Cancer Genetics 238 (2019) 69–75

Contents lists available at ScienceDirect

Cancer Genetics

journal homepage: www.elsevier.com/locate/cancergen

Case Report

Cytogenomic characterization of double minute heterogeneity in therapy related acute myeloid leukemia

Prasad Koduru*, Weina Chen, Barbara Haley, Kevin Ho, Dwight Oliver, Kathleen Wilson

Department of Pathology, and Division of Hematology and Oncology, Department of Medicine, UT Southwestern Medical Center, Dallas, USA

ARTICLE INFO

Article history:
Received 20 May 2019
Revised 24 July 2019
Accepted 6 August 2019

Keywords:
Breast cancer
Double minutes
Secondary AML
MYC
KMT2A
Amplification

ABSTRACT

Breast cancer patients treated with adjuvant chemotherapy regimens containing alkylating agents and anthracyclines are at an increased risk for secondary myeloid malignancies, either acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Complex genomic changes (karyotypes and/or gene amplification) accompany the development of the secondary neoplasms. Here we present a unique case of a breast cancer patient who developed secondary AML within 18 months of treatment with trastuzumab, pertuzumab, docetaxel, carboplatin (TCHP) and radiation. Leukemia cells had catastrophic alterations in chromosomes 8, 11, and 17. Genetic abnormalities in the leukemia cells included amplification of *MYC* and *KMT2A* as double minutes, and deletion and mutational inactivation of *TP53* Concurrent amplification of different genes at different levels and on different double minutes, we have named "double minute heterogeneity." Clinically, this case highlights the need to identify genes amplified in secondary myeloid malignancies by cytogenomic microarray (CMA) analysis since these may have therapeutic implications.

Introduction

Somatic perturbations such as translocation, mutation, deletion, gene amplification and epigenetic modification in proto-oncogenes and/or tumor suppressor genes may result in unscheduled expression of proto-oncogenes, or altered function of tumor suppressor genes, or cause gene overexpression in tumors. Gene amplification is a unique form of genomic instability in the cancer genome and is often considered a biomarker for aggressive disease that is resistant to therapy. While low level amplification is represented by copy number gains (trisomy or tetrasomy), high level amplification may manifest morphologically as a homogeneously staining region (HSR) at a particular chromosome band (intra-chromosomal) or as small light staining paired chromatin structures called double minutes (dmin) scattered in the nucleoplasm [1]. The number of dmin vary from one per cell to several per cell in the same tumor [2]. Dmin and/or HSR have been reported frequently in advanced stage tumors concurrently with complex karyotypes both in diagnostic specimens and in specimens obtained in-therapy or post-therapy [3]. Occasionally dmin are reported as one of the primary genetic aberrations in leukemia [4–6].

Gene amplification is relatively frequent in solid tumors, but it is infrequent in hematopoietic tumors [7]. In AML, or MDS the mechanism for gene gain is most commonly gain of an entire chromosome such as chromosome 8, 11, or 21, whereas *KMT2A* (*MLL*), *MYC* and *RUNX1* are amplified infrequently [8–11].

KMT2A encodes a transcriptional coactivator that regulates global gene expression during early embryogenesis and hematopoiesis [12]. Rearrangements (translocations and/or deletions) in this gene are relatively common in hematopoietic proliferations and more than 130 genomic partners have been identified [13–15]. High level amplification of *KMT2A* is infrequent but has been reported in AML, MDS and in therapy related B-acute lymphoblastic leukemia [10,16–18]. Amplification of this gene resulting in overexpression has been associated with cell proliferation, increased survival and differentiation arrest [19].

MYC plays a crucial role in signal transduction pathways that promote the cell cycle [20]. *MYC* is frequently rearranged in B-cell neoplasms, and amplified in solid tumors [21]. Myeloid neoplasms are commonly characterized by trisomy 8 resulting in a copy number gain of *MYC*, but it is rarely amplified. Ectopic expression of *MYC* has been shown to result in inducing AML in mice [22]. Nevertheless, whether amplification of *MYC* or other genes in the amplicon at 8q24.1 contributes to aggressive oncogenic proliferation remains controversial [23].

Earlier studies of dmin in hematological tumors with FISH probes or CMA identified incorporation of *MYC* or *KMT2A* or neither in dmin [3]. Here we present a case of secondary AML with dmin some with amplified *MYC* and the others with amplified *KMT2A* concurrently, a phenomenon of double minute heterogeneity not reported previously.

* Corresponding author.
E-mail address: prasad.koduru@utsouthwestern.edu (P. Koduru).

https://doi.org/10.1016/j.cancergen.2019.08.001
2210-7762/

Materials and methods

Morphology and flow cytometry

Peripheral blood (PB) and bone marrow (BM) aspirate smears were stained with Wright-Giemsa. Core biopsy and clot section were processed and stained with hematoxylin and eosin (HE) and by immunohistochemistry (IHC). Flow cytometric immunophenotyping was performed on the BM aspirate using 10-color (BD FACSCanto^{TM}) flow cytometer (Becton Dickinson, San Jose, CA) and analyzed using cluster analysis with Cytopaint Classic Software (Leukobyte, CA). Samples were processed and stained with a panel of myeloid, lymphoid and immature cell markers.

Cytogenetics and fluorescence in situ hybridization (FISH)

A BM aspirate obtained at diagnosis and at relapse was harvested for metaphases directly and after culturing for 24 hrs following standard protocols (culturing in RPMI1640 supplemented with 10% FCS and 1% L-glutamine). Metaphase spreads were G-banded, analyzed and karyotypic nomenclature given following ISCN [24].

The probes for 5/5q-, 7/7q-, cep 8, 20q-, *KMT2A*, and the probes for *RUNX1T1*/*RUNX1, ABL1*/*BCR, PML*/*RARA*, and *CBFB* were used to detect MDS and AML related genetic alterations. A dual-color break-apart probe covering *MYC*, and a tricolor probe for *FGFR1* were used additionally to further characterize rearrangements in chromosome 8. Probes for centromere 17 and *TP53* were used to establish loss of the short arm of 17. All probes but *FGFR1* were purchased from Abbott molecular (Des Plaines IL); *FGFR1* probe was purchased from Cytocell (Tarrytown, NY).

