partial chromosome losses and chromosome gains, such as 5q-, -7 and +8, are predominantly observed, whereas balanced translocations are relatively rare in MDS [1, 2]. Unbalanced translocations are also frequently found, and they are usually detected as a part of complex karyotypes, associated with loss of chromosomal material, and related to disease progression [1, 3].

Deletions or translocations of chromosome band 13q14, the locus of the retinoblastoma (*RB1*) gene, are observed in a variety of hematological malignancies including myelofibrosis (MF), MDS, acute myeloblastic leukemia (AML), chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL) [4-6]. Recently, it has been shown that deletions of 13q14 are detected at a high frequency (more than 40%) in cases of CLL and multiple myeloma (MM) by fluorescence *in situ* hybridization (FISH) analyses [7, 8]. The microsatellite locus *D13S319*, which is located at 13q14.3 and telomeric to the *RB1* gene, is the most commonly deleted marker in CLL and MM [7, 8]. It is suggested that the deletion of a candidate tumor suppressor gene in the vicinity of *D13S319* is a significant event in the tumorigenesis of these lymphoid malignancies. On the other hand, in myeloid malignancies including MDS, deletions or translocations of 13q14 have been also analyzed in association with *RB1* and *D13S319* [9-13], but the pathological roles of these abnormalities, especially of 13q14 translocations, remain to be completely elucidated.

We describe here a novel translocation der(13)t(7;13)(p13;q14) involving 13q14 in MDS. The results suggest that the *RB1* gene and/or another

tumor suppressor gene near *D13S319* may be involved in the pathogenesis of MDS with 13q14 translocations by monoallelic deletion.

2. Materials and methods

2.1. Case History

A 66-year-old woman was admitted to our hospital for precise examination of progressive anemia in December 2003. As of 1986, she had been already diagnosed as mild macrocytic anemia in another hospital, but the definite diagnosis could not be obtained. In June 2002, her hemoglobin level decreased to below 7.0 g/dl and the treatment with transfusion of red blood cells was started once a month. Chromosome analyses have not been performed during the clinical course before admission to our hospital.

Peripheral blood on admission showed hemoglobin 6.4 g/dl, platelets 189 x 10^9/l and white blood cells 2.6 x 10^9/l with 1% blasts, 1% bands, 35% segmented neutrophils, 4% monocytes, 5% eosinophils, 2% basophils and 52% lymphocytes. Bone marrow was hypercellular with 7.4% blasts. Dysplastic changes including cytoplasmic hypogranulation of neutrophils, megaloblastic morphology of erythroblasts and micromegakaryocytes, were observed in the bone marrow cells. Surface marker analysis by three-color flow cytometry with CD45 gating revealed that the blasts were positive (more than 20%) for CD4 (73.7%), CD10 (63.1%), CD16 (59.0%), CD13 (96.2%), CD14 (56.2%), CD33 (97.4%), CD41a (64.4%) and HLA-DR (26.1%), but negative for CD34 (9.1%). She was diagnosed as MDS, refractory anemia with excess of blasts (RAEB) in the French-American-British (FAB) classification, or RAEB-I in the World Health Organization (WHO) classification. She received non-myeloablative cord blood transplantation in March 2004, but the disease relapsed soon after. She died of disease progression and pneumonia in May 2004.

2.2. Chromosome analysis and Spectral Karyotyping (SKY)

Chromosome analyses were performed on short-term culture of the cells

obtained from bone marrow on admission (December 2003) and before transplantation (February 2004) by the G-banding technique. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995) [14]. Spectral karyotyping (SKY) was carried out with SkyPaint[TM] kit (Applied Spectral Imaging, Migdal Ha'Emek, Israel) according to the manufacturer's instructions. A total of 5 metaphase spreads obtained on admission were analyzed for spectral karyotyping.

2.3. Fluorescence in situ hybridization (FISH) analysis

Probes used in FISH analyses were LSI 13 (*RB1*) SpectrumGreen Probe and LSI *D13S319* (13q14.3) SpectrumOrange Probe (Vysis, Downers Grove, IL, USA). The LSI 13 DNA probe hybridizes to band 13q14, spanning the *RB1* region of human chromosome 13. In a normal cell hybridized with the *RB1* probe, the expected signal pattern is two distinct signals. In a cell with a *RB1* deletion, one signal will be observed. The LSI *D13S319* DNA probe hybridizes to the band 13q14.3, locus of *D13S319* located telomeric to the *RB1* gene, of human chromosome 13. In a normal cell, the expected pattern for the *D13S319* probe is the two distinct signals. In a hybridized abnormal cell containing the deletion, one signal pattern will be observed. FISH analyses were performed on 20 metaphase spreads and 100 interphase nuclei obtained on admission.

