AML1/MTG16 fusion gene from a t(16;21)(q24;q22) translocation in treatment-induced leukemia after breast cancer

We present a new case of secondary acute myeloid leukemia (sAML) with a t(16;21)(q24;q22)-AML1/MTG16 documented by conventional and molecular cytogenetics. Clinical and cytogenetic data of all other published cases are reviewed. The t(16;21)(q24;q22) is the most frequent balanced translocation of 21q22/AML1 in sAML. Topoisomerase II inhibitors play a critical role in inducing the AML1/MTG16 fusion gene.

The q22 band of chromosome 21 carries AML1 (CBFA2), which is a critical gene for hematopoiesis, undergoing fusion with ETO (MTG8) gene at 8q22 in approximately 10-15% cases of acute myeloid leukemia (AML). AML1 also fuses to ETV6 in the most frequent translocation of childhood acute lymphocytic leukemia (ALL), the t(12;21)(p13;q22). Other chromosomal rearrangements involving AML1 in myelodysplastic syndromes (MDS) and AML are a t(1;21) (p36;q22), a t(3;21)(q26;q22), a t(5;21)(q13;q22) and a t(17;21) (q11;q22). The t(16;21)(q24;q22) is a rare abnormality described in therapy-related leukemia and MDS in which the fusion partner of AML1, at 16q24, is the MTG16 gene.[1] It belongs to the same gene family as MTG8 with which it shares significant homology.

A 62-year old woman was admitted in July 1999 because of fatigue and fever. In 1984 she underwent surgery for breast cancer. In 1990 and in 1996 she received chemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil, mitomycin, mitoxanthrone, cisplatin, and VP16) because of lung metastases. Hematologic investigations showed: hemoglobin 8.2 g/dL, white cell count 2.9×10^9/L (24% neutrophils, 72% lymphocytes, 4% monocytes), platelet count 23×10^9/L. Bone marrow aspirate and biopsy were consistent with a diagnosis of AML (>30% of blasts) and trilineage myelodysplasia. Massive infiltration from plasma cells was noted. The secondary AML was not treated because of severe respiratory failure due to development of pneumonia. Bone marrow karyotype at diagnosis was as follows: 46,XX [3 cells], 46,XX, t(16;21)(q24;q22) [2], 47,XX,+8,t(16;21)(q24;q22) [10].

Results from M-FISH (multitarget fluorescence *in situ* hybridization) (Spectravysion) and paintings for chromosome 16, labeled with digoxigenin, and chromosome 21, labeled with biotin, are illustrated in Figure 2A and 2B. FISH results with a mixture of two PAC clones, pac70A4 and pac122F9 for the MTG16 gene at 16q24 (Dr. F Hosoda, National Cancer Center Research Institute, Tokyo, Japan), labeled with biotin, and PAC 1107L6 for the AML1 gene at 21q22 (Dr. M. Rocchi, University of Bari, Italy) labeled with digoxigenin, are shown in Figure 2C The t(16;21) is a subtle change and only locus-specific probes definitively proved the MTG16/AML1 fusion. Neither painting nor M-FISH unravelled the exchange as a reciprocal one.

Cytogenetic and clinical data in Table 1 show that the t(16;21) is typically seen in iatrogenic MDS and AML with a median latency of three years between drug exposure and appearance of the secondary disease (Table 1).[2,3-6] The translocation is always associated with additional chromosomal changes, especially trisomy 8 (5 cases), which is striking in AML after breast cancer (3/3). Rearrangements at 21q22 are known as consistent changes in secondary disorders. Two balanced translocations involving AML1, t(8;21) and t(3;21), were included by Pui and Rellings among epipodophyllotoxin-induced aberrations.[7] Both these translocations, however, have also been related to protocols containing anthracyclines.[8,9] Recently, Hromas *et al.* focused on radiation as a critical genotoxic insult leading to rearrangements of 21q22 and AML1 gene.[10] All nine cases with t(16;21) received topoisomerase II inhibitors while radiotherapy and/or alkylating agents were present in the treatment protocols of six cases. Moreover the schedule of patient 4 (etoposide+carboplatin) suggests that topoisomerase II inhibitors are sufficient to induce the t(16;21)-associated secondary leukemia. Interestingly, in the treatment of this patient there was also a platinum component which is thought to potentiate the leukemogenic effect of topoi-

Table 1. Reported cases with t(16;21)(q24;q22).

