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Case report

FLT3 mutation and *AML/ETO* in a case of Myelodysplastic syndrome in transformation corroborates the two hit model of leukemogenesis

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Abstract

The aim of this report is to present a case of Myelodysplastic syndrome (MDS) who presented, during AML transformation, a step-wise genetic progression that corroborates the two hit model of leukemogenesis. A RCDM-RS (WHO)/RARS (FAB) patient with normal karyotype at diagnosis, evolved into AML after six months of follow up. At transformation, AML/ETO fusion was detected, although marrow blast cells were not increased until 21 days later, when FLT3-ITD was also demonstrated pointing out that the overgrowth of the FLT3/ITD clone was concomitant with the outburst of marrow blasts. These findings corroborates the two hit model of leukemogenesis in which one class of mutations (Class I) (FLT3/ITD) confers a proliferative or survival advantage to cells, and a second class of mutations (Class II) (AML/ETO) interferes with hematopoietic differentiation.
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1. Introduction

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by ineffective hematopoiesis, peripheral cytopenias and an additional risk to evolve to acute leukemia (AL) in up to 30% of the cases [1]. Chromosomal abnormalities represent the single most important prognostic factor for predicting acute leukemia transformation [2]. Clonal chromosomal abnormalities have been reported in 30–50% of primary MDS patients while patients with normal karyotype comprise a heterogeneous group with an unpredictable outcome [1]. Cytogenetics and molecular data provided evidence that MDS has a step-wise genetic progression [1]. In adult MDS, P53 mutations, hypermethylation of P15 and internal tandem duplication of *FLT3* are associated with disease progression and evolution to AML [1,3].

FMS-like tyrosine kinase 3 (FLT3) gene, a member of the class 3 receptor tyrosine kinase family is expressed on hematopoietic progenitor cells and mediates stem cell proliferation and differentiation [3]. Recently, an internal tandem duplication (ITD) in the *FLT3* region encoding the justamembrane domain has been reported in AML patients with poor prognosis [3]. This mutation results in constitutive activation of FLT3 kinase with subsequent activation of proliferative pathways [4]. In addition, point mutations of tyrosine kinase domain of FLT3 have also been associated with a gain of function phenotype [3].

The aim of this report is to present a case of MDS [5] who presented, during AML transformation, a step-wise genetic progression that corroborates the two hit model of leukemogenesis in which one class of mutations (Class I) (in this case, FLT3) confers a proliferative or survival advantage

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to cells, and a second class of mutations (Class II) (in this case, AML-ETO) interferes with hematopoietic differentiation [14]. At the sixth month of follow up, during routine evaluation, bone marrow smears showed normal blast cell counts but karyotype presented a rare finding of *t*(8;21) plus dup(9q) with AML1/ETO rearrangement confirmed by FISH. At the seventh month of follow up, the patient demonstrated a FLT3-ITD mutation and bone marrow smears showed 60% of myeloid blasts.

2. Case report

A 60-year-old woman sought for medical attention due to progressive dyspnea. Blood counts revealed: hemoglobin = 9.6 g/dL, MCV = 100 fl, MHC = 30 pg, normal reticulocyte count, white blood cell count (WBC) = 2200/uL with neutrophils = 1672/uL (76%), lymphocytes = 374/uL (17%) and monocytes = 154/uL (7%), platelet count of 133.000/uL. Bone marrow aspiration showed a normocellular marrow with G/E ratio = 1/1, erythroid hyperplasia with moderate dysplasia, dysmegakaryocytopoiesis with micromegakaryocytes and blast cell count of 1.0%. Prussian blue stain demonstrated 20% of ring sideroblasts. Bone marrow biopsy showed a hypercellular marrow with mild dysplasia and fibrosis grade I. Bone marrow karyotype was 46,XX[20]. The diagnosis of refractory cytopenia with multilineage dysplasia (RCDM) and ringed sideroblasts (WHO) or refractory anemia with ringed sideroblasts (RARS) (FAB) was established. The International Prognostic Scoring System risk (IPSS) [2] was assigned Intermediate I. At sixth month of routine follow up, symptoms, blood counts, bone marrow blast cell count and bone marrow biopsy were unchanged, except for bone marrow karyotype that showed: 46,XX,*t*(8;21)(q22;q22),dup(9q)(q13)[5]/46,XX[13]. FISH for AML/ETO confirmed the rearrangement in 46% of nuclei interphase cells, but did not show any rearrangement in the sample kept from diagnosis (fixed in Carnoy‘s medium), ruling out an undetected translocation at diagnosis. FLT3-ITD mutation was investigated and resulted negative at this moment. According to WHO classification, transformation was considered due to the occurrence of *t*(8;21). Chronic renal insufficiency precluded more aggressive treatment. Twenty-one days later, WBC was 26,000/uL with 60% of myeloid blast cells. A new bone marrow biopsy showed