3. Results

Chromosome analysis on admission showed the complex karyotypes as follows (Fig. 1): 46,XX,del(6)(q?),-7,der(13)t(7;13)(p13;q14),+mar1 [20]. The same karyotype was also detected just before cord blood transplantation. To identify the origin of deleted and marker chromosomes, we applied SKY analysis on metaphase spreads of the same sample preparation. SKY analysis revealed that the marker chromosome was derived from chromosome 7 with unbalanced translocations involving chromosome 6 and 13. Deletion of 6q was found to be resulting from unbalanced translocation with chromosome 7. Furthermore, the structure of der(13)t(7;13)(p13;q14) was confirmed. The der(13)t(7;13)(p13;q14) was generated by the translocation of the segment 7p13-7pter onto 13q14, whereas the chromosome 13q material was translocated onto the 6q fragment of the der(7)t(6;7)(q?;q11), but not 7p13. As a result, the karyotype shown by G-banding was revised as follows (Fig. 2): 46,XX,der(6)t(6;7)(q11;?),der(7)del(7)(p13?)t(6;7)(q?;q11)t(6;13)(q?;q?),der(13)t(7;13)(p13;q14)[5].

G-banding and SKY analyses identified the der(13)t(7;13)(p13;q14), suggesting that the *RB1* gene and *D13S319* at 13q14 may be involved in the translocation. Hence, we performed FISH analyses with these probes on metaphase spreads and interphase nuclei. FISH with *RB1* showed that only a single signal was observed on normal chromosomes 13 in all 20 metaphase spreads analyzed (Fig. 3A). On the other hand, FISH on interphase nuclei revealed that 59% and 41% of 100 non-dividing cells exhibited one and two *RB1* signals, respectively (Fig. 3B). FISH with *D13S319* also showed similar results. Only one *D13S319* signal was detected on normal chromosome 13 in all 20 metaphase spreads examined (Fig. 3C), whereas 65% and 35% of 100 cells demonstrated one and two *D13S319* signals by

interphase FISH, respectively (Fig. 3D). These results indicated that one allele of *RB1* and *D13S319* was deleted following the translocation between 13q14 and 7p13.

4. Discussion

On the basis of G-banding and SKY analyses, we defined an unbalanced translocation der(13)t(7;13)(p13;q14) in a case of MDS. Furthermore, FISH analyses revealed monoallelic loss of *RB1* and *D13S319* in all metaphase spreads and in approximately 60% of interphase cells. This discrepancy in FISH analyses may be due to the higher ability of cells with *RB1*/*D13S319* deletions to divide in culture than cells without deletions. Translocations between 7p13 and 13q14 have never been described in human malignancies [15], then this is the first case with the der(13)t(7;13)(p13;q14). This translocation appeared as a part of three-way translocations involving chromosomes 6, 7 and 13, but we could not completely identify the breakpoints on the der(6)t(6;7) and the der(7)t(6;7)t(6;13) because of the complexity of the translocations. In view of the structures of three derivative chromosomes, it is supposed that the translocations evolved from a primary translocation involving chromosomes 6 and 7, followed by the second exchange between the long arm of chromosome 13 and the der(7)t(6;7)(q?;q11). Alternatively, it is possible that these complex translocations occurred simultaneously. However, considering that the translocations were identified at the initial examination and in all metaphase spreads analyzed, it is difficult at present to elucidate whether the der(13)t(7;13)(p13;q14) is a primary genetic event or an additional change during disease progression of MDS.

In myeloid malignancies including MDS, interstitial deletions involving 13q14 are recurrently found [4-6], and it is naturally expected that the *RB1* gene at 13q14 may be deleted. In fact, it has been shown that *RB1*, *D13S319* and *D13S25*, which is located more downstream of *D13S319*, are deleted in almost all cases of myeloid malignancies with 13q14 deletions examined, especially in cases of agnogenic myeloid metaplasia (AMM)/MF,

AML and MDS [9-12]. Furthermore, Nagamura et al. [16] revealed by colony-forming assay and interphase FISH that the majority of hematopoietic progenitor cells of MDS with del(13)(q12q14) lacked one allele of the *RB1* gene and suggested an important role of the *RB1* gene in abnormal hematopoiesis.

On the other hand, myeloid malignancies with 13q14 translocations have been less evaluated about *RB1* deletions. Juneau et al. [9] reported loss of the *RB1* gene in 2 cases of AMM with 13q14 translocations. La Starza et al. [10] delineated a commonly deleted region covering *RB1*, *D13S319* and *D13S25* loci in 3 cases of AML/MDS with translocations involving 13q13-q14. Sinclair et al. [12] reported that 2 MF patients with balanced translocations involving 13q14 exhibited loss of *RB1*, *D13S319* and *D13S25*. Tanaka et al. [11] investigated 7 cases of myeloid malignancies with 13q14 translocations and showed allelic loss of these 3 loci in 3 cases of AML/MDS and loss of the *RB1* gene only in 2 cases of AML. However, these 3 loci were intact in 2 cases of AML/CML. Coignet et al. [13] also reported that 6 cases of AML/CML with t(12;13)(p12;q14) retained the *RB1* gene and their breakpoints at 13q14 were clustered centromeric of the *RB1* gene. As a result, breakpoints and deleted regions were heterogeneous in myeloid malignancies with 13q14 translocations, although several cases showed monoallelic loss of *RB1* and *D13S319*.

