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A new complex karyotype in a unique de novo myelodysplastic syndrome case involving ten chromosomes and monoallelic loss of *TP53*

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ABSTRACT

Myelodysplastic syndrome (MDS) is a group of clonal stem cell disorders characterized by cytopenia, dysplasia in one or more cell lineages, and ineffective hematopoiesis. MDS is associated with high risk of progression to acute myeloid leukemia. At initial diagnosis, clonal cytogenetic aberrations are present in 40–70% of patients with de novo (primary) MDS and in 65–95% of patients with therapy-associated ones (t-MDS). Most commonly observed abnormalities present in MDS are monosomy 5 and 7, trisomy 8, deletions of long arm of chromosome 20 as well as complex karyotypes. Loss or gain of chromosomal material is known to be related to disease development and progression.

In a de novo adult MDS case banding cytogenetics, refined by array-proven multicolor banding (aMCB) revealed a unique complex karyotype involving ten chromosomes that include del(5q), del(7q), deletions in parts of both chromosomes 10, and a dic(7;17). Interestingly, the dic(7;17) leads to monosomy of the tumor suppressor gene *TP53*. The patient had an immunophenotype consistent with refractory anemia with excess blasts in transformation (RAEB-t) according to French–American–British (FAB) classification.

To the best of our knowledge, a comparable adult MDS case associated with such a complex karyotype and loss of *TP53* was not previously reported. As most of complex aberrations were found simultaneously in all studied malignant cells this may be a hint on an initial one step development of this complex rearrangement by chromothripsis.

1. Introduction

Myelodysplastic syndromes (MDS), a group of clonal stem cell disorders, are characterized by cytopenia, dysplasia in one or more cell lineages, and ineffective hematopoiesis. They are associated with significant morbidity and mortality due to bone marrow failure or evolution to acute myeloid leukemia (AML). Overall, there is a short survival time to be observed in 20 to 40% of MDS patients (Aul et al., 1995). According to the World Health Organization (WHO), MDS is an age associated disorder, occurring predominantly in elderly patients with a median age of 70 years and is rarely seen before the age of 50 years (Brunning et al., 2001).

Cytogenetic findings are among the few independent variables correlated with clinical outcome in MDS, and they form the cornerstone of the International Prognostic Scoring System (IPSS) (Olney and Le Beau, 2001). At diagnosis, clonal cytogenetic aberrations are present in 40 to 70% of patients with de novo (primary) MDS and in 65 to 95% of patients with therapy-associated MDS (t-MDS) (Fenaux et al., 1996).

Common recurrent cytogenetic abnormalities in order of decreasing frequency, include −7/del(7q), −5/del(5q), +8, del(20q), —Y, i(17q) or t(17p), −13/del(13q), del(11q), del(12p) or t(12p); besides complex karyotypes are frequently described in MDS cases (Aul et al., 1995; Fenaux et al., 1996). Unbalanced rearrangements are frequent in MDS and they can be detected as a part of complex karyotypes. Chromosomal deletions leading to loss of multiple genes, putatively tumor suppressor genes (TSGs) (Fenaux et al., 1996; Heim, 1992) and gain of chromosomal material may activate oncogenes, thus both may be related to disease progression (Aul et al., 1995; Pedersen et al., 2000; Slovak et al., 2010).

Here we report a highly complex case of an adult de novo MDS with a unique complex karyotype including a stable dicentric derivative chromosome and monoallelic loss of the TSG *TP53*.

2. Material and methods

2.1. Case report

A 52-year-old male patient without familial medical background of malignancy presented with a 1 month history of rhinorrhagia, fatigue, loss of weight, and fever. Physical examination revealed