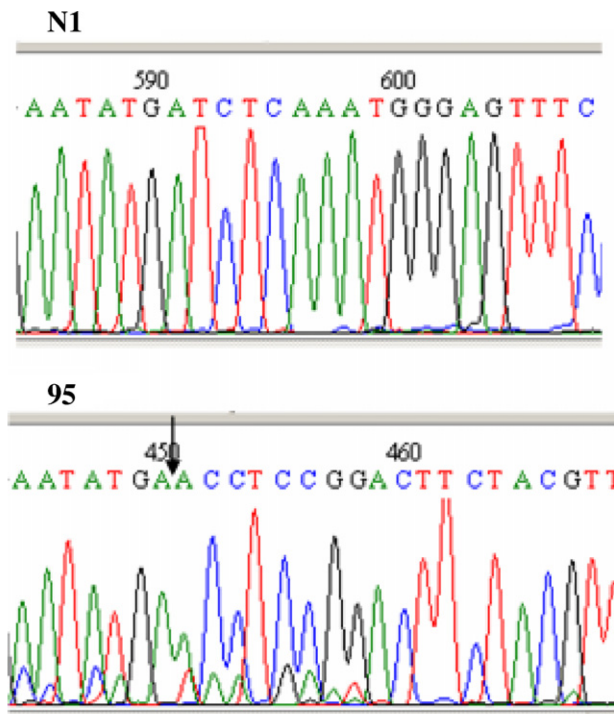

Fig. 1. Sequencing confirmed the ITD with 34 bp. N1 = normal control.

an increase of myeloid blast cells with fibrosis grade I, compatible with transformation into AML. The imunophenotype profiling was CD13, CD33, CD34, CD117, DR, CD7 and CD56 positive. FLT3-ITD was detected and confirmed by DNA sequencing (Fig. 1). Karyotype analysis showed the same abnormality, but now in 9 metaphases: 46,XX,*t*(8;21)(q22;q22),dup(9q)(q13)[9]. The patient died due to sepsis (Fig. 2).

3. Discussion

The case presented here demonstrates a step-wise progression in a myeloid disorder: a RCDM-RS (WHO)/ RARS (FAB) patient with normal karyotype at diagnosis, evolved into AML after six months of follow up. At transformation, AML/ETO fusion was detected, although marrow blast cells were not increased until 21 days later, when FLT3-ITD was also demonstrated pointing out that the overgrowth of the

Diagnosis ⇓	Sixth Month Follow up ⇓	Seventh Month ⇓
Blasts = 1%	Blasts =1%	Blasts = 60%
KT = normal	KT=t(8;21),dup(9q)[5]	KT=t(8;21),dup(9q)[9]
FISH AML/ETO = negative	FISH AML/ETO = positive	FISH AML/ETO=positive
FLT3-ITD = absence	FLT3-ITD = absence	FLT3-ITD = Presence

Fig. 2. At the MDS diagnosis, the karyotype and FISH were normal and FLT3-ITD was absent. At the sixth month, the karyotype presented a *t*(8;21) and dup(9q) which were confirmed by FISH, but marrow blasts were just 1%. At the seventh month, the cytogenetics abnormalities were the same, but the patient presented 60% of blast cells and the presence of FLT3-ITD. KT: karyotype (G-banding).

FLT3/ITD clone was concomitant with the outburst of marrow blasts.

To exclude the possibility of an AML with *t*(8;21) not diagnosed at presentation, FISH in the fixed stored sample at diagnosis was negative for AML/ETO rearrangement [6–8].

The translocation *t*(8;21) is one of the most frequent chromosomal abnormalities, detected in 12% of all AML patients and in up to 40% of those classified as FAB-M2 [9] while in our institution the incidence is of 10.5% [10]. The translocation targets AML1 (RUNX1), a member of the RUNX family characterized by a DNA-binding *Runt* domain at the amino terminus that is retained in the fusion gene [9]. This domain is necessary for DNA binding and heterodimerization of AML1 with CBFB, the non-DNA-binding subunit of the complex. Although the AML1/CBFB complex seems to be a key regulator of definitive hematopoiesis, it is not completely understood how the AML1-ETO fusion gene contributes to leukemogenesis yet. One key mechanism seems to be the suppression of AML1- and C/EBPα-dependent activation of genes responsible for myeloid development [9]. However, numerous murine "in vivo" models showed that AML1-ETO on its own is not able to induce leukemia and additional steps are necessary to transformation [11].

Likewise, Kelly et al. [12], reported the induction of an oligoclonal myeloproliferative disease in a FLT3-ITD mouse model, but an AML phenotype was not created. The mutant FLT3 appears to present an activity that is similar to other constitutively activated tyrosine kinases such as BCR/ABL, TEL/PDGFBR and TEL/JAK2^{V617F}, all of them associated with a myeloproliferative phenotype in humans [3].