As shown in Table 1, a total of 14 cases of MDS with translocations involving 13q14 have been described in the literature to date [10-11, 17-25]. Almost all cases had complex karyotypes. Balanced and unbalanced translocations were observed in 7 cases and 7 cases, respectively. In 3 cases (No. 3, 7, 8), the 13q14 translocations were not detected in the stem line, suggesting that these aberrations might be acquired during disease progression and have different roles from others. Subtypes of MDS were various and common clinical features in these cases could not be found, because only

limited information was available. Three cases (No. 3, 9, 10) exhibited the same translocation t(12;13)(p13;q14), suggesting that t(12;13)(p13;q14) is one of the non-random aberrations in MDS. Deletions of *RB1* and *D13S319* were examined in 5 and 4 cases, respectively and loss of these 2 loci was detected in 4 cases (No. 10-12, 14). Only one case with t(12;13)(p13;q14) (No. 9) retained the *RB1* gene, whereas it was deleted in another case (No. 10). That is, molecular mechanisms of t(12;13)(p13;q14) in MDS appear to be not equivalent. Other three cases (No. 10-12) lacked *D13S25* as well as *RB1* and *D13S319*, although we could not perform FISH with *D13S25* in the present case. These findings indicate that submicroscopic deletions accompanying balanced or unbalanced 13q14 translocations are recurrent cytogenetic aberrations in MDS and that commonly deleted regions at 13q14 extend from *RB1* to at least *D13S319* and may prolong to *D13S25* loci. It is possible that unidentified tumor suppressor gene(s) located in the vicinity of *D13S319* as well as *RB1* may be implicated in the pathogenesis of MDS having 13q14 translocations associated with cryptic deletions.

The RB protein acts as a cell cycle regulator which blocks the transition of normal cells from G0/G1 into S phase of the cycle. Mice with homozygous disruption of the *RB1* alleles resulted in an overall normal development but had lethal anemia, suggesting a critical role of the *RB1* gene in erythropoiesis [26]. In the present case, anemia was predominantly observed during the clinical course, while white blood cells and platelets counts were relatively preserved. This impaired erythropoiesis might be related to monoallelic loss of the *RB1* gene. In several solid tumors carrying 13q14 deletions of one allele, the *RB1* gene is usually inactivated by point mutations and/or gross rearrangements of the other allele. Unfortunately, we could not examine the mutation of the *RB1* gene in the remaining allele of the present case. Tanaka et al. [11] tested for *RB1* gene mutations in 2 MDS patients with 13q14 translocations (No. 11 and 12 in Table 1) and 4

AML patients with 13q14 deletions. However, no detectable alterations of five exons (exons 12 to 16) of the *RB1* gene encoding pocket A in RB protein was demonstrated. In general, the abnormalities of the *RB1* gene and its expression appear to be a rare event in MDS [27]. These findings may support the possibility that deletions or translocations of 13q14 affect other tumor suppressor gene(s) distinct from the *RB1*, for instance the *RFP2* gene near *D13S319*, as suggested in CLL and MM [28].

Chromosome rearrangements at 7p are often detected in hematological malignancies including MDS and AML, but recurrent aberrations involving 7p13 have not been identified [15, 29]. It is unlikely that chromosome region 7p13 preferentially contains well-known oncogenes or genes involved in tumorigenesis. Recently, the *PURB* gene was identified at 7p13 with *PURA* gene at 5q31 and deletions of these genes were detected in MDS and AML [30]. The Purβ protein functions in a complex with Purα, which is an RB-binding protein and implicated in cell cycle control. It is possible that the *PURB* gene at 7p13 might be involved in the der(13)t(7;13)(p13;q14) in association with *RB1*. Further cytogenetic and molecular analyses for more cases are needed to elucidate the pathogenesis of der(13)t(7;13)(p13;q14) and the pathological roles of *RB1* and *D13S319* in MDS.

References

[1] Fenaux P, Morel P, Lai JL. Cytogenetics of myelodysplastic syndromes. Semin Hematol 1996;33:127-138.

[2] Mecucci C. Molecular features of primary MDS with cytogenetic changes. Leuk Res 1998;22:293-302.

[3] Pedersen B, Norgaard JM, Pedersen BB, Clausen N, Rasmussen IH, Thorling K. Many unbalanced translocations show duplication of a translocation participant. Clinical and cytogenetic implications in myeloid hematologic malignancies. Am J Hematol 2000;64:161-169.

[4] Johnson DD, Dewald GW, Pierre RV, Letendre L, Silverstein MN. Deletions of chromosome 13 in malignant hematologic disorders. Cancer Genet Cytogenet 1985;18:235-241.