Cytogenomic microarray (CMA)

CMA analysis was performed with the DNA extracted from BM using an Agilent CGH+SNP 180k microarray platform (Agilent GGXChip + SNP v1.0 4-plex CGH 180k) according to the manufacturer's protocol (Agilent Technologies, Santa Clara, CA). Data interpretation was performed using Genoglyphix software (Perkin Elmer, Waltham, MA). Gain or loss of three contiguous probes was considered as a genomic aberration and annotated according to the human genome build GRCh37/hg19.

Molecular genetics

For *TP53* analysis, exons 4–11 were Sanger sequenced using DNA extracted from formalin-fixed paraffin embedded sections of the bone marrow aspirate using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). Amplicons generated using lab-developed M13-tailed PCR primers for each exon were treated with ExoSAP IT (ThermoFisher, Austin, TX) and then bi-directionally sequenced using BigDye v3.1 chemistry (ThermoFisher). Sequencing products were harvested with XTerminator purification reagents (ThermoFisher) and run on an ABI 3730xL DNA analyzer. All DNA sequences were manually reviewed.

Results

The patient was a 63 year old female diagnosed in August 2015 with a HER 2-positive stage ll invasive ductal breast carcinoma and treated with TCHP chemotherapy for 6 cycles followed by surgery and breast radiation.

Eighteen months post diagnosis, she complained of fatigue and the exam was remarkable for skin pallor and petechiae. Blood counts revealed a WBC 13,320 with 46% blasts, Hgb 7.6, Hct 23.6, and 29,000 platelets. Bone marrow confirmed an AML with 80% marrow blasts.

Three months later she began 7 + 3 induction therapy with cytarabine and idarubicin. Repeat BM one month later showed persistent leukemia with 80% blasts. A month later tumor lysis syndrome developed with uric acid 8.8 mg and creatinine > 2. Aggressive hydration, allopurinol, rasburicase, transfusions, and leukemia treatment with dacogen and hydroxyurea failed to control the WBC. Acidosis, acute kidney injury and respiratory failure developed. Despite antibiotics, antifungals, steroids, fluids, respiratory and dialysis support, the patient status worsened. The family requested transition to comfort care and she expired 21 months from diagnosis.

Morphologic and immunophenotypic findings

A complete blood smear analysis at admission showed anemia (Hgb7.6 g/L), leukocytosis (white blood cells 13.28×10^3/ul) with left-shifted granulocytes, increased myeloblasts (46%), and thrombocytopenia (29×10^3/ul). BM aspirate and clot section/core biopsy showed hypercellularity (90% cellularity) with a myeloid blast predominance (~80%). Blasts were medium to large in cell size with fine chromatin, prominent nucleoli, variable eosinophilic granules, and moderate cytoplasm (Figs. 1a and 1b). Immunohistochemical stains showed that blasts expressed *MYC* (Fig. 1c) and *TP53* (Fig. 1d). Flow cytometric immunophenotypic analysis of BM aspirate revealed myeloid blasts with the following immunophenotype: CD4(dim+), CD13(predominantly -), CD14(-), CD15(variably+), CD19(subset partial+), CD33(+), CD34(subset +), CD36(few+), CD38(+), CD45(+), CD56(-), CD64(partial+), CD79a(-), CD117(variably+), HLA-DR(variably +), MPO(+), and TdT(partial +). Given the morphologic and immunophenotypic findings and the history of prior chemotherapy and radiation, a diagnosis of therapy-related AML was rendered.

Cytogenetics

All 20 metaphases analyzed from the diagnostic specimen had an abnormal karyotype constituting two clones, a near diploid clone and a near tetraploid clone; all metaphases had dmin. G-banded karyotype was described as 45,XX,add(7)(p22),−8,idic(11)(q11),add(17)(p11.2),dmin[*cp*15]/90<4n>,idemx2[cp5] (Fig. 2). A karyotype similar to the first clone was obtained from the specimen obtained at first relapse.

FISH results

Since the patient had pancytopenia at presentation, interphase FISH was requested and performed to detect chromosomal abnormalities specific to MDS (deletion 5q or monosomy 5, deletion 7q or monosomy 7, rearrangement or deletion of *KMT2A*, trisomy 8, deletion 20q), and specific to AML [t(8;21), t(15;17), t(9;22), inv(16)] using standard probes. Probes for 8 centromere (D8Z2), *RUNX1T1* (8q22) and *KMT2A* (11q23) showed aberrant signal pattern. All other probes showed normal signal pattern. The D8Z2 probe showed one normal signal and one diminished signal in interphase nuclei (Supplemental Figure 6). This suggested the presence of a partially deleted chromosome 8 centromere in the genome. *RUNX1T1* probe showed one signal suggesting loss of one copy of this gene in the leukemia cells. On metaphases the D8Z2 probe showed one normal signal on the normal chromosome 8 and a diminished signal on the short arm of the add(7)(p22) chromosome; this suggests that the additional material at 7p22 was derived from chromosome 8 (Fig. 3a). In order to further characterize this abnormal chromosome we hybridized metaphases with *FGFR1* probe (8p11.23p11.22) and with *RUNX1T1*