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hepatosplenomegaly without lymph node involvement. Initial complete blood count revealed white blood cells count (WBC) of 2.5 × 10^9/l, red blood cells (RBC) 2.48 × 10^6/mm^3, hemoglobin (Hb) level of 6.4 g/dl, and platelet count was 22.8 × 10^9/l. Blasts in bone marrow aspiration were more than 20%. Serum lactate dehydrogenase (LDH) value was 1358 U/l (normal value up to 480 U/l), serum aspartate aminotransferase level was 62 U/l (normal up to 45 U/l) and alanine aminotransferase level 39 U/l (normal up to 45 U/l). Total serum protein was within normal range at 5.1 g/dl (normal value 6.4–8.3 g/dl) but serum albumin was 3.1 g/dl (normal value 3.2–5 g/dl). The patient was treated immediately according to a standard AML protocol, before MDS was diagnosed based on immunophenotyping. After one day of the therapeutic treatment his hematological parameters were WBC 1 × 10^9/l, RBC 2.6 × 10^6/mm^3, Hb level 8.6 g/dl, platelet count 19 × 10^9/l, and LDH value 642. Three days later, the patient succumbed due to unknown causes. His brother agreed with scientific evaluation of the case and the study was approved by the ethical committee of the Atomic Energy Commission, Damascus, Syria.

2.2. Chromosome analysis

Chromosome analysis using GTG-banding according to standard procedures (AL-Achkar, Wafa, Nweder, 2007) was performed before the treatment. A minimum of 20 metaphase cells derived from unstimulated bone marrow culture were analyzed. Karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 2013) (Shaffer et al., 2013).

2.3. Molecular cytogenetics

Fluorescence in situ hybridization (FISH) using a specific probe for 17p13 (*TP53*) (Q-Biogene, USA), centromere-specific probes (CEP) for chromosome 7, 9 and 17, and a subtelomeric (ST) probe for 9qter (Abbott Molecular/Vysis, Abbott Park, IL, USA) were applied according to the manufacturer's instructions (AL-Achkar, Wafa, Nweder, 2007). Multicolor FISH (M-FISH) using all 24 human whole chromosome paints as probes and array-proven multicolor banding (aMCB) probes sets based on microdissection derived region-specific libraries for chromosomes 3, 5, 6, 7, 10, 15 and 18 were performed as previously reported (Liehr et al., 2002). A minimum of 10 metaphase spreads were analyzed, using a fluorescence microscope (AxioImager.Z1 mot, Carl Zeiss Ltd., Hertfordshire, UK) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes plus the counterstain DAPI (4′,6-diamino-2-phenylindole). Image capture and processing were performed using an ISIS imaging system (MetaSystems).

2.4. Flow cytometric immunophenotype

Immunophenotyping was performed using a general panel of fluorescent antibodies against the following antigens typical for different cell lineages and cell types: CD1a, CD2, CD3, CD4, CD5, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD32, CD33, CD34, CD38, CD41a, CD45, CD56, CD57, CD64, CD103, CD117, CD123, CD138, CD209, CD235a and CD243; in addition antibodies to Kappa and Lambda light Chains, IgD, sIgM, and HLADr were tested. All antibodies were purchased from BD Biosciences. Samples were analyzed on a BD FACSCalibur™ flow cytometer. Autofluorescence, viability, and isotype controls were included. Flow cytometric data acquisition and analysis were conducted by BD Cellquest™ Pro software.

3. Results

Prior to the treatment GTG-banding revealed a karyotype of 39–42,XY,-5,-7,-15,-18,der(3)t(3;10)(?:?),der(6)t(5;6)(?:?),dic(7;17)(?:?),der(9)t(3;9)(?:?),del(10)(q?),del(10)(p?),der(18)t(15;18)(?:?) [18];the [18] means number of cells/39–41,X,-5,-7,-15,-18,-Y,der(3)t(3;10)(?:?),der(6)t(5;6)(?:?),dic(7;17)(?:?),der(9)t(3;9)?:?),del(10)(q?),del(10)(p?),der(18)t(15;18)(?:?) [2]; the [2] means number of cells (Fig. 1). Further studies were performed based on molecular cytogenetics (Figs. 2–3). Dual-color-FISH (D-FISH) using specific CEP probes for chromosomes 7 and 17 revealed one green signal on normal chromosome 7, one red signal on normal chromosome 17, and one red and one green signal on the dic(7;17), respectively (Fig. 2). A probe-set specific for ST 9q-region characterized one red signal to be on normal chromosome 9 and the second red signal on the dic(7;17) (Fig. 2). The locus-specific probe 17p13 (*TP53*) confirmed the absence of the 17p on the dic(7;17) (Fig. 3). M-FISH showed many unbalanced rearrangements involving overall ten chromosomes including both chromosomes 10. aMCB, using probes for most of the derivative chromosomes revealed the following final karyotype:

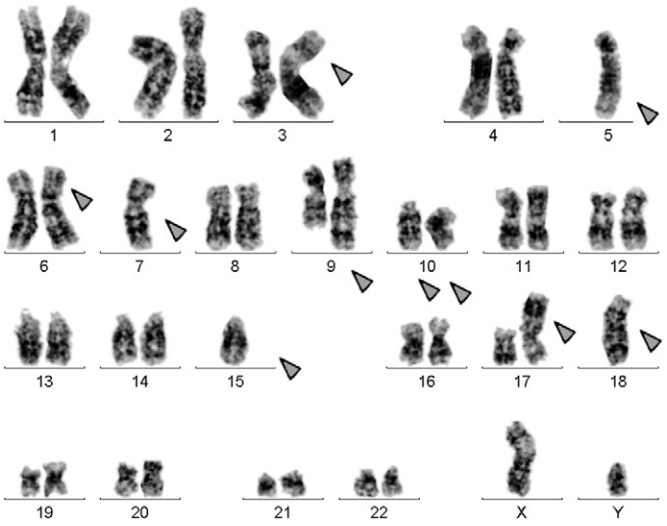

Fig. 1. GTG-banding revealed a complex karyotype in 18/20 metaphases which was resolved by FISH (see Fig. 2) as: 39–42,XY,-5,-7,-15,-18,der(3)t(3;10)(p21.3;p11.2),der(6)t(5;6)(6pter- > 6p22::5p15.3- > 5p13.3::6p22- > 6qter),dic(7;17)(17qter- > 17p11.2::7p11.2- > 7q11.2::10q23- > 10q26.3::7q11.2- > 7q36::9q34- > 9qter),der(9)t(3;9)(p21.3;q34),del(10)(q23),del(10)(p11.2),der(18)t(15;18)(18pter- > 18q23::15q21.2- > 15q23::18q12- > 18pter). All derivative chromosomes are marked and highlighted by arrow heads.

39–42,XY,-5,-7,-15,-18,der(3)t(3;10)(p21.3;p11.2),der(6)t(5;6)(6pter- > 6p22::5p15.3- > 5p13.3::6p22- > 6qter),dic(7;17)(17qter- > 17p11.2::7p11.2- > 7q11.2::10q23- > 10q26.3::7q11.2- > 7q36::9q34- > 9qter),der(9)t(3;9)(p21.3;q34),del(10)(q23),del(10)(p11.2),der(18)t(15;18) (18pter- > 18q23::15q21.2- > 15q23::18q12- > 18pter) [18];the [18] means number of cells/39–41,X,-5,-7,-15,-18,-Y,der(3)t(3;10)(p21.3;p11.2),der(6)t(5;6)(6pter- > 6p22::5p15.3- > 5p13.3::6p22- > 6qter),dic(7;17)(17qter- > 17p11.2::7p11.2- > 7q11.2::10q23- > 10q26.3::7q11.2- > 7q36::9q34- > 9qter),der(9)t(3;9)(p21.3;q34),del(10)(q23),del(10)(p11.2),der(18)t(15;18) (18pter- > 18q23::15q21.2- > 15q23::18q12- > 18pter) [2]; the [2] means number of cells.

Immunophenotyping on bone marrow specimen characterized this case as RAEB-t according to FAB classification. Granulocytes and monocytes showed low side scatter profile. The blast cell population was positive for $CD45^{dim}$, CD34, CD33 and expressed CD13, MPO, HLA-DR, CD117 heterogeneously. This cell population was negative for CD10, CD235a, CD41a and lymphocytes markers.

4. Discussion

We report a cytogenetically highly complex de novo adult MDS case with a karyotype involving ten chromosomes, a dicentric derivative derived from parts of chromosomes 7 and 17 leading to partial monosomy 17p including TSG *TP53*.