[5] Morgan R, Hecht F. Deletion of chromosome band 13q14: A primary event in preleukemia and leukemia. Cancer Genet Cytogenet 1985;18:243-249.

[6] Fitchett M, Griffiths MJ, Oscier DG, Johnson S, Seabright M. Chromosome abnormalities involving band 13q14 in hematologic malignancies. Cancer Genet Cytogenet 1987;24:143-150.

[7] Corcoran MM, Rasool O, Liu Y, Iyengar A, Grander D, Ibbotson RE, Merup M, Wu X, Brodyansky V, Gardiner AC, Juliusson G, Chapman RM, Ivanova G, Tiller M, Gahrton G, Yankovsky N, Zabarovsky E, Oscier DG, Einhorn S. Detailed molecular delineation of 13q14.3 loss in B-cell chronic lymphocytic leukemia. Blood 1998;91:1382-1390.

[8] Chang H, Bouman D, Boerkoel CF, Stewart AK, Squire JA. Frequent monoallelic loss of *D13S319* in multiple myeloma patients shown by interphase fluorescence *in situ* hybridization. Leukemia 1999;13:105-109.

[9] Juneau AL, Kaehler M, Christensen ER, Schad CR, Zinsmeister AR,

Lust J, Hanson C, Dewald GW. Detection of RB1 deletions by fluorescence in situ hybridization in malignant hematologic disorders. Cancer Genet Cytogenet 1998;103:117-123.

[10] La Starza R, Wlodarska I, Aventin A, Falzetti D, Crescenzi B, Martelli MF, Van den Berghe H, Mecucci C. Molecular delineation of 13q deletion boundaries in 20 patients with myeloid malignancies. Blood 1998;91:231-237.

[11] Tanaka K, Arif M, Eguchi M, Guo SX, Hayashi Y, Asaoku H, Kyo T, Dohy H, Kamada N. Frequent allelic loss of the *RB*, D13S319 and D13S25 locus in myeloid malignancies with deletion/translocation at 13q14 of chromosome 13, but not in lymphoid malignancies. Leukemia 1999;13:1367-1373.

[12] Sinclair EJ, Forrest EC, Reilly JT, Watmore AE, Potter AM. Fluorescence *in situ* hybridization analysis of 25 cases of idiopathic myelofibrosis and two cases of secondary myelofibrosis: monoallelic loss of RB1, D13S319 and D13S25 loci associated with cytogenetic deletion and translocation involving 13q14. Br J Haematol 2001;113:365-368.

[13] Coignet LJA, Lima CSP, Min T, Streubel B, Swansbury J, Telford N, Swanton S, Bowen A, Nagai M, Catovsky D, Fonatsch C, Dyer MJS. Myeloid- and lymphoid-specific breakpoint cluster regions in chromosome band 13q14 in acute leukemia. Genes Chromosomes Cancer 1999;25:222-229.

[14] Mitelman F, editor. An international system for human cytogenetic nomenclature. Basel: S. Karger, 1995.

[15] Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations in cancer. Updated on November 29, 2004. Available at: http://cgap.nci.nih.gov/Chromosomes/Mitelman. Accessed December 20, 2004.

[16] Nagamura F, Takabe T, Takahashi S, Ohno N, Uchimaru K, Ogami K,

Iseki T, Tojo A, Asano S. One allele deletion of the *RB1* gene in a case of refractory anemia with del(13)(q12q14): a fluorescence in situ hybridization study of the *RB1* gene. Cancer Genet Cytogenet 2003;146:77-80.

[17] Taniwaki M, Horiike S, Inazawa J, Nishida K, Misawa S, Takino T, Abe T. Cytogenetic studies in 32 patients with myelodysplastic syndrome: insights to specific chromosomal abnormalities and prognosis. Jpn J Clin Oncol 1987;17:141-150.

[18] Pinkerton PH, London B, Dube ID, Senn JS. Trisomy 14q in myelodysplastic syndromes. Cancer Genet Cytogenet 1990;49:113-116.

[19] Abeliovich D, Yehuda O, Ben-Neriah S, Matzner Y. Therapy-related myelodysplastic syndrome. Two cytogenetically unrelated abnormal clones in a patient with multiple myeloma. Cancer Genet Cytogenet 1993;70:117-119.

[20] Grigg AP, Gascoyne RD, Phillips GL, Horsman DE. Clinical, haematological and cytogenetic features in 24 patients with structural rearrangements of the Q arm of chromosome 3. Br J Haematol 1993;83:158-165.

[21] Toze CL, Barnett MJ, Klingemann HG. Response of therapy-related myelodysplasia to low-dose interleukin-2. Leukemia 1993;7:463-465.

[22] Parlier V, van Melle G, Beris Ph, Schmidt PM, Tobler A, Haller E, Jotterand Bellomo M. Hematologic, clinical, and cytogenetic analysis in 109 patients with primary myelodysplastic syndrome. Prognostic significance of morphology and chromosome findings. Cancer Genet Cytogenet 1994;78:219-231.