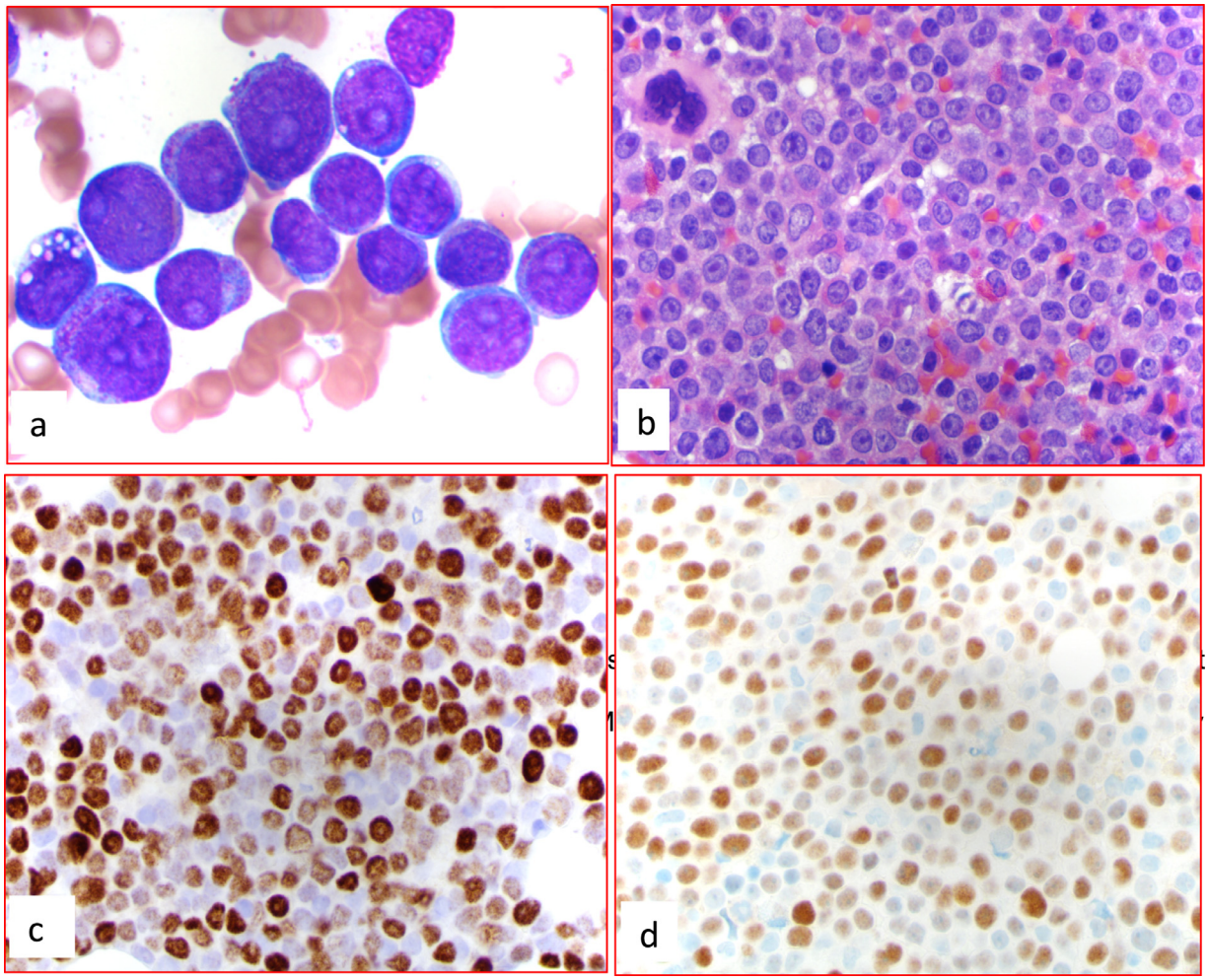

Fig. 1. (a): Medium-sized to large myeloid blasts in BM aspirate; (b): sheets of blasts in BM clot section; (c): blasts variably strongly positive for MYC (~80% of blasts) by IHC; (d): blasts variably positive for TP53 (~50% of blasts) by IHC.

(8q22) probe. *FGFR1* showed a signal in the middle of the added material at 7p22 (Fig. 3b), whereas the *RUNX1T1* had no signal on this chromosome. *MYC* was also absent on this chromosome (results shown later in Fig. 6b). Thus FISH results for different probes on chromosome 8 suggested complex alterations leading to loss, deletion, and rearrangement of different segments in one homologue.

The *KMT2A* probe showed amplification and partial deletion in interphase nuclei (Fig. 4a) (Supplemental Figure 1). On metaphases it showed a signal in the pericentromeric region of the idic(11) chromosome and cryptic insertion of the 3′-region into 17p11 (Fig. 4b).

CMA results

Since only some dmin were positive with the *KMT2A* probe, we performed CMA to detect the origin of the remaining dmin. Genomic CMA profiling identified six clinically significant losses and four clinically significant gains involving chromosomes 8, 11 and 17. The chromosome 8 profile showed four regions of genomic losses and one gain with the following karyotype: arr[GRCh37] 8q11.1q11.21(46923323_50491701)x1, 8q13.2q22.2(70115645_100654309)x1, 8q22.3q24.13(104314771_126137830)x1,8q24.13q24.21 (126170606_130917133) amp,8q24.21q24.3 (130958847_143242529)x1 (Fig. 5a). This was further confirmed by FISH with *MYC* break-apart probe (Fig. 6). Two of the regions of loss surrounded the amplified region which was 4.75 Mb and contained *MYC* (log ratio of >2.5). IHC staining for *MYC* showed strong positivity suggesting over expression of this gene (Fig. 1c). The chromosome 11 profile showed the following array karyotype: arr[GRCh37] 11p15.5p11.2 (205827_48388756)x3, 11q12.1q23.3(55896790_117046281)x1, 11q23.3(117109943_118471354) amp, 11q23.3(118563828_118924588)x3 (Fig. 5b). In the long arm the large 11q12.1q23.3 deletion was centromeric to *KMT2A*, and the region of gain at 11q23.3 contained the amplified *KMT2A* (log ratio of 2.5). The chromosome 17p loss encompassed the entire short arm: arr[GRCh37] 17p13.3p11.2(48858_22192306)x1 (Supplemental Figures 3, 4). From the combined results of G-band karyotype, FISH and CMA the karyotype of the leukemia cells was revised to 45,XX, der(7)(7qter->7p22::8q13->8q11.22::8p10->8pter),der(11)(11pter->11q12.1::11q23.3->11q25::11p11.2->11pter),add(17)(p11.2),dmin.

Molecular analysis

TP53 sequencing revealed a C141Y mutation (cysteine to tyrosine at amino acid 141) in exon 5, within the DNA binding domain of *TP53* (Supplemental Figure 5). This mutation is classified as Pathogenic/Likely Pathogenic in the ClinVar database and was previously shown to decrease expression of p53-regulated genes such as *BAX* and *p21* in osteosarcoma transfection assays [25].

