Myelodysplastic syndrome transforming to atypical chronic myeloid leukemia shows changes in its mutation allele frequency and acquisition of new mutations

Hakim NM a, Tam W b, Philipovsky A a,b, Tonk V c, Orazi A a,*

a Texas Tech University Health Sciences Center, El Paso, TX, USA
b Weill Cornell Medicine, New York, NY, USA
c Texas Tech University Health Sciences Centre, Lubbock, TX, USA

ABSTRACT

Although acute transformation to acute myeloid leukemia represents a well-established form of disease progression in myelodysplastic syndromes (MDS), the progressive development of proliferative features with a phenotypic shift to a myelodysplastic/myeloproliferative neoplasm such as chronic myelomonocytic leukemia developing from a prior MDS has also been observed. However, transition from a MDS to an atypical chronic myeloid leukemia (aCML) is exceptionally rare. Herewith we report one such case, describing its clinical, morphologic and molecular correlates. The observed molecular progression which paralleled the phenotypic shift, partially elucidates the pathogenetic mechanisms involved in this rare type of disease progression.

1. Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic diseases characterized by cytopenia(s) associated with ineffective hematopoiesis, morphologic dysplasia, immunophenotypic aberrancy, recurrent cytogenetic abnormalities and somatic mutations, and increased risk of developing acute myeloid leukemia (AML) [1]. Although acute transformation to AML represents the commonest form of disease progression in MDS, the progressive development of proliferative features with a phenotypic shift from MDS to a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) such as chronic myelomonocytic leukemia (CMMML) has also been observed. However, transition from a MDS to atypical chronic myeloid leukemia BCR-ABL1 negative (aCML) is exceptionally rare. Herewith we describe one such case and its molecular correlates and discuss the pathogenetic role involved in this rare type of disease progression.

2. Case report

We report a case of an 81 year old male with a history of diabetes mellitus and hypertension who presented with generalized weakness, fatigue, and an 18 kgs (40-lb) weight loss over two years. Initial CBC revealed severe pancytopenia: WBC: 0.85 × 10^9/L, HB: 5.6 g/dl, HCT: 17.7%, platelets: 68 × 10^9/L. The differential count showed: Neutrophils 55%, Bands 6%, Lymphocytes 32%, Monocytes 3%, Eosinophils 3% and Basophils 1%. He underwent bone marrow (BM) examination which yielded suboptimal specimens. The hemodiluted BM aspirate showed occasional dysplastic megakaryocytes and 2% blasts. BM karyotype showed 47,XY,+8[6]/46,XY[14]. Because his serum erythropoietin (EPO) level was less than 500 mU/mL (normal 4–26 mU/mL), injections of 40,000 EPO units every two weeks were prescribed. In addition, he received six cycles of Azacitidine (75 mg/m^2). His-CBC improved and he became transiently transfusion independent. A repeated BM examination two months later, showed normal for age BM cellularity (30%), 2% blasts and significant (>10%) dysplastic changes in the megakaryocytic series (Fig. 1). The diagnosis was MDS unclassifiable (MDS with single lineage dysplasia but with pancytopenia, per WHO 2017) [1]. BM karyotype showed 47,XY,+8,del(9)(q22q32)[10]/46,XY[10]. Mutation analysis with a target-sequencing panel of myeloid genes was performed: U2AF Q157P, GATA2 frameshift mutation were found associated with several subclonal mutation in GATA2 and KDM6A (Table 1). He received supportive care only. In the next five months, in the presence of persistent anemia (HB: 5.2 g/dl; HCT: 16.2%) and thrombocytopenia (platelets: 45 × 10^9/L), he developed neutrophilic leukocytosis with WBC gradually reaching 24.61 × 10^9/L; Neutrophils 77%, Bands 7%, Blasts 2%, Metamyelocytes 6%, Myelocytes 2%, Monocytes 0% and Lymphocytes 6%. The neutrophil granulocytes showed severe dysplasia (Fig. 2). A third BM examination was performed that showed a hypercellular BM (90% cellularity) with a marked neutrophilic proliferation characterized by

* Corresponding author.
https://doi.org/10.1016/j.lrr.2021.100248
Received 22 February 2021; Received in revised form 28 April 2021; Accepted 10 May 2021
Available online 18 May 2021
2213-0489/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license

https://creativecommons.org/licenses/by-nc-nd/4.0/
Fig 1. A, B, and C: (A) blood smear showing anisopoikilocytosis and one morphologically unremarkable neutrophil; B and C: bone marrow biopsy showing an erythroid predominance associated with dysmegakaryopoiesis, the latter better appreciated at higher magnification (C).