[23] Wang P, Spielberger RT, Thangavelu M, Zhao N, Davis EM, Iannantuoni K, Larson RA, Le Beau MM. dic(5;17): A recurring abnormality in malignant myeloid disorders associated with mutations of *TP53*. Genes Chromosomes Cancer 1997;20:282-291.

[24] Fugazza G, Cerri R, Bruzzone R, Patrone F, Sessarego M. Duplication of the der(13)t(12;13)(p13;q14) in chronic myelomonocytic leukemia. Haematologica 1997;82:336-337.

[25] Mohr B, Bornhauser M, Thiede C, Schakel U, Schaich M, Illmer T, Pascheberg U, Ehninger G. Comparison of spectral karyotyping and conventional cytogenetics in 39 patients with acute myeloid leukemia and myelodysplastic syndrome. Leukemia 2000;14:1031-1038.

[26] Lee EYHP, Chang CY, Hu N, Wang YCJ, Lai CC, Herrup K, Lee WH, Bradley A. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 1992;359:288-294.

[27] Preudhomme C, Vachee A, Lepelley P, Vanrumbeke M, Zandecki M, Quesnel B, Cosson A, Fenaux P. Inactivation of the retinoblastoma gene appears to be very uncommon in myelodysplastic syndromes. Br J Haematol 1994;87:61-67.

[28] Elnenaei MO, Hamoudi RA, Swansbury J, Gruszka-Westwood AM, Brito-Babapulle V, Matutes E, Catovsky D. Delineation of the minimal region of loss at 13q14 in multiple myeloma. Genes Chromosomes Cancer 2003;36:99-106.

[29] Mecucci C, van Orshovens A, Boogaerts M, Michaux JL, Van den Berghe H. Characterization of deletions of chromosome 7 short arm occurring as primary karyotypic anomaly in acute myeloid leukaemia. Br J Haematol 1989;71:13-17.

[30] Lezon-Geyda K, Najfeld V, Johnson EM. Deletions of *PURA*, at 5q31, and *PURB*, at 7p13, in myelodysplastic syndrome and progression to acute myelogenous leukemia. Leukemia 2001;15:954-962.

.

Figure legends

Fig. 1. G-banded karyotype of the bone marrow cells. The karyotype is 46,XX,del(6)(q?),-7,der(13)t(7;13)(p13;q14),+mar1. Arrows indicate rearranged chromosomes.

Fig. 2. Spectral karyotypes of the metaphase spread after spectra based classification (left side, reverse DAPI; right side, SKY). Chromosomes were assigned a pseudocolor according to the measured spectrum. The revised karyotype is 46,XX,der(6)t(6;7)(q11;?),der(7)del(7)(p13?)t(6;7)(q?;q11)t(6;13)(q?;q?),der(13)t(7;13)(p13;q14). Arrows indicate rearranged chromosomes.