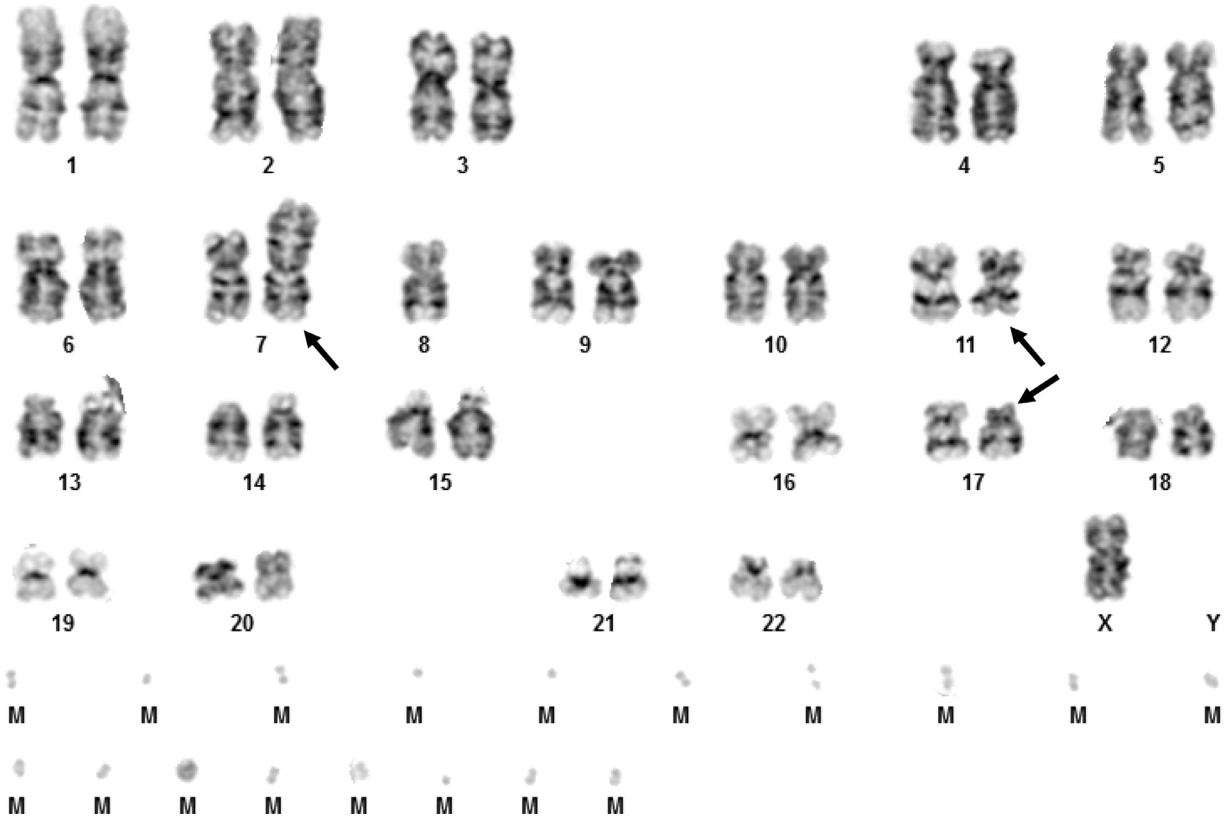

Fig. 2. G-banded karyotype of leukemia cells. Note - loss of second copy of X in this metaphase was non-clonal.

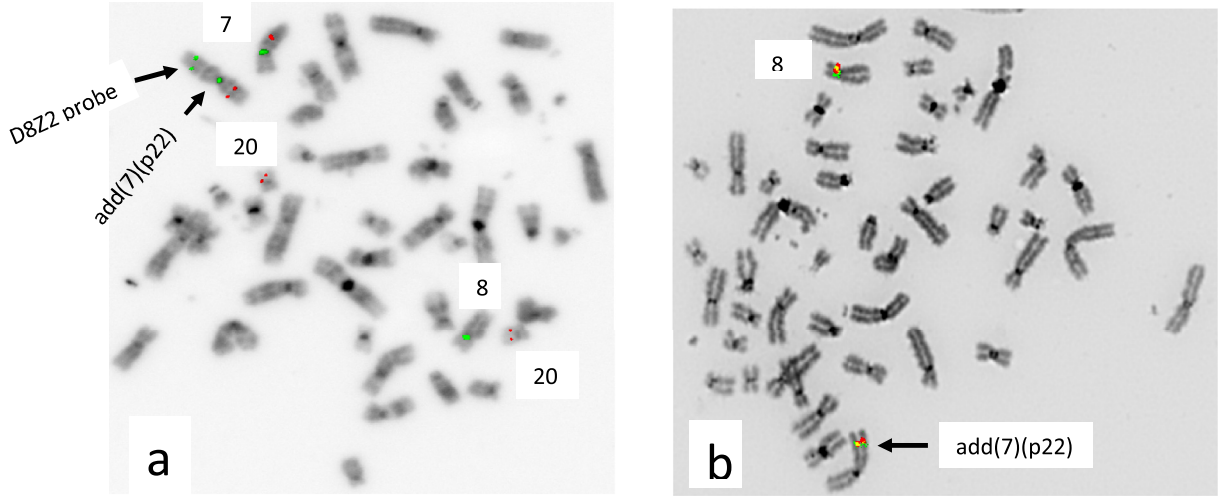

Fig. 3. Metaphase FISH. a. Probes for cep 7 (D7Z1 green), 7q31 (D7S486 red), cep 8 (D8Z2 green), and 20q11 (D20S108 red) hybridized simultaneously. The small green signal in the middle of the short arm of the add(7) chromosome probably was cep8 (arrow). b. FISH with *FGFR1* (break apart) – the signal was present in the middle of the add(7)(p22) chromosome (arrow).