Table 1
Genetic alterations identified by target sequencing in the patient’s MDS and aCML.

| GENE  | Chr | Position | Type           | HGVS DNA         | HGVS Protein      | Effect               | MDS VAF | aCML VAF |
|-------|-----|----------|----------------|------------------|-------------------|----------------------|---------|----------|
| ASXL1 | 20  | 31022441 | Insertion      | c.1934_1935insG  | p.Gly646Trpfs*12  | frameshift           | 20.5%   | 42.3%    |
| GATA2 | 3   | 128200140| Deletion       | c.1160_1165delCCATGA | p.Thr387_Met388del | in-frame deletion    | 26.2%   | 46.0%    |
| GATA2 | 3   | 128204690| Insertion      | c.750_751insC    | p.Ser251Leufs*31  | frameshift           | 3.4%    | 35.0%    |
| GATA2 | 3   | 128204771| Substitution   | c.670G>T         | p.Glu224*         | nonsense             | 2.1%    | 6.1%     |
| GATA2 | 3   | 128204878| Deletion       | c.563delC        | p.Thr188Argfs*30  | frameshift           | 2.6%    | ND*      |
| KDM6A | X   | 44879857 | Substitution   | c.446C>A         | p.Ala149Glu       | Missense             | 14.2%   | NC*      |
| NRAS  | 1   | 115258747| Substitution   | c.35G>C          | p.Gly12Ala        | Missense             | ND      | 14.3%    |
| NRAS  | 1   | 115258745| Substitution   | c.37G>C          | p.Gly13Arg        | Missense             | ND      | 4.3%     |
| PTPN11| 12  | 112888165| Substitution   | c.181G>C         | p.Asp61His        | Missense             | ND      | 9.1%     |
| U2AF1 | 21  | 44514777 | Substitution   | c.470A>C         | p.Gln157Pro       | Missense             | 20.5%   | 49.5%    |

*ND, not detected; *NC, not covered.

Fig 2. A, B, and C: (A) blood smear showing red blood cells with anisopoikilocytosis and neutrophilic leukocytosis with dysplastic neutrophils displaying a combination of hypo-segmented nuclei (i.e., pseudo-Pelger-Huet anomaly) and abnormal nuclear hyper-segmentation, in addition to 10% immature granulocytic forms (note one blast and one myelocyte); B: bone marrow aspirate showing myeloid predominance with dysplasia. The marrow is markedly hypercellular (90%) due to a granulocytic proliferation. Presence of dysmegakaryopoiesis can be appreciated in the corresponding biopsy (C).
dysgranulopoiesis (>10%) and dysmegakaryopoiesis (>10%) (Fig. 2). The blast count was 4%. The diagnosis was that of an atypical neutrophilic progression resembling the MDS/MPN subtype of aCML.

BM karyotype showed 46-47,XY,+mar[cp2]/46,XY[28]. BM fluorescent in situ hybridization (FISH) for trisomy 8 performed on interphase cells using the 8centromere/9centromere probes showed normal results. Sequential metaphase FISH using the 8cen/9cen probe set further confirmed that the marker chromosome seen in both the metaphases was not derived from either of them. In addition, BM FISH for BCR-ABL1 showed normal result for BCR/ABL/ASS1(CML) panel (nuc ish (ABL,ASS1)x2)[192/200],[BCRx2][191/200]. Mutational analysis by target sequencing was performed in a different laboratory on a peripheral blood sample and results are shown in Table 1. The U2AF Q57P, GATA2 in-frame deletion and ASXL1 frameshift mutations were also found but now at a higher allele variant frequencies. In addition, novel PTPN11 and NRAS mutations were identified (Table 1). The patient refused additional supportive care and died due to sepsis two months later, approximately one and half year after the beginning of his disease symptoms.

3. Discussion

MDS is a group of diseases characterized by marrow failure caused by ineffective hematopoiesis. MDS lack a proliferative features except for the presence of isolated thrombocytosis which is mostly limited to the cytogenetically defined subgroups of MDS with isolated del5q or MDS with inv(3)(q21.3) [1]. The MDS/MPN group of diseases although they share with MDS several features e.g. morphologic dysplasia and cytopenia (more often anemia), differ from MDS by having some proliferative features i.e. the elevation of at least one of the non-erythroid blood cell categories (leukocytosis, monocytosis or thrombocytosis) [1]. In adult patients, the MDS/MPN include CMML, aCML, and MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) [1]. Up to one-third of MDS patients will eventually transform to AML, the risk of such transformation being strongly dependent on the number of blasts and cytogenetic abnormalities as well as the presence of specific mutations [1].