Fig. 3. FISH analyses with the *RB1* probe (A, B) and the *D13S319* probe (C, D) on metaphase spreads (A, C) and interphase nuclei (B, D). The arrow indicates (A) one *RB1* signal on normal chromosome 13, (B) single *RB1* signal, (C) one *D13S319* signal on normal chromosome 13, and (D) single *D13S319* signal.
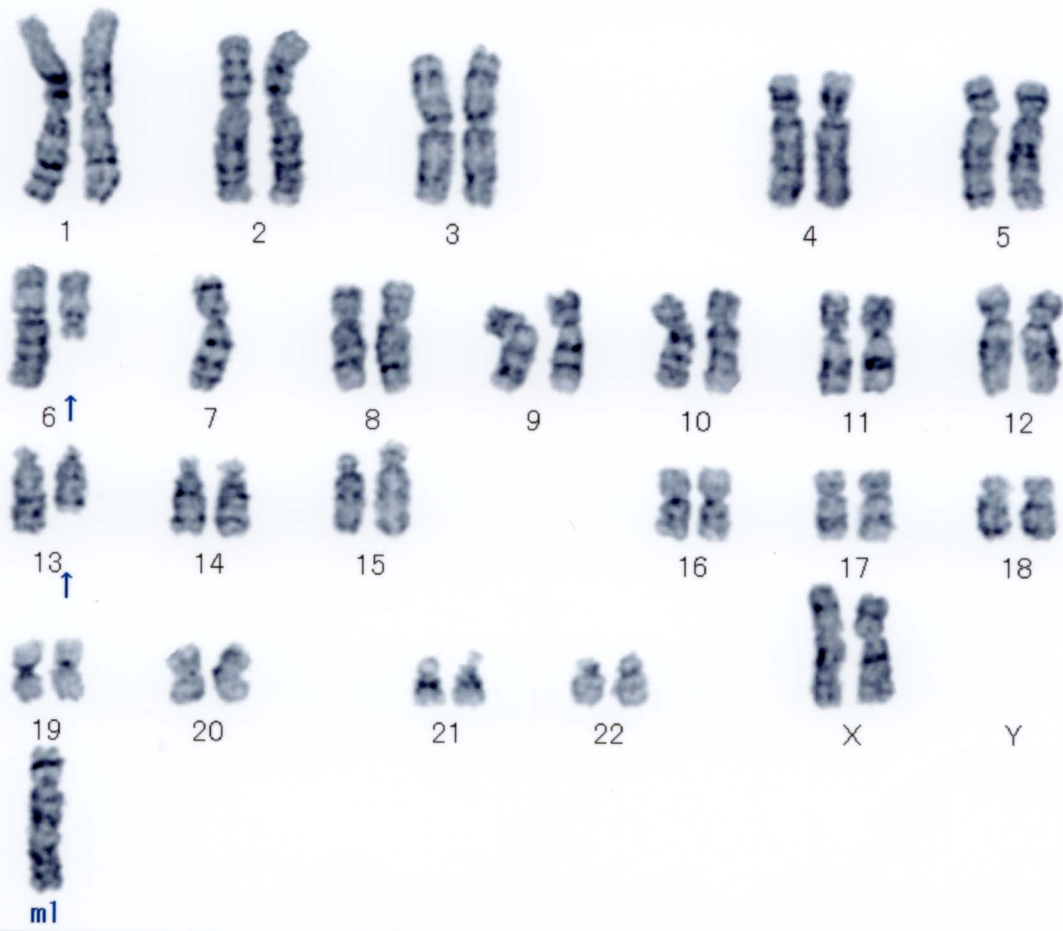
Table 1. Reported cases of myelodysplastic syndrome with translocations involving 13q14
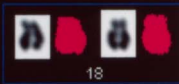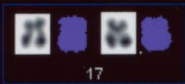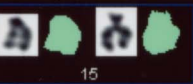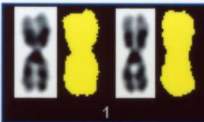
Case No.	Age/ Sex	Diagnosis	Karyotypes	RB1 FISH	D13S319 FISH	References
1	61/M	sMDS	44,X,t(Y;12;18)(p11;q12;q12),t(1;5)(q42;q13),t(2;10;17)(p13;p12;p11),**der(3)t(3;13) (p31;q14)**,der(4)t(4;5)(p11;q11),-5,t(7;22)(q36;q11),-13,del(16)(q22)[36]	ND	ND	Taniwaki *et al*, 1987 [17]
2	40/F	RAEB	46,XX,**t(1;13)(p36;q14)**[8]/47,XX,+14[4]/46,XX[5]	ND	ND	Pinkerton *et al*, 1990 [18]
3	69/M	sMDS	46,XY,del(5)(q13q35),del(7)(q22)[16]/46,idem,**t(12;13)(p13;q14)**[4]/46,XY,t(1;1)(p13;p36) [4]/46,XY[3]	ND	ND	Abeliovich *et al*, 1993 [19]
4	88/F	RAEB-t	45,XX,del(3)(q21),-5,add(7)(q21),**der(7)t(7;13)(q22;q14)**,-12,add(13)(q22),+mar[23]/ 46,XX[2]	ND	ND	Grigg *et al*, 1993 [20]
5	31/F	RAEB	44-46,XX,**t(1;13)(p22;q14)**,t(1;20)(q25;p13),-5,-7,+8,add(17)(p11),-21[13]/46,XX[17]	ND	ND	Toze *et al*, 1993 [21]
6	80/M	CMML	43,X,-Y,**der(2)t(2;13)(p23-25;q12-14)**,-3,-5,del(7)(q22q33-34),add(12)(p11.2-12),-13, del(16)(q12-13q24),+r[10]/42,idem,-r[3]/44,idem,+13[3]/43,idem or +Y,-r[11]/46,XY[19]	ND	ND	Parlier *et al*, 1994 [22]
7	30/F	RA	46,XX,add(8)(q24)[9]/46,idem,**t(6;13)(q23;q14)**[3]/46,XX[5]	ND	ND	Parlier *et al*, 1994 [22]
8	64/F	sMDS	45,XX,-7[3]/45,XX,dic(5;17)(q11;p11)[11]/44,XX,dic(5;17),**der(7)t(7;13)(q11;q14)**, add(13)(q14),-17[5]/44,XX,-3,dic(5;17),der(7)t(3;7)(p14;q32)[6]/	ND	ND	Wang *et al*, 1997 [23]