Discussion

Genomic regions can amplify in-situ and produce an HSR at the site of amplification. Alternatively a genomic region can excise from the original site, form an episome by fusing the broken ends of the DNA double strand and amplify; this mechanism produces dmin in the nucleoplasm [26–29]. In the episomal model, one allele remains intact and the second allele is deleted and forms an episome. The degree of amplification may vary from a few copies per nucleus to many suggesting continued progression of amplification as the tumor cells multiply. Cases with gene amplification have complex genomic abnormalities and often have loss of *TP53* and/or have inactivating mutations in it [30]. Thus, generalized genomic instability induced by prior treatment may be a critical factor in the origin of gene amplification in tumors. The amplified segments, HSR or dmin, possibly harbor genes that confer resistance to standard therapy and promote aggressive clones and hence a poor prognosis. For example, in breast cancer amplification of HER2 is associated with an inferior prognosis [31], and in neuroblastoma amplification of *MYCN* is associated with advanced

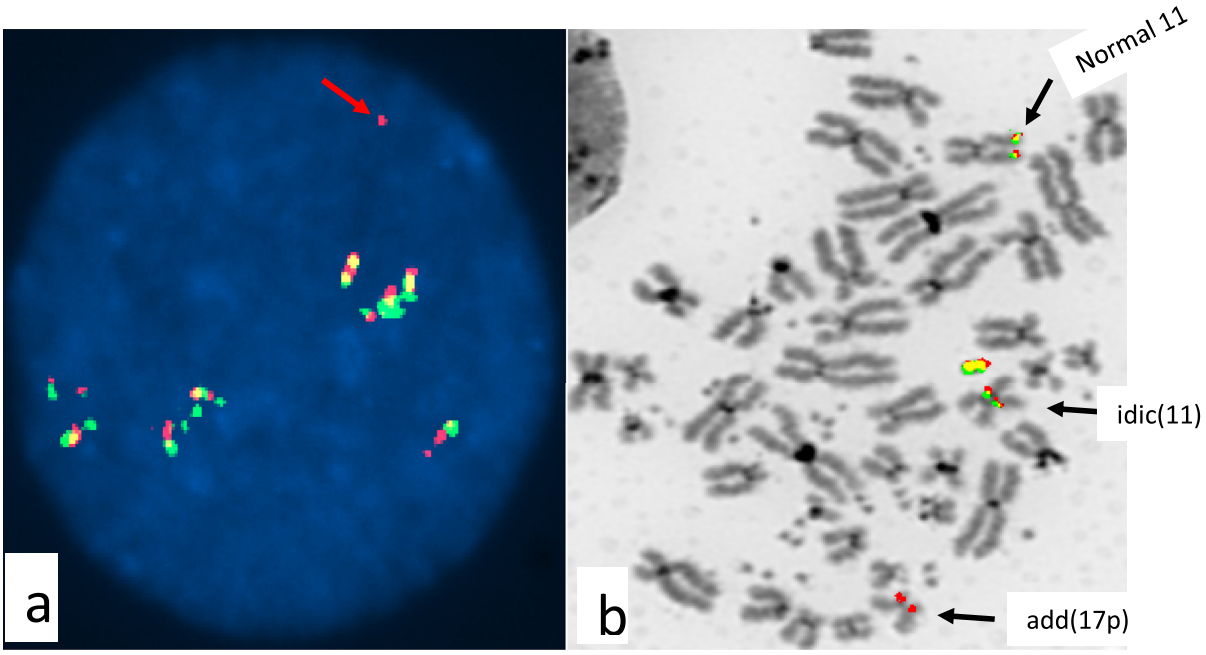

Fig. 4. FISH with *KMT2A* probe. a. An interphase nucleus showing amplification and partial deletion (arrow), b. metaphase FISH showing signal on the idic(11) chromosome, on add(17) chromosome and on a dmin.

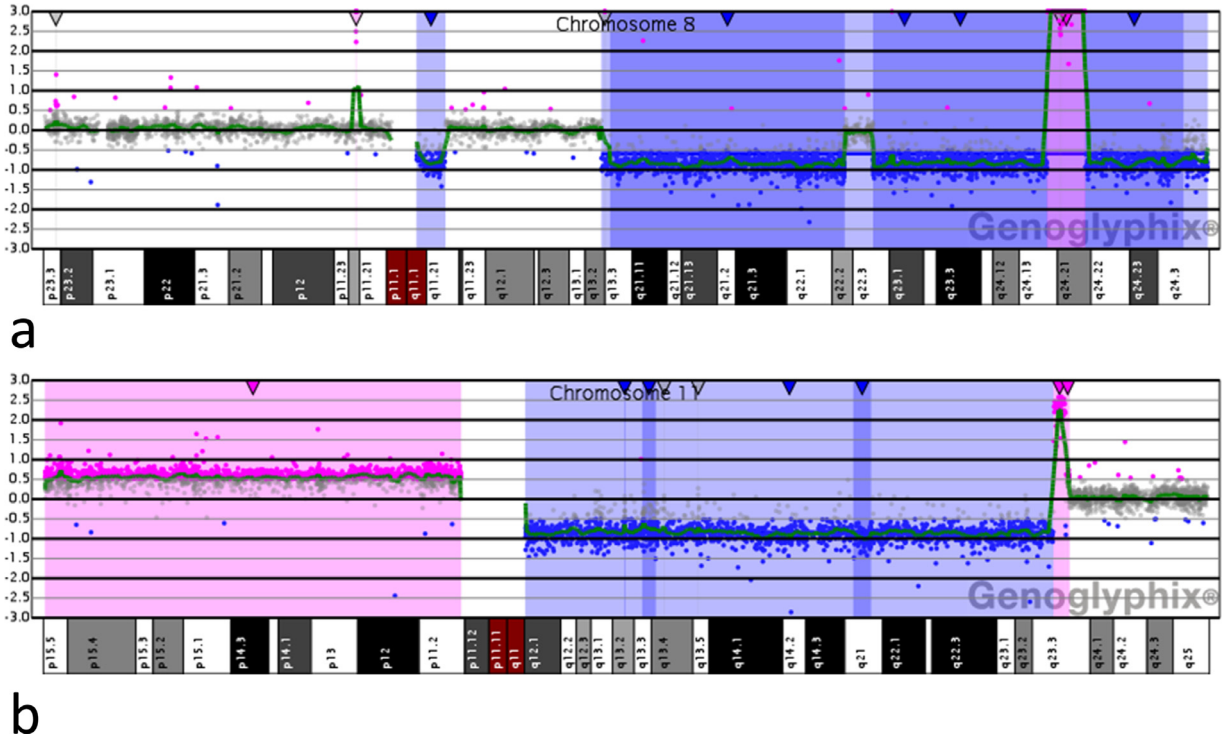

Fig. 5. CMA plots. a. chromosome 8 showing loss of most of 8q in different segments, and amplification of 8q24.13 region. b. chromosome 11 showing one copy gain for entire short arm, loss of most of long arm and amplification of 11q23.3 region (see text for details).