Although transformation to AML is a relatively common and widely recognized form of disease progression in MDS, its evolution to an MDS/MPN is much less frequent. Published evidence has shown that some CMML cases had evolved from a preexisting MDS [2–5]. In these older reports prior to the NGS era, no genetic changes were found to be associated with this types of progression. More recently, Singh ZN et al. who studied three cases of MDS followed by CMML showed no evidence of cytogenetic changes at the time of progression, although a JAK2 V617F mutation was documented in one case [6]. Unfortunately, no prior JAK2 test result was available, thus is impossible to conclude whether the mutation was preexistent or acquired at the time of progression. However, the acquisition of JAK2 V617F mutation may have been relevant because in cases of MDS with ring sideroblasts the occurrence of this mutation has been considered instrumental to their transformation to a MDS/MPN-RS-T [7].

In contrast to the evolution to MDS/MPN-RS-T or to CML, transition from MDS to aCML is exceptionally rare. In 1998, Del Canizo et al. published a series of 80 MDS in which 6 showed a MDS/MPN type progression with 5 evolved to CMML and 1 to aCML [3]. An additional case was published in 2016 by Grisaev SV et al., [8] a therapy-related MDS which transformed to aCML. Its transformation was associated with the acquisition of additional cytogenetic abnormalities including monosomy 17.

The presence of isolated trisomy 8 as identified in our case is intriguing in view of a recent report from a French Study group describing a subset of patients with MDS and isolated trisomy 8 which showed at the time of diagnosis or during the course of disease, one or more of the following “myeloproliferative neoplasm-like features”: WBC >10 × 10⁹/L; circulating immature granulocytes mostly myelocytes and metamyelocytes; ≥2% palpable splenomegaly [9]. These results seem to be congruent with the notion that the presence of trisomy 8 is capable of producing an enhanced proliferative activity with increased colony formation in vitro [10,11]. The disappearance of the trisomy 8 seen in our third BM sample, confirmed by FISH analysis although a rare event, has previously been described in the literature. In one study based on serial BM of five MDS patients, there was a significant variation in the proportion of trisomy 8 positive cells in repeated samples obtained during the course of the disease and in one patient, the chromosomal anomaly eventually disappeared completely [12]. The fluctuation in the degree of positivity and the observed disappearance were not in relation to treatment or disease progression. In our case which also showed a similar fluctuating frequency, one could hypothesize a subclonal role of the trisomy 8 which never reached above a 50% frequency and finally disappeared.

At the molecular level, MDS/MPN diseases are more likely to carry gene mutations associated with the activation of growth factor signaling pathways in conjunction with mutations in epigenetic regulators or splicing factors associated with morphologic dysplasia [13]. In our patient, although no cytogenetic progression was observed, the transformation was paralleled by the acquisition of secondary mutations. In the MDS sample, the U2AF Q57P likely represents the main clonal alteration. Its association with trisomy 8 is infrequent, found in only 1 of 75 (1.3%) patients with myeloid malignancies [14]. Several subclonal mutation in GATA2, ASXL1 and KDM6A were also seen in the MDS sample. GATA2 mutations can be germline and associated with a predisposition to MDS/AML, or somatically acquired. Somatic GATA2 mutations are not common and only seen in less than 5% of the MDS [2]. The GATA2 variants seen in our case include one more dominant variant involving an in-frame deletion at the second zinc finger of GATA2 and three minor frameshift or nonsense variants that can result in a truncated protein devoid of the two DNA-binding zinc fingers. All these GATA2 variants are somatic and pathogenic. Comparing the mutation profiles of the prior MDS with that of the subsequent aCML, 3 of the 4 GATA2 variants (except p.T188Rfs*30) were shared between the two specimens, as were the ASXL1 and U2AF1 mutations. Unfortunately, it is not possible to determine the status of the KDM6A mutation seen in the MDS sample since the KDM6A gene was not covered in the panel used for analysis of the aCML sample. Overall, however, based on the variant allele frequencies (VAF) of the different variants, we can hypothesize that a minor pre-existing clone harboring the GATA2 p.T384_M388del and p.S251Lfs*31 mutations, as well as the ASXL1 and U2AF1 mutations in the MDS becomes the dominant tumor clone at the time of tumor progression (to aCML). The subclonal acquisition of novel PTPN11 and NRAS mutations, known indicators of adverse prognostic significance, has likely been an additional important contributing factor in the observed progression, in line with the previous observation that implicated RAS mutations in the evolution of the myelodysplastic-type chronic myelomonocytic leukemia (CMML) to its myeloproliferative variant [15]. In addition, PTPN11 and RAS mutations can be acquired during disease progression in MDS [16,17]. Mutations in these genes are likely to result in activation of growth signaling pathways, leading to increased cell proliferation with transition to an MDS/MPN type disease phenotype.