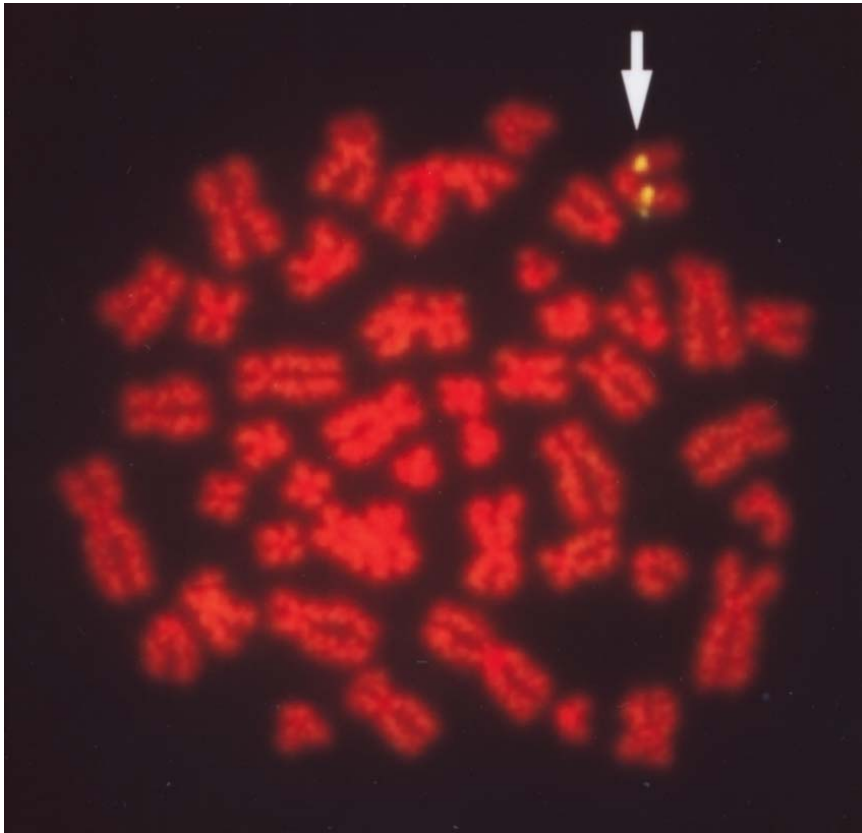|						
			44,XX,-3,der(5)t(5;7)(q11;p1?5),dic(5;17),der(7)t(7;16)(p1?5;p13)t(3;7), der(16;17)t(16;17)(p13;p11)ins(16;5)(p13;q11q11),add(21)(q22)[3]			
9	70/F	CMML	50,XX,+i(1)(q10),+8,+10,+11,**t(12;13)(p13;q14)**[4]/51,idem,+**der(13)t(12;13)(p13;q14)**[25]/ 46,XX[3]	der(12)	ND	Fugazza *et al*, 1997 [24]
10	73/M	MDS	43,X,-Y,del(5)(q13q21),der(7)t(7;20)(p11;p11),**t(12;13)(p13;q14)**,-17,der(19)t(17;19) (q21;q13),-20[9]/44,idem,+**der(13)t(12;13)(p13;q14)**[5]/44,idem,+13[2]	Del	Del	La Starza *et al*, 1998 [10]
11	63/M	MDS→ AML M6	47,XY,**t(2;13)(q27;q14)**,add(3)(q11),der(4)t(4;8)(q21;q?22),add(5)(q15),del(7)(q22),t(12;20) (p11;q11),add(14)(q24),-16,-18[16]	Del	Del	Tanaka *et al*, 1999 [11]
12	63/M	MDS	46,XY,der(2)t(2;11)(q31;p11),del(7)(q13q25),**der(11)inv(11)(p11q24)t(2;11)(q31;p11) t(11;13)(q21;q14),der(13)t(11;13)(p11;q14)**,del(17)(p11)[25]/46,XY[2]	Del	Del	Tanaka *et al*, 1999 [11]
13	74/F	RARS	46,XX,**der(6)t(6;13)(p21;q14)**,der(13)t(6;13;22)(p21p?;q?;q1?1),t(20;22)(q11;q1?1)[8]/ 46,XX[1]	ND	ND	Mohr *et al*, 2000 [25]
14	66/F	RAEB	46,XX,der(6)t(6;7)(q11;?),der(7)del(7)(p13?)t(6;7)(q?;q11)t(6;13)(q?;q?), **der(13)t(7;13)(p13;q14)**[5]	Del	Del	Present case