disease and poor prognosis [32]. Patients with amplified genes may be benefited by agents that target these genes. The dmin may also include proto-oncogenes, whose unscheduled expression is associated with tumorigenesis and/or progression [33,34].

The emergence of acute leukemia after breast cancer treatment has been reported and is usually associated with the use of type II topoisomerase inhibitors or alkylating agents [35–37]. But, patients treated with docetaxel and cyclophosphamide are not at an increased risk for secondary leukemia or MDS [38]. The leukemogenic potential of these two drugs in breast cancer is rarely reported. In the present case, the relatively acute onset of AML18 months after treatment with chemotherapy and radiation for the primary breast cancer mirrors the reports of acute leukemia after type II topoisomerase inhibitors and alkylators.

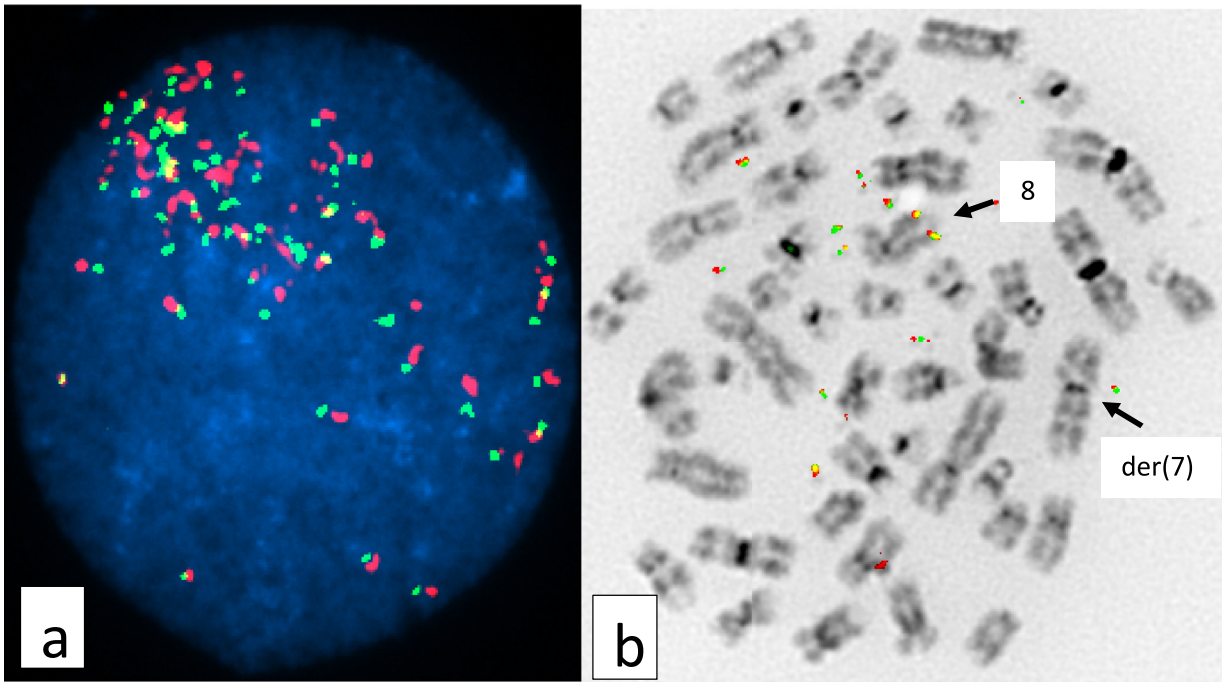

Fig. 6. FISH with *MYC* break-apart probe on a interphase nucleus, and (b) on a metaphase. Signal was present on the normal 8 and on some dmin, but not on the der(7).

In hematological neoplasms dmin are reported in de-novo and in treatment related AML. Frequently *MYC* or infrequently *KMT2A* are incorporated in the dmin. This is the first report in which both *MYC* and *KMT2A* are concurrently amplified in the same tumor but on different dmin. Common features in patients with *KMT2A* amplification include older age (about 67 years), complex karyotypes, deletion or mutational inactivation of *TP53*, poor response to treatment and short survival [3,39,40]. Clinical and genetic architecture of leukemic cells of this patient concur with these previous reports.

Catastrophic genetic events

Although the karyotype of the leukemia cells was complex, only three chromosomes (8, 11, and 17) were affected by a multitude of abnormalities that led to rearrangement, or deletion, or amplification of three important genes, namely *MYC, KMT2A* and *TP53*. The G-banded karyotype, FISH and CMA results all indicate catastrophic genetic events in one homologue of chromosomes 8 and chromosome 11. Chromosome 8 was monosomic for the most of 8q due to large segmental deletions; two of the four deletions on 8q surrounded the region of gain that contained *MYC*, which was amplified in the dmin. This suggests that a segment of 8q24.13 excised from its home-site, circularized into an episome, amplified, and overexpressed as detected by the IHC. This is in contrast to one study in which *MYC* present in dmin was not expressed [23]. Deletion in the long arm of chromosome 8 has been reported in AML patients with *MYC* amplification [23,41]. Since trisomy 8 in AML provides a mechanistic platform for higher expression of genes on this chromosome [42], the significant oncogenic event in this case was *MYC* amplification and over expression, which in turn promotes higher proliferation, genomic instability and poor outcome.