Recently, Schessl et al. [13] demonstrated, for the first time that retrovirally engineered coexpression of *AML1-ETO* and *FLT3-ITD* potently synergized to trigger the development of aggressive leukemia in a murine transplantation model. Mice engrafted with AML1-ETO/FLT3-ITD-coexpressing bone marrow cells succumbed to an aggressive acute leukemia after a median latency time of 233 days post-transplantation (according to this model, these two abnormalities were demonstrated as being able of inducing acute leukemia).

These findings corroborates the two hit model of leukemogenesis in which one class of mutations (Class I) (as example, FLT3/ITD) confers a proliferative or survival advantage to cells, and a second class of mutations (Class II) (AML/ETO) interferes with hematopoietic differentiation [14]. These data do not preclude the possibility that additional mutations may be required for the acute leukemia, but suggest that at least two classes of mutations are necessary. In the present case, the AML/ETO interfered with hematopoietic differentiation and FLT3-ITD provided the proliferative phenotype which was demonstrated by the outburst of blast cells. Besides the *t*(8;21), the patient also showed a rare duplication of the long arm of chromosome 9 (dup(9q)), which could be associated with an increased activity of possible oncogenes.

Notwithstanding, it has been shown in murine models that AML1/ETO may exert its leukemogenic function in cooperation with the expression of different genes as WT1 [15], N-RAS [13] or c-KIT [16], due to point mutations in different hematopoieteic tyrosine kinases that contribute to the pathogenesis of the remaining cases without FLT3-ITD.

We believe that new reports studying these mutations in a prospective way, as here presented, could encourage the systematic screening of activating mutations in MDS patients, in an attempt to elucidate the steps involved in acute leukemia transformation.

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References

[1] List AF, Vardiman J, Issa JP, DeWitte TM. Myelodysplastic syndromes. Hematology (Am Soc Hematol Educ Prog) 2004:297–317.

[2] Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluation of prognosis in Myelodysplastic syndromes. Blood 1997;89(6):2079–88.

[3] Kiyoi H, Naoe T. FLT3 in human hematologic malignancies. Leuk Lymphoma 2002;43(8):1541–7.

[4] Frohling S, Schelenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, et al. Prognostic significance of activating FLT3 mutation in younger adults (16–60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML study group Ulm. Blood 2002;100:4372–80.

[5] Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997. Ann Oncol 1999;10(12):1419–32.

[6] Hagemeijer A, de Klein A, Wisjman J, van Meerten E, de Greef GE, Sacchi N. Development of an interphase fluorescent in situ hybridization (FISH) test to detect *t*(8;21) in AML patients. Leukemia 1998;12(1):96–101.

[7] Klaus M, Haferlach T, Schnittger S, Kern W, Hiddemann W, Schoch C. Cytogenetic profile in the novo acute myeloid leukemia with FAB subtypes M0,M1 and M2: a study based on 652 cases analysed with morphology, cytogenetics and fluorescence in situ hybridization. Cancer Genet Cytogen 2004;155(1):47–56.

[8] Kozlov I, Beason K, Yu C, Herghson M. Cd79a expression in acute myeloid leukemia with *t*(8;21) and the importance of cytogenetics in the diagnosis of leukemias with immunophenotypic ambiguity. Cancer Genet Cytogenet 2005;163(1):62–7.

[9] Kuchenbauer F, Feuring Buske M, Buske C. ML1-ETO needs a partner: new insights into the pathogenesis of *t*(8;21) leukemia. Cell Cycle 2005;4(12):1716–8.

[10] Chauffaille MLLF. A importância da citogenética em leucemia mielóide aguda e síndrome mielodisplásica [Tese de livre docência]. São Paulo: Universidade Federal de São Paulo; 2003.

[11] Moore MA. Converging pathways in leukemogenesis and stem cell self-renewal. Exp Hematol 2005;33(7):719–37.

[12] Kelly LM, Liu Q, Kutok JL, Williams JR, Boulton Cl, Gilliland DG. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. Blood 2002;99: 310–8.

[13] Schessl C, Rawat VP, Cusan M, Deshpande A, Kohl TM, Rosten PM, et al. The AML1–ETO fusion gene and the FLT3 length mutation collaborate in inducing acute leukemia in mice. J Clin Invest 2005;115(8):2159–68.

[14] Gilliland DG. Hematologic malignancies. Curr Opin Hematol 2001;8:189–91.

[15] Nishida S, Hosen N, Shirakata T, Kanato K, Yanagihara M, Nakatsuka S, et al. AML1-ETO rapidly induces acute myeloblastic leukemia in cooperation with the Wilms tumor gene, WT1. Blood 2006;107(8): 3303–12.

[16] Beghini A, Peterlongo P, Ripamonti CB, Larizza L, Cairoli R, Morra, et al. C-kit mutations in core binding factor leukemias. Blood 2000;95(2):726–7.