No.	Gender/Age	FAB subtype	Additional aberrations	Previous malignancy	Treatment	Interval between treatment and malignant hemopathy (years)	Survival after AML diagnosis (months)	Ref.
1	F/<15	AML-M1	no	-	-	-	-	3
2	F/55	AML-M1	+8	T-NHL	CHOP, radiotherapy	4	1.5	4
3	M/42	Hypoplastic MDS	no	no	no		12	2
4	F/73	MDS	+8	Lung cancer	etoposide, carboplatin	3	8	2
5	F/53	MDS	add(7q), del(13q), del(1q)	Oviductal ca.	carboplatin, doxorubicin, cyclophosphamide, tegafur	2	12	2
6	F/60	AML	del(7q)	T-NHL	etoposide, mitoxanthrone cyclophosphamide	1	12+	5
7	F/42	AML-M2	+8	Breast ca.	mitoxanthrone cyclophosphamide, 5-fluorouracil, vincristine, radiotherapy	3	30+	6
8	F/39	AML-M2	+8	Breast ca.	mitoxanthrone, cyclophosphamide, 5-fluorouracil, vincristine, tamoxifen. radiotherapy	4	24*	6
9	F/62	AML-M2	+8	Breast ca.	cyclophosphamide, methothrexate, 5-fluorouracil, mitomycin, mitoxanthrone, etoposide, cisplatin radiotherapy	9	1	Present

*F, female; M, male; ca.: cancer; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; * The patient died from metastases of the previous malignancy, being in complete remission from her leukemia; Ref, references; -, not available; +, alive.*

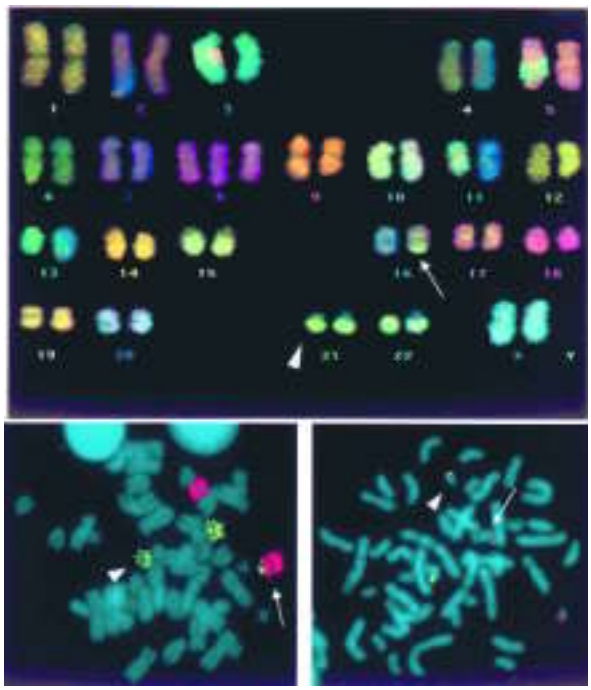

Figure 2. A) Example of M-FISH showing trisomy 8 and the presence of extra material belonging to chromosome 21 on one chromosome 16 (arrow). Material from chromosome 16 is not detected in the der(21) (arrowhead). B) FISH with painting chromosome 16 (red) and painting chromosome 21 (green). The normal chromosome 16 is red whereas the der(16) (arrow) has both the red and the green signals. Both normal 21 and der(21) (arrowhead) show only green signals. C) FISH with a mixture of pac70A4 and 122F9 (MTG16 gene) in green and pac 1107L6 (AML1 gene) in red. Both der (16) (arrow) and der(21) (arrowhead) show a red/green fusion signal.

somerase II inhibitors. Our case showed a peculiar morphologic finding in the bone marrow, that is massive plasmacytic infiltration classified as a reactive phenomenon on the basis of polyclonal immunoglobulin production and absence of trisomy 8 in bone marrow plasma cells (data not shown). Reactive plasmacytosis has been recognized in about 6% of adult AML, including secondary disorders. The pathogenetic mechanism leading to this phenomenon is unclear. Paracrine production of interleukin-6 by leukemic blasts and immunologic response to the tumor have been put forward as possible explanations.

This is the first report of t(16;21) associated with reactive bone marrow plasmacytosis.

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