Chromosome 11 had multiple aberrations: a gain of the short arm, loss of most of 11q, and cryptic rearrangements involving the 11q23.3 region encompassing *KMT2A*. One event led to deletion of the 5′-region and cryptic insertion of the 3′-region of *KMT2A* into the short arm of abnormal 17. In the second event, chromatin containing *KMT2A* was excised and amplified as dmin. Two distinct tumorigenic perturbations in *KM2TA* have been implicated in leukemogenesis. The most frequent type of change both in de novo and in therapy related leukemias in children and in adults is rearrangement with different partners in the genome, forming fusion genes and producing fusion proteins. The second type of change, which is rare, is amplification reported in therapy related leukemia [43]. Alterations in *KM2TA* in secondary leukemia have been attributed to DNA damage induced by either chemotherapy or radiation therapy, or other cellular stress mechanisms [13].

The third event was loss of most of 17p and cryptic insertion of *KMT2A* into this abnormal chromosome. Loss of 17p lead to loss of one copy of *TP53*. Although tumor cells had one normal copy of 17, the *TP53* allele on this chromosome had mutations which has been reported in hematological malignancies [44,45]. Loss by deletion or mutational inactivation of *TP53* is more frequent (up to 40%–60%) in AML with complex karyotypes and gene amplification [3,40,45]. Furthermore, loss of *TP53* is documented to lead to genomic instability and gene amplification in tumors [46,47].

In summary, genomically this case had several novel findings not reported previously. The pattern of chromosomal losses and gain around 8q24.13 involving only one chromosome (chromosome 8) suggests excision and episomal amplification of *MYC*. In contrast, the pattern of losses and gains at 11q23.3 suggests that two mechanisms were involved: intrachromosomal transposition and extrachromosomal amplification in dmin. Segmental losses and gains in chromosome 8 and in chromosome 11 and concurrent amplification of *MYC* and *KMT2A*, and loss of 17p at the diagnosis of secondary AML suggests catastrophic genomic aberrations affecting chromosomes 8 and 11 in a leukemogenic progenitor cell. This insult to the genome may have had occurred during treatment of the previous malignancy. This is the first report of concurrent amplification of *MYC* and *KMT2A* on different dmin in a hematologic malignancy. We propose the name “double minute heterogeneity” for this previously undescribed phenomenon. This case also illustrates the need to perform CMA in cases of MDS and/or AML with complex abnormal karyotype and dmin, HSR or ring chromosomes in elderly patients to identify amplified genes. This information is useful in tailoring therapeutic regimen that is effective in disease management.

Acknowledgments

All authors report no conflict of interest or financial interest. The work in this manuscript was not supported by any funding.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.08.001.