Thus our case illustrates that mutational profiles can change during follow-up of MDS and that repeated molecular testing can be helpful in confirming disease progression and may at least partially explain the morphologically and phenotypic shift which can be observed in a subset of these cases.

Declaration of Competing Interest

None of the authors has any conflict of interest.
References

[1] S.H. Swerdlow, E. Campo, N.L. Harris, et al., (Eds.), WHO Classification of Tumours of Haematopoietic and Lymphoid tissues, Updated 4th Edition, IARC Press, Lyon, France, 2017.

[2] Y. Hasegawa, N. Sakai, M. Toyama, et al., Chronic myelomonocytic leukemia transformed from refractory anemia with ring sideroblasts with a rare abnormal chromosome, inv(12), Rinsho Ketsueki 31 (1) (1990) 75–79.

[3] M.C. Del Canizo, A. Bruufau, A. Mota, et al., The value of cell cultures for the diagnosis of mixed myelodysplastic/myeloproliferative disorders, Haematologica 83 (1) (1998) 3–7.

[4] G.E. Verhoef, H. Desmyttere, P. Zachee, et al., Myelodysplastic syndrome evolving into a myeloproliferative disorder: one disease or two [letter]? Leukemia 8 (2) (1994) 714–715.

[5] S.A. Wang, N. Galili, J. Cerny, et al., Chronic myelomonocytic leukemia evolving from preexisting myelodysplasia shares many features with de novo disease, Am. J. Clin. Pathol. 126 (5) (2006) 789–797.

[6] Z.N. Singh, G.R. Post, E. Kiwan, A.M. Maddox, Cytopenia, dysplasia, and monocytosis: a precursor to chronic myelomonocytic leukemia or a distinct subgroup? Case reports and review of literature, Clin. Lymphoma Myeloma Leuk. 11 (3) (2011) 293–297.

[7] L. Malcovati, M.G. Della Porta, D. Pietra, et al., Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis, Blood 114 (17) (2009) 3538–3545.

[8] S.V. Gritsaev, I.I. Kostroma, I.M. Zapreev, et al., Transformatiya vtorichnogo mielodisplasticheskogo sindroma v atipichniy khronicheski mieloleikoz u bol’noi ostym mieloidnym leikozom (Transformation of secondary myelodysplastic syndrome to atypical chronic myeloid leukemia in a female patient with acute myeloid leukemia), Ter Arkh 88 (7) (2016) 104–108. Russian.

[9] L. Drevon, A. Marceau, O. Maarek, et al., Myelodysplastic syndrome (MDS) with isolated trisomy 8: a type of MDS frequently associated with myeloproliferative features? A report by the Groupe Francophone des Myelodysplasies, Br. J. Haematol. 182 (6) (2018) 843–850.

[10] E.M. Sloand, L. Pfannes, G. Chen, et al., CD34 cells from patients with trisomy 8 myelodysplastic syndrome (MDS) express early apoptotic markers but avoid programmed cell death by up-regulation of antiapoptotic proteins, Blood 109 (6) (2007) 2399–2405.

[11] S.R. Yousef, M.M. Ismail, E. Abd Al Wahed, H Al Dessoky, Growth advantage of CD34+ cells in trisomy 8 high-risk myelodysplastic syndrome despite enhanced apoptotic signals, East Mediterr. Health J. 18 (10) (2012) 1065–1071.

[12] A. Iwabuchi, K. Ohyashiki, J.H. Ohyashiki, et al., Trisomy of chromosome 8 in myelodysplastic syndrome. Significance of the fluctuating trisomy 8 population, Cancer Genet. Cytogenet. 62 (1) (1992) 70–74.

[13] M.M. Patnaik, T.L. Lasho, Genomics of myelodysplasia/myeloproliferative neoplasm overlap syndromes, Hematol. Am. Soc. Hematol. Educ. Program 2020 (1) (2020) 450–459.

[14] S.Y. Kim, K. Kim, B. Hwang, et al., The high frequency of the U2AF1 S34Y mutation and its association with isolated trisomy 8 in myelodysplastic syndrome in Asians, but not in Caucasians, Leuk. Res. 61 (10) (2017) 96–103.

[15] C. Ricci, E. Fermo, S. Corbi, et al., RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant, Clin Cancer Res 16 (8) (2010) 2246–2256.

[16] C.Y. Chen, L.I. Lin, J.L. Tang, et al., Acquisiton of JAK2, PTPN11, and RAS mutations during disease progression in primary myelodysplastic syndrome, Leukemia 20 (6) (2006) 1155–1158.

[17] R. Bejar, What biologic factors predict for transformation to AML? Best Pract. Res. Clin. Haematol. 31 (4) (2018) 341–345.