Abbreviations: M, male; F, female; ND, not done; Del, deletion; sMDS, secondary myelodysplastic syndrome; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation; CMML, chronic myelomonocytic leukemia; AML, acute myeloblastic leukemia. Bold letters show 13q14 chromosomal translocations.
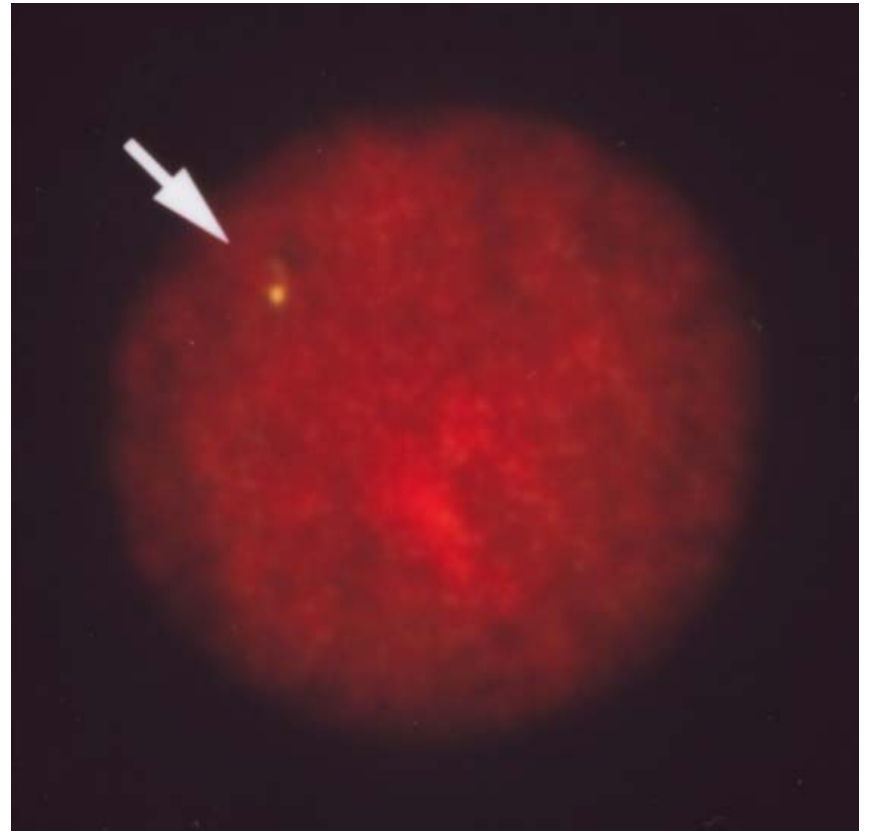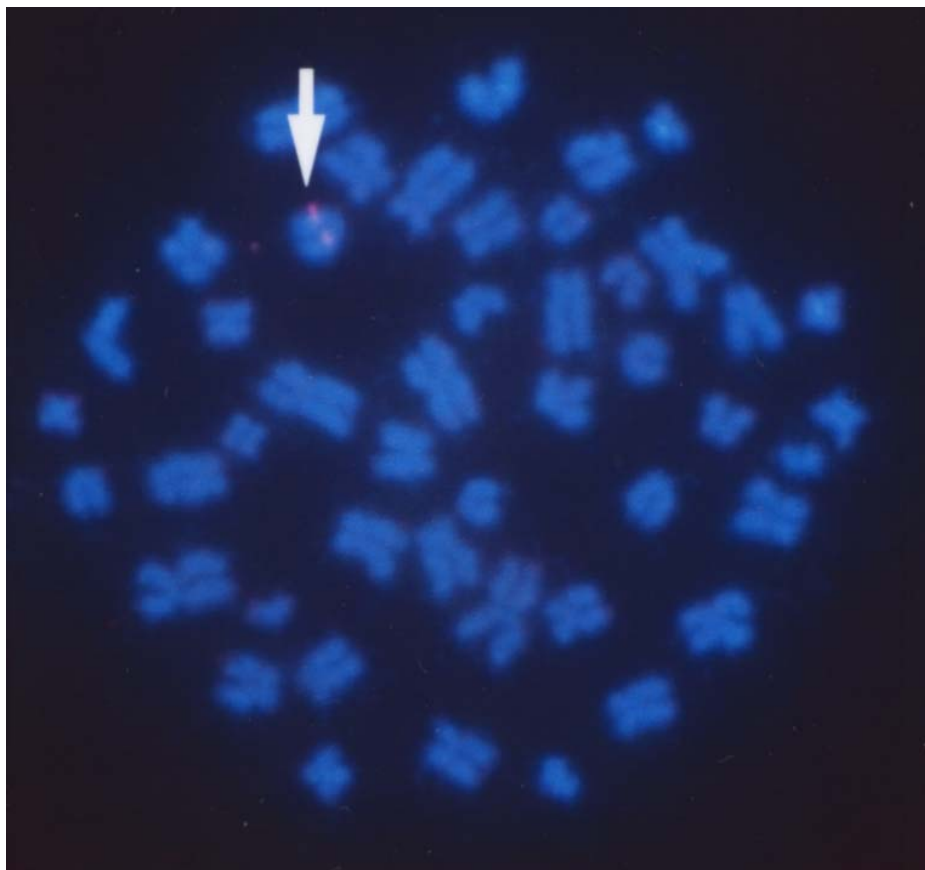
1 2 3 4 5

6 ↑ 7 8 9 10 11 12

13 ↑ 14 15 16 17 18

19 20 21 22 X Y

m1

A   B