References

[1] Cowell JK. Double minutes and homogeneously staining regions: gene amplification in mammalian cells. Ann Rev Genet 1982;16:21–59.
[2] Marinello MJ, Bloom ML, Doeblin TD, Sandberg AA. Double minute chromosomes in human leukemia. N Engl J Med 1980;303:704.
[3] Huh YO, Tang T, Talwalker SS. Double minute chromosomes in acute myeloid leukemia, myelodysplastic syndrome, and chronic myelomonocytic leukemia are associated with micronuclei, *MYC* or *MLL* amplification and complex karyotype. Cancer Genet 2016;209:13–320.
[4] Reddy KS, Sulcova V. c-myc amplification in a preleukemia patient with trisomy 4 and double minutes: review of the unique coexistence of these two chromosome abnormalities in acute myelogenlous leukemia. Cancer Genet Cytogenet 1997;95:206–9.
[5] Govberg IJ, Wolf JL, Cotter PD. Trisomy 4 and double minutes in acute myeloid leukemia: further evidence that double minutes can occur as the primary cytogenetic abnormality. Cancer Genet Cytogenet 2000;121:212–15.
[6] Receveur A, Ong J, Merlin L, Azgui Z, Merle-Béral H, et al. Trisomy 4 associated with double minute chromosomes and MYC amplification in acute myeloblastic leukemia. Ann Genet 2004;47:423–7.
[7] Rayeroux KC, Campbell JJ. Gene amplification in myeloid leukemias elucidated by fluorescence in situ hybridixation. Cancer Genet Cytogenet 2009;193:44–53.
[8] Li YS. Double minutes in acute myeloid leukemia. Int J Cancer 1983;32:45–459.
[9] Bruckert P, Kappler, et al. Double minutes and c-MYC amplification in acute myelogenous leukemia: Are they prognostic factors? Cancer Genetic Cytogenet 2000;120:73–9.
[10] Streubel B, Valent P, Jager U, et al. Amplification of the MLL gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. Genes Chromosomes Cancer 2000;27:380–6.
[11] Andersen MK, Christiansen DH, Pedersen-Bjergaard J. Amplification or duplication of chromosome band 21q22 with multiple copies of the AML1 gene and mutation of the TP53 gene in therapy-related MDS and AML. Leukemia 2005;19:197–200.
[12] Hess JL, Yu BD, Li B, Hanson R, et al. Defects in yolk sac hematopoiesis in MLL-null embryos. Blood 1997;90:1799–806.
[13] Gole B, Wiesmüller L. Leukemogenic rearrangements at the mixed lineage leukemia gene (MLL)-multiple rather than a single mechanism. Front Cell Dev Biol 2015;25(3):41 eCollection 2015. doi:10.3389/fcell.2015.00041.
[14] Afrin S, Zhang CRC, Meyer C, Stinson CL. Targeted next-generation sequencing for detecting MLL gene fusions in leukemia. Mol Cancer Res 2017 Nov 13. doi:10.1158/1541-7786.MCR-17-0569.
[15] Meyer C, Burmeister T, Gröger D, Tsaur G, et al. The MLL recombinome of acute leukemias in 2017. Leukemia 2017 Jul 13. doi:10.1038/leu.2017.213.
[16] Avet-Loiseau H, Godon C, Li JY, Daviet A, et al. Amplification of the 11q23 region in acute myeloid leukemia. Genes Chromosomes Cancer 1999;26:166–70.
[17] Michaux L, Wlodarska I, Stul M, et al. MLL amplification in myeloid leukemias: a study of 14 cases with multiple copies of 11q23. Genes Chromosomes Cancer 2000;29:40–7.
[18] Andersen MK, Christiansen DH, Kirchhoff M, et al. Duplication or amplification of chromosome band 11q23, including the unrearranged MLL gene, is a recurrent abnormality in therapy-related MDS and AML, and is closely related to mutation of the TP53 gene and to previous therapy with alkylating agents. Genes Chromosomes Cancer 2001;31:33–41.
[19] Popovic R, Riesbeck LE, Velu CS, et al. Regulation of mir-196b by MLL and its overexpression by MLL fusions contributes to immortalization. Blood 2009;113:3314–22.
[20] Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008;8:976–90.
[21] George DL. Amplification of cellular proto-oncogenes in tumors and tumor cell lines. Cancer Surv 1984;3:497–513.
[22] Luo H, Li Q, O'Neal J, Kreisel F, et al. c-Myc rapidly induces acute myeloid leukemia in mice without evidence of lymphoma-associated antiapoptotic mutations. Blood 2005;106:2452–61.
[23] Storlazzi CT, Fioretos T, Paulsson K, Strömbeck B, et al. Identification of a commonly amplified 4.3 mb region with overexpression of C8FW, but not MYC in MYC-containing double minutes in myeloid malignancies. Hum Mol Genet 2004;13:1479–85.
[24] ISCN. An international system for human cytogenetic nomenclature. An international system for human cytogenetic nomenclature. MCGowman-Jordan J, Simons A, Schmid M, editors. Basel: Karger; 2016.
[25] Xu H, El-Gewely MR. Differentially expressed downstream genes in cells with normal or mutated p53. Oncol Res 2003;13(6-10):429–36 PMID12725534].
[26] Wahl GM. The importance of circular DNA in mammalian gene amplification. Cancer Res 1989;49:1333–40.
[27] Von Hoff DD. New mechanisms of gene amplification in drug resistance (the episome model). Cancer Treat. Res 1991;57:1–11.
[28] Vogt N, Lefèvre SH, Apiou F, Dutrillaux AM, et al. Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. Proc Natl Acad Sci U S A 2004;101:11368–73.
[29] Storlazzi CT, Lonoce A, Guastadisegni MC, Trombetta D, et al. Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. Genome Res 2010;20:1198–206.
[30] Christiansen DH, Andersen MK, Pedersen-Bjergaard J, et al. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 2001;19:1405–13.
[31] López-Guerrero JA, Llombart-Cussac A, Noguera R, et al. HER2 amplification in recurrent breast cancer following breast-conserving therapy correlates with distant metastasis and poor survival. Int J Cancer 2006;118:1743–9.
[32] Schwab M, Amler LC. Amplification of cellular oncogenes: a predictor of clinical outcome in human cancer. Genes Chromosomes Cancer 1990;1:181–93.
[33] Karube K, Ying G, Tagawa H, et al. BCL6 gene amplification/3q27 gain is associated with unique clinicopathological characteristics among follicular lymphoma without BCL2 gene translocation. Mod Pathol 2008;21:973–8.
[34] Zou D, Yi S, Cui R, Liu W, et al. BCL-2 and MYC gain/amplification is correlated with central nervous system involvement in diffuse large b cell lymphoma at leukemic phase. BMC Med Genet 2017;18(16):16. doi:10.1186/s12881-017-0381-z.
[35] Patt DA, Duan Z, fang S, et al. Acute myeloid leukemia after adjuvant breast cancer therapy in older women: understanding risk. J Clin Oncol 2007;25:3871–6.
[36] Wolff AC, Blackford AL, Viswanathan K, et al. Risk of marrow neoplasms after adjuvant breast cancer therapy: the national comprehensive cancer network experience. J Clin Oncol 2015;33:340–8.
[37] Freedman RA, Seisler DK, Foster JC, et al. Risk of acute myeloid leukemia and myelodysplastic syndrome among older women receiving anthracycline-based adjuvant chemotherapy for breast cancer on moderan cooperative group trials (Alliance A151511). Breast Cancer Res Treat 2017;161:363–73.
[38] Rosenstock AS, Niu J, Giordano SH, et al. Acute myeloid leukemia and myelodysplastic syndrome after adjuvant chemotherapy: A population-based study among older breast cancer patients. Cancer 2018. doi:10.1002/cncr.31144.
[39] Dolon M, McGlennen, Hisrch B. MLL amplification in myeloid malignancies: clinical, molecular, and cytogenetic findings. Cancer Genet Cytogenet 2002;134:93–101.
[40] Tang G, Dinardo C, Zhang L, et al. MLL amplification in acute myeloid leukemia and myelodysplastic syndrome is associated with characteristic clinicopathological findings and TP53 gene mutation. Hum Pathol 2015;46:65–73.
[41] Christacos NC, Sherman L, Roy A, et al. Is the cryptic interstitial deletion of 8q24 surrounding MYC a common mechanism in the formation of double minute chromosome? Cancer Genet Cytogenet 2005;161:90–2.
[42] Schoch C, Kohlmann A, Dugas M, et al. Impact of trisomy 8 on expression of genes located on chromosome 8 in different AML subgroups. Genes Chromosomes Cancer 2006;45:1164–8.
[43] Fernandes CM, Hamerschlak N, Kishimoto RK, et al. Radiotherapy-related acute myeloid leukemia with *KMT2A* amplification. Br J Haematol 2016;172:653.
[44] Koduru P, Raju K, Vadmal V, et al. Correlation between mutation in P53, p53 expression, cytogenetics, histologic type, and survival in patients with B-cell non-Hodgkin's lymphoma. Blood 1997;90:4078–91.
[45] Zatkova A, Merk S, Wendehack M, et al. AML/MDS with 11q/MLL amplification show characteristic gene expression signature and interplay of DNA copy number changes. Genes Chromosomes Cancer 2009;48:510–20.
[46] Livingstone LR, White A, Sprouse J, et al. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 1992;70:923–35.
[47] Yin Y, Tainsky MA, Bischoff FZ, et al. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 allele. Cell 1992;70